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Impact, host range and chemical ecology of the lily beetle, *Lilioceris lilii*

by

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DECLARATION

I hereby declare that I have composed this thesis. The work of this thesis is a record of my own work; any collaborative work has been specifically acknowledged.

Andrew Salisbury
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ABSTRACT

The lily beetle (*Lilioceris lilii,* Coleoptera: Chrysomelidae) has a univoltine life cycle and a limited host range (*Lilium, Fritillaria* and *Cardiocrinum*). A survey of 237 professional growers indicates that *L. lilii* is a problem for many in the UK lily industry.

A three-year field trial using six different *Lilium* indicated that the species *Lilium regale* is more resistant to *L. lilii* than the hybrids *L.* 'Tiber', *L.* 'Brindisi', *L.* 'Conca d'Or', *L.* 'Eliganzer' and *L.* 'Golden Joy'. Phenology observations between the trial and an established population of the beetle have provided base-line data to which further observations can be compared. Future host susceptibility trials should use a standard lily such as *L. regale* against which others can be compared.

Behavioural bioassays using a linear-track olfactometer demonstrated that the responses of *L. lilii* to hosts and conspecifics are at least in part odour-mediated. Significantly more diapaused females moved into airstreams containing the odour of intact hosts over clean air, to that of hosts and beetles combined over that of undamaged or manually-damaged plants, and into airstreams from intact plants over larval-infested plants. Pre-diapause males moved into the airstreams of intact hosts in preference to *L. lilii*-infested plants.

Using air entrainment, gas chromatography (GC) and coupled gas chromatography-mass spectrometry (GC-MS), headspace volatiles from beetle-infested host plants have been collected and identified. From these compounds, methyl salicylate, nonanal, *cis*-jasmone, linalool, 6-methyl-5-hepten-2-one and β-pinene elicited electrophysiological responses from *L. lilii* using electroantennography (EAG) and coupled GC-EAG. Bioassays indicate that diapaused female beetles move into the airstream of clean air in preference to *cis*-jasmone.

Investigations from all areas of the project have progressed our understanding of the ecology of *L. lilii* but further studies are needed before more effective control strategies can be developed.

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ABBREVIATIONS

ANOVA	Analysis of variance
DEFRA	Department for Environment, Food and Rural Affairs, UK Government
d.f.	Degrees of freedom
EAG	Electroantennogram
GC	Gas chromatography
MS	Mass spectrometry
HDC	Horticultural Development Council
LTO	Linear track olfactometer
NS	Not significant
RHS	Royal Horticultural Society
RI	Retention Index
SD	Standard deviation
SED	Standard error of the difference

GLOSSARY

Descriptions of the following terms were taken from Dethier (1960), Nordlund and Lewis (1976), Pasteur (1982), Hokkanen (1991), Pickett et al. (1997) and Cox (2007).

Allomone: A chemical that mediates interactions between individuals of different species, where the producing organism benefits but not the emitter.

Antifeedant: (Deterrent) A chemical which inhibits feeding when present in a place where insects would in its absence, feed.

Arrestant: A chemical that causes insects to aggregate in contact with it, the mechanism of aggregation being kinetic or having a kinetic component.

Attractant: A chemical which causes insects to make orientated movements towards its source.

Kairomone: A chemical that mediates interactions between individuals of different species, where the receiving organism benefits but not the emitter.

Pheromone: A chemical that mediates an interaction between organisms of the same species.

Push-pull: An approach to pest management where a crop is protected by semiochemicals, some making it less attractive to a pest whilst at the same time, other semiochemicals are used to make a trap crop or trap more attractive so that pathogens or pesticides can be deployed there to control the pest.

Repellent: A chemical which causes insects to make orientated movements away from its source.

Semiochemical: A chemical involved in the chemical interaction between organisms.

Thanatosis: The feigned death response shown by some terrestrial animals.

Trap crop: Plant stands grown to attract pests away from the main crop where management can be carried out more economically.

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CHAPTER 1. INTRODUCTION: THE BIOLOGY OF THE LILY BEETLE, *Lilioceris lilii* (Scopoli) (COLEOPTERA: CHRYSOMELIDAE)

The lily beetle, *Lilioceris lilii* (Scopoli) is a bright red leaf beetle (Chrysomelidae: Criocerinae) which has become a pest of lilies (*Lilium*: Liliaceae) in the UK and parts of North America (Salisbury 2003b, Casagrande and Kenis 2004). This review outlines the biology and distribution of *L. lilii*, gives current management practices and discusses avenues of research which could improve management prospects for the beetle.

Figure 1.1. *Lilioceris lilii* adult.

1.1. DESCRIPTION

Adult *L. lilii* are typical of the criocerine form (Labeyrie 1963), being approximately 8 mm long, bright red with a black head and legs (Figure 1.1). *Lilioceris lilii* is one of 142 described *Lilioceris* species, the largest concentration of which occurs in China (Berti and Rapilly 1976). Two other species of *Lilioceris* occur in Central Europe, the Onion beetle *L. merdigera* (L.) and *L. tibialis* (Villa). *Lilioceris merdigera* is similar in appearance to *L. lilii* but has a red head and legs (with the exception of joints and tarsi, Berti and Rapilly 1976). *Lilioceris merdigera* F. is a synonym of *L. lilii* (Scop.) (Fowler 1890), which can lead to confusion in some older publications; for example Stephens (1839) refers to *Crioceris merdigera* L. although the beetle described feeds on *Lilium* and is black with red thorax and elytra, and so is clearly *L. lilii* (Scop.). *Lilioceris tibialis* is found on wild *Lilium* in the Alps and is distinguished from *L. lilii* by its red tibiae (Berti and Rapilly 1976). In this review the assumption has been made that references to *L.* (*Crioceris*) *lilii* or *merdigera* feeding on *Lilium* or *Fritillaria* refer to the lily beetle and

those concerning a pest of *Allium* spp., *L. merdigera*. Some difficulty remains with articles referring to *Lilioceris* on other plant genera (see section 1.3). Adult *L. lilii* can fly (Cox 2001) but additional literature on this behaviour has not been encountered.

The eggs of *L. lilii* are approximately 1.0 x 0.5 mm (Figure 1.2). Immediately after oviposition they are bright orange and covered in a orange-red sticky layer, which aids adherence to the leaf surface; as they mature the eggs darken (Reinecke 1910, Müller and Rosenberger 2006).

Figure 1.2. *Lilioceris lilii* eggs on a *Lilium* leaf.

Lilioceris lilii larvae (Figure 1.3) are a rather humped eruciform type, dirty orange-red with a dark head and legs, and when mature they are 8 to 10 mm in length (Fox Wilson 1942). The abdomen has regulary disposed dark areas each with two bristles and first instar larvae have an egg bursting spine laterally on the first abdominal segment (Cox 1994). The larvae are normally covered in their own mucilaginous excreta derived from a dorsally situated anal opening (Balachowsky and Mensil 1936), which is distributed over the dorsal surface of the larva using abdominal bristles (Emmel 1936). The four larval instars can be identified by the size of the head capsule (Livingston 1996).

Pupation occurs in the soil beneath the host plant where a ‘silken’ cocoon incorporating soil particles is constructed (Nolte 1939). The pupa is orange-red and glabrous with a densely microspiculate abdominal cuticle, Cox (1996) gives a detailed description.

Figure 1.3. *Lilioceris lilii* larvae on *Lilium regale*.

1.2. LIFE CYCLE

Adult *L. lilii* have been recorded in every month of the year (Cox 2001) and it is this stage that overwinters among plant debris, in soil and similar hibernacula but not necessarily near host plants (Fox Wilson 1942, Haye and Kenis 2004). Adults have been observed on hosts from early spring (Lataste 1931, Haye and Kenis 2004), but may continue to emerge until June (Halstead 1989). Mating behaviour is not well studied, however adults appear to detect one another over long distances and approach each other whilst oscillating their antennae which may indicate the presence of a pheromone (Emmel 1936). A preoviposition period of two weeks following diapause occurs under laboratory conditions (Haye and Kenis 2004). In the field gravid females and eggs have been observed from late March (Cox 2001). Mating may occur before each oviposition (Nolte 1939) which can occur until September (Haye and Kenis 2004). Females can produce 200 to 367 eggs in one season (Lataste 1932, Fox Wilson 1942). It was thought adults could produce eggs in a second season (Lataste 1932) but this has been shown to be untrue (Haye and Kenis 2004).

Eggs are laid in linear groups of 2-16 on the ventral leaf surface parallel to the leaf veins (Emmel 1936, Müller and Rosenberger 2006). Eclosion occurs after 4-10 days (Balachowsky and Mensil 1936, Haye and Kenis 2004), although incubation times of three weeks have been noted (Cox 2001).

Hatchling larvae feed together on the ventral epidermis of the leaf, leaving the dorsal epidermis intact. Later instars consume the entire leaf, usually from the margin and move upwards to undamaged leaves as foliage is devoured (Nolte 1939, Haye and

Kenis 2004). Larvae will also feed on flowers, seed capsules and the epidermis of the stem and after heavy attacks only the desiccated stem remains (Fox Wilson 1942, Brown 1946). Larvae feed for 10-24 days before entering the soil and constructing silken cocoons at a depth of 3 to 4 cm (Reinecke 1910, Haye and Kenis 2004). Pupation takes place in the cocoon after a mean of 8.9 days at 22 °C and the entire subterranean phase lasts a mean of 20.2 days at 22 °C (Haye and Kenis 2004).

New generation adults are said to occur from mid May to October, but may be most numerous in July (Balachowsky and Mensil 1936, Cox 2001). However, it is not clear how these authors distinguished 'new' adults from those that had previously overwintered. A rigorous study indicates that the first new adults emerge in July (Haye and Kenis 2004). It has been suggested that new adults produce a second generation and that three generations are possible in a year (Lataste 1932, Balachowsky and Mensil 1936), but observations (Fox Wilson 1942, Halstead 1989) and laboratory rearing (Haye and Kenis 2004) indicate that diapause is obligatory before copulation and oviposition.

Much of the available works on the life cycle of *L. lilii* are based on observations made during the early part of the 20th century and the information provided is often unsubstantiated and contradicted in later publications (Halstead 1989, Cox 2001, Haye and Kenis 2004). Several early misconceptions on *L. lilii* life cycle are still widely reported in pest control literature including references to two generations a year (e.g. Alford 1995). It is clear that additional work is required to clarify the phenology of *L. lilii* under UK field conditions.

1.3. HOST RANGE

At least one life stage of *L. lilii* has been reported on 23 plant genera (Table 1.1). However, a distinction should be made between the plants on which the adults have been observed or are able to feed and those on which eggs are laid and development can be completed. Adult *L. lilii* are often observed on plants with no damage occurring (e.g. *Hemerocallis*), or damage has been wrongly attributed to *L. lilii*: Fox Wilson (1942) shows a photograph of *Polygonatum* apparently damaged by *L. lilii*, but is more likely to have been caused by slugs or snails (A. Halstead, pers. com, 2004). Additional inaccuracies may have occurred due to confusion with *L. merdigera* (section 1.1), whose hosts include *Convallaria, Allium* and *Polygonatum* (Labeyrie 1963). In non-choice laboratory tests, adult *L. lilii* will feed on 13 plant genera. However, with the exception of a single larva (out of 45) surviving to adult on *Streptopus amplexifolius* (L.) (Ernst et al. 2007), complete development has only been observed on *Lilium* and

Fritillaria (Tables 1.1, 1.3). Assuming the plants in the above reports are representative of the genera, most of the hosts listed in the literature can be rejected. Only *Lilium* and *Fritillaria* should be considered true hosts, with *Cardiocrinum* included as a larval host as extensive damage has been observed on this genus by several authors (Table 1.3).

Table 1.1. Plant genera on which *Lilioceris lilii* has been observed.

O = Observed (no feeding damage noted); Y = Will feed; N = No feeding damage in non-choice tests (Livingston 1996, Scarborough 2002, Ernst et al. 2007, pers. obs); ? = No observation. *In non-choice tests one larva out of 45 completed development to adult (Ernst et al. 2007).

Plant family	*Genus*	Adult	Larva	Additional reference(s)
Amaryllidaceae	*Narcissus*	Y	?	Livingston (1996)
Alstroemeriaceae	*Alstromeria*	Y	N	Coghill (1946)
Campanulaceae	*Campanula*	N	N	Casagrande and Livingston (1995)
Convallariaceae	*Convallaria*	Y	N	Reinecke (1910)
	Maiamthemum	Y	?	LeSage (1983)
	Polygonatum	Y	N	Temperé (1926), Fox Wilson (1942)
	Streptopus	Y	N*	
	Tricyrtis	Y	?	RHS data
Hostaceae	*Hosta*	Y	?	
Hemerocallidaceae	*Hemerocallis*	O/ N	N	Cox (2001)
Hyacinthaceae	*Muscari*	Y	?	
Iridaceae	*Crocus*	Y	?	
	Iris	Y	?	Cox (2001)
Liliaceae	*Cardiocrinum*	Y	Y	See Table 1.3
	Fritillaria	Y	Y	See Table 1.3
	Lilium	Y	Y	See Table 1.3
	Medeola	Y	N	
	Nomocharis	Y	Y	Fox Wilson (1943)
	Smilax	Y	?	
	Tulipa	Y	?	
Melanthiaceae	*Trillium*	Y	N	
Solanaceae	*Nicotiana*	O/ N	?	Cox (2001)
	Solanum	Y	N	Temperé (1926)

Lilioceris lilii has been observed on 57 hybrid *Lilium*, 30 *Lilium,* one *Cardiocrinum* and five *Fritillaria* species. Within *Lilium* the beetle has been observed on species and hybrids from all major taxonomic groups and hybrid divisions, with the exception of division III (Tables 1.2, 1.3, 1.4). Casual observation is the source for much of the information on these hosts: Fox Wilson (1943) and Halstead (1990) use data provided by Royal Horticultural Society (RHS) members and the largest contributions to the list were made at the RHS Garden Wisley between 2000 and 2003 by casual observation (Cox 2001, pers. obs). This type of data can indicate which plants are hosts for *L. lilii*, particularly if larvae are present, but cannot give any indication of resistance. C. Conjin (pers. com, 2000) recorded percentage foliar damage in laboratory and field trials to determine differences in the susceptibility of several *Lilium,* and concluded that some cultivars are less susceptible to attack than others, although all *Lilium* could be attacked by adult *L. lilii* (Table 1.4). In other studies eggs were laid on *L. henryi* and *L. speciosum* but these did not hatch or larva died soon after ecdysis and it was concluded that these lilies were less susceptible to attack than Oriental hybrids and *L. lancifolium* (Livingston 1996); larval survival has been found to be lower on *L.* 'Black Beauty' than on *L.* 'Oriental Pink' and Asiatic hybrids (Casagrande and Tewksbury 2007a). However, other authors have recorded larvae on these plants but give no indication of survival (Table 1.3). There are approximately 100 *Lilium* species and more than 8000 hybrids (McRae 1998), three *Cardiocrinum* species (Synge 1980) and at least 100 species of *Fritillaria* (Pratt and Jefferson-Brown 1997). It is clear that additional work and a more systematic approach is required to assess the resistance of any *Lilium* or *Fritillaria* to *L. lilii*.

Table 1.2. Lily species groups and hybrid divisions after Comber (1949) and the lily register (Leslie 1982, Matthews 2007)

Species groups		Hybrid divisions	
1	*martagon*	I	Asiatic hybrids
2	American	II	Martagon hybrids
3	*candidum*	III	Euro-Caucasian hybrids
4	Oriental	IV	American hybrids
5	Asian	V	Longiflorum hybrids
6	Trumpet	VI	Trumpet and Aurelian hybrids
7	*dauricum*	VII	Oriental hybrids
		VIII	Other hybrids
		IX	Species and cultivars of species

Table 1.3. Part 1 of 3. Observations made on the presence of *Lilioceris lilii* on *Lilium, Fritillaria* and *Cardiocrinum*. * see Table 1.2. 1 = Beitrag (1932), 2 = Barton (1941), 3 = Fox Wilson (1943), 4 = Coghill (1946), 5 = Southgate (1959), 6 = Livingston (1996), 7 = Cox (2001), 8 = Anderson and Bell (2002), 9 = Salisbury (2003b), 10 = pers. obs. (1999-2004) , 11 = Haye and Kenis (2004), 12 = Ernst et al. (2007).

***Species*/ hybrid**	**Group***	**Adults**	**Eggs/ Larvae**
Cardiocrinum giganteum (Wallich)	n/a	4, 9, 11	8, 11
Fritillaria imperialis L.	n/a	8, 9, 11	9, 9
F. meleagris L.	n/a	8, 9	8, 9
F. pontica Wahlenberg	n/a	8	-
F. pyrenaica L.	n/a	8	-
Lilium hansonii Moore	1	9, 10	9, 10
L. martagon L.	1	1	1, 7, 9, 10, 11
L. tsingtauense Gilg	1	10	9, 10
L. occidentale Purdy	2	9	9
L. pardalinum Kellogg	2	4, 7, 9, 10	7, 9, 10
L. philadelphicum	2	12	12
L. superbum L.	2	9, 10	9, 10
L. bulbiferum L.	3	11	11
L. candidum L.	3	2, 4, 5, 9	5, 9, 10
L. monadelphum Bieberstein	3	9	9
L. pomponium L.	3	9	9
L. pyrenaicum Gouan	3	9	-
L. auratum Lindley	4	5, 7	5
L. rubellum Baker	4	10	9, 10
L. speciosum Thunberg	4	6, 10	9, 10
L. concolor Salisbury	5		10
L. davidii Elwes	5	7, 9, 10	9, 10
L. duchartrei Farnchet	5	9	9, 10
L. henryi Baker	5	4, 5, 6, 9, 10	4, 5, 9, 10
L. lancifolium Thunberg	5	3, 5, 6, 7, 9, 10	3, 5, 6, 9,
L. leichtlinii Hooker	5	10	10
L. nepalense Don	5	9	9
L. pumilum de Candole	5	5	5
L. formosanum Wallace	6	9, 10	9, 10

Table 1.3. Part 2 of 3. Observations made on the presence of *Lilioceris lilii* on *Lilium, Fritillaria* and *Cardiocrinum*. * see Table 1.2.

Species/ hybrid	Group*	Adults	Eggs/ Larvae
L. leucanthum Baker	6	9, 10	9, 10
L. sulphureum Baker	6	9	-
L. dauricum Ker-Gawler	7	5	-
L. 'Amber Gold'	I	9	9
L. 'Butter Pixie'	I	9, 10	9, 10
L. 'Connecticut King'	I	6, 8	6
L. 'Enchantment'	I	6, 7, 9, 10	6, 9, 10
L. 'George Soper'	I	10	-
L. 'Karen North'	I	10	9, 10
L. 'Karmen'	I	10	10
L. 'King Pete'	I	9	-
L. 'Ladykiller'	I	9, 10	-
L. 'Marie North'	I	9	-
L. 'Matchless'	I	10	-
L. 'Mont Blanc'	I	6	6
L. 'Montreaux'	I	6	6
L. 'Nutmegger'	I	10	10
L. 'Orange Pixie'	I	-	9
L. 'Orange Triumph'	I	10	10
L. 'Pandora'	I	-	9, 10
L. 'Peggy North'	I	9	9
L. 'Pink Tiger'	I	9	9
L. 'Prins Constatjn'	I	9	4, 5
L. 'Red Lion'	I	9	-
L. 'Rosemary North'	I	9, 10	9, 10
L. 'Santorin'	I	9, 10	9, 10
L. 'Vanguard'	I	9	9
L. 'Yellow Blaze'	I	9, 10	9, 10
L. x *hollandicum*	I	10	10
L. 'Brocade'	II	9, 10	9, 10
L. 'Mrs R.O. Backhouse'	II	9	9, 10
L. x *dalhansonii*	II	9	9, 10
L. 'Afterglow'	IV	9	9

Table 1.3. Part 3 of 3. Observations made on the presence of *Lilioceris lilii* on *Lilium, Fritillaria* and *Cardiocrinum*. * see Table 1.2.

Species/ hybrid	Group*	Adults	Eggs/ Larvae
L. 'Kirschroter Tänzer'	IV	-	10
L. Bellingham Group	IV	9, 10	9, 10
L. San Gabrial Group	IV	9, 10	9, 10
L. 'Casa Rosa'	V	6	6
L. 'Bright Star'	VI	9, 10	9, 10
L. 'Green Magic'	VI	9	9
L. 'Moonlight'	VI	9	10
L. Pink Perfection Group	VI	9	9, 10
L. 'Thunderbolt'	VI	-	9, 10
L. 'Vico Queen'	VI	10	10
L. Golden Splendor Group	VI	9, 10	9, 10
L. 'Arthur Grove'	VII	9, 10	9, 10
L. 'Cover Girl'	VII	9	9
L. 'Mona Lisa'	VII	6	6
L. 'Showbiz'	VII	9, 10	10
L. 'Star Gazer'	VII	6, 7	6, 7
L. Everest Group	VII	-	9
L. 'Smoky Mountain'	VIII	9	9

Table 1.4. Lilies investigated for resistance to *Lilioceris lilii* (after C. Conjin, pers. com 2000). * see Table 1.2.

Most resistant	Group*	Most susceptible	Group*
L. henryi	5	*L. auratum*	4
L. 'Black Beauty'	VII	*L.* 'Acapulco'	VII
L. 'Donau'	VII	*L.* 'African Queen'	VI
L. 'Lollypop'	I	*L.* 'Berlin'	VII
L. 'Reinesse'	I	*L.* 'Casa Blanca'	VII
		L. 'Grand Cru'	I

1.4. WORLDWIDE DISTRIBUTION

Lilioceris lilii can be found almost anywhere lilies grow (natural or cultivated) in the northern hemisphere and is the most widely distributed of the *Lilioceris* species (Nolte 1939, Berti and Rapilly 1976, Cox 2001, Gold 2003, Figure 1.4.). The origin of *L. lilii* is unclear: It has been speculated that the beetle originated in China, but records from China are sparse and a centre of origin there is now considered unlikely (Lu and Casagrande 1998, Yu et al. 2001). *Lilioceris lilii* is an established alien in the UK (Fox Wilson 1942) and North America (Brown 1946, Casagrande and Livingston 1995)

In North America, *L. lilii* was established in Montreal, Canada, by 1945 (Brown 1946). The beetle remained restricted to Montreal Island until 1978, then in 1981 it was reported from Ottawa and by 2002 had been reported across the Canadian provinces of Québec, Ontario, Nova Scotia, Manitoba and New Brunswick (LeSage 1984, Casagrande and Kenis 2004). *Lilioceris lilii* was first reported in the USA from Cambridge, Massachusetts in 1992 (Casagrande and Livingston 1995). *Lilioceris lilii* spread rapidly, in 1995 occurring over 50 miles from the original infestation and by 2007 being firmly established in seven states in north-eastern USA (Casagrande and Kenis 2004, Casagrande and Tewksbury 2007b). It is thought that *L. lilii* could become much more widely distributed in North America, based on its Eurasian distribution and the establishment of other Criocerinae of European origin (Haye and Kenis 2000, Gold et al. 2001).

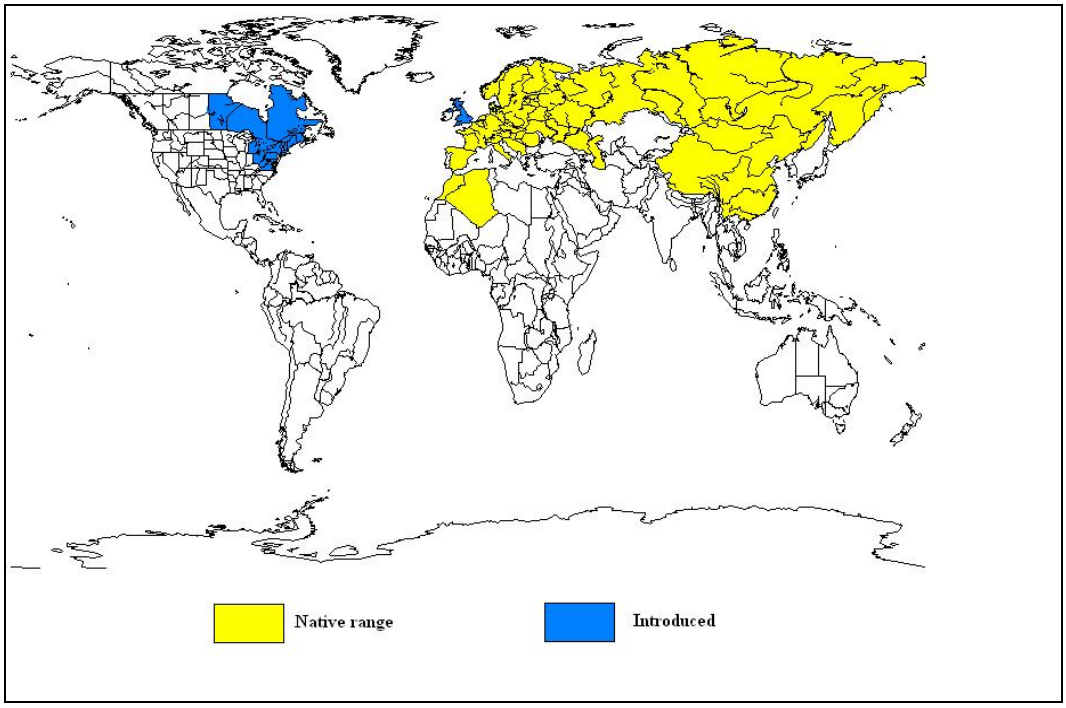


Figure 1.4. Worldwide distribution of *Lilioceris lilii*.

1.5. DISTRIBUTION IN THE UK

Before 1900, *L. lilii* was recorded from London, Swansea and Chatterden, Kent and considered very rare (Stephens 1839, Turner 1895), subsequently it was not reported in the UK for another 44 years. In 1939 "numbers" of *L. lilii* were recorded at Chobham, Surrey (Barton 1940, Fox Wilson 1942), in 1940 a single adult *L. lilii* was recorded in Carlisle (Richards 1943), in 1945 infestations were reported in Liverpool (Anon 1954) and at a nursery in Flintshire; the Flintshire infestation may have originated with bulbs imported from Holland and was probably destroyed by applications of DDT (Coghill 1946). The lack of records from Carlisle, Liverpool and Flintshire between the 1940s and 1989 indicates a failure to establish in these areas at that time (Halstead 1989).

In addition to Chobham, by 1943 *L. lilii* had been reported from two sites in Surrey and one in Middlesex (Fox Wilson 1943). By 1959 *L. lilii* was widespread in Surrey and occurred in the surrounding areas of Hampshire and Berkshire (Southgate 1959). The continued presence of *L. lilii* in Chobham and its apparent spread outwards from the town indicates that this was probably the site of establishment in the UK (Halstead 1990, Salisbury 2003b). By the late 1970s *L. lilii* was established in four south-eastern counties adjoining Surrey (Salisbury 2003b). During the 1980s the range of *L. lilii* extended to most counties in south-east England, although virtually all records fell within a 40 km radius of Chobham (Halstead 1989). By 2000, *L. lilii* was present in almost every county in southern England and reported as far north as Cheshire and Lincolnshire (Cox 2001). *Lilioceris lilii* was reported from Scotland (Glasgow) and Northern Ireland (Belfast) in 2002, in both cases it is likely to have been present for at least a year before being reported (Anderson and Bell 2002). *Lilioceris lilii* continues to survive and appears to be spreading in Scotland and Northern Ireland (RHS data). The distribution of *L. lilii* in England and Wales continues to expand: by the end of 2006 *L. lilii* had been recorded in almost every English county and was becoming widespread in Wales (Figure 1.5).

1.6. GENERALIST PREDATORS

Nolte (1939) observed a *L. lilii* larva killed by a nymph of the Brassica shield bug, *Eurydema oleracea* (L.) (Hemiptera: Pentatomidae). A lacewing (Neuroptera) larva and an anthocorid nymph (Hemiptera) have been observed feeding on *L. lilii* larvae (A. Salisbury, unpublished). Simple non-choice tests with carabid beetles (*Abax parallelepipedus* (Pill. & Mitt.), *Carabus nemoralis* Müll. and *Nebria brevicollis* (F.)) have been carried out (pers. obs). Adult *L. lilii* were not consumed in these tests but a low level of egg predation was observed with *A. parallelepipedus.*

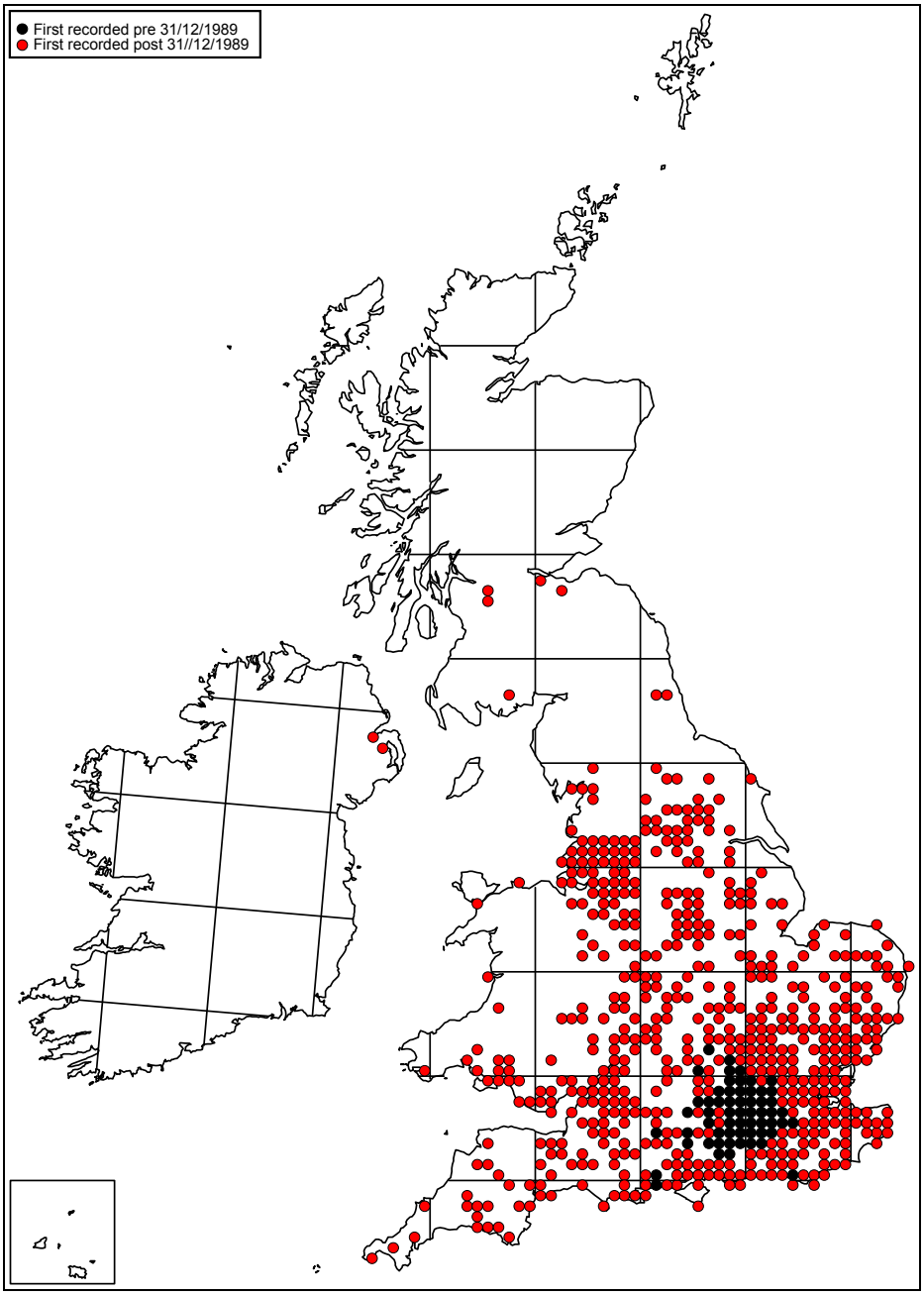


Figure 1.5. Post 1939 10 km dot distribution map of *Lilioceris lilii* from records held by the RHS (at October 2007). Produced using DMAP©.

1.7. PARASITOIDS

It was not until 1996 that four hymenopteran larval parasitoids, one hyperparasitoid, one egg parasitoid and some generalist tachinid (Diptera) parasitoids of *L. lilii* were confirmed (Table 1.5). Despite extensive surveillance, parasitoids of adult *L. lilii* have not been found (Haye and Kenis 2004). Combined, the parasitoids infect 25% to 94% of *L. lilii* larvae in mainland Europe (Haye and Kenis 2000, Kenis et al. 2002a, Haye and Kenis 2004). None of the larval parasitoid kills *L. lilii* larvae before they are mature. All three ichneumonid parasitoids are solitary; superparasitism and

multiparasitism occur frequently but only one larva completes development (Casagrande and Kenis 2004). The dominant parasitoid in most of Northern and Western Europe (including the UK) is *Lemophagus errabundus* (Figures 1.6 and 1.7), *Diaparsis jucundus* is dominant in Southern Europe and *Tetrastichus setifer* (Figures 1.8 and 1.9) in Germany (Haye and Kenis 2000, 2004). The parasitoids are distributed temporally: *T. setifer* infects larvae throughout the summer; *L. errabundus* is an early season parasitoid; the other species occur mainly in July (Kenis et al. 2002a, Haye and Kenis 2004). There is some evidence that habitat can affect the distribution of the parasitoids: *D. jucundus* has a lower rate of parasitism in *L. lilii* populations on cultivated lilies (50%) compared to *L. lilii* populations on natural *Lilium martagon* L. (up to 90%) (Haye and Kenis 2004).

The first confirmed report of *T. setifer* in the UK was from East Kent in 1997 (Cox 2001). This species has since been recorded from South Essex, Surrey, Sussex, Kent, Middlesex, Suffolk, Cambridge and East Yorkshire (Cox 2001, Salisbury 2003a, RHS data, Figure 1.11). *Tetrastichus setifer* may therefore be as widely distributed as its host as it is present in areas where *L. lilii* has only recently become established (section 1.5). *Lemophagus errabundus* was reared from *L. lilii* larvae collected from Essex in 1998, and has been recorded in Surrey, Sussex and Middlesex (Salisbury 2003a, RHS data, Figure 1.11). The hyperparasitoid *Mesochorus lilioceriphilus* (Figure 1.10) has been recorded in Surrey from *L. lilii* larvae collected in June (Salisbury 2004). None of the parasitoids can be native to the UK as they are specific to the genus *Lilioceris* (Gold et al. 2001, Kenis et al. 2002a): *L. lilii* is the only representative of the genus in the UK and is an established alien (section 1.5).

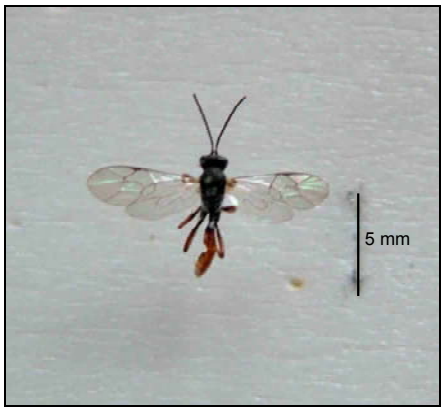


Figure 1.6. *Lemophagus errabundus* female.

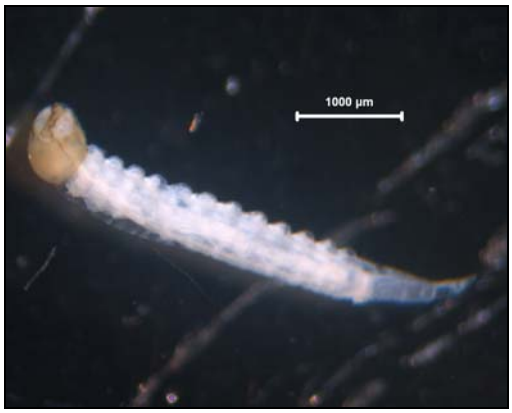


Figure 1.7. Larva of *Lemophagus errabundus.*

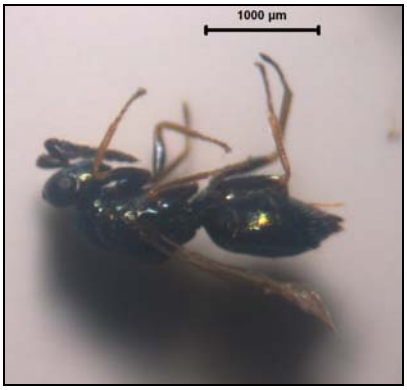


Figure 1.8. *Tetrastichus setifer* female. **Figure 1.9. *Tetrastichus setifer* larvae**

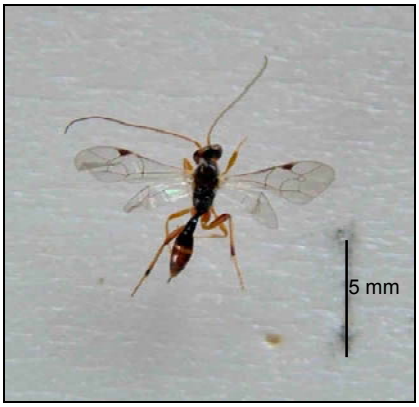


Figure 1.10. *Mesochorus lilioceriphilus* female.

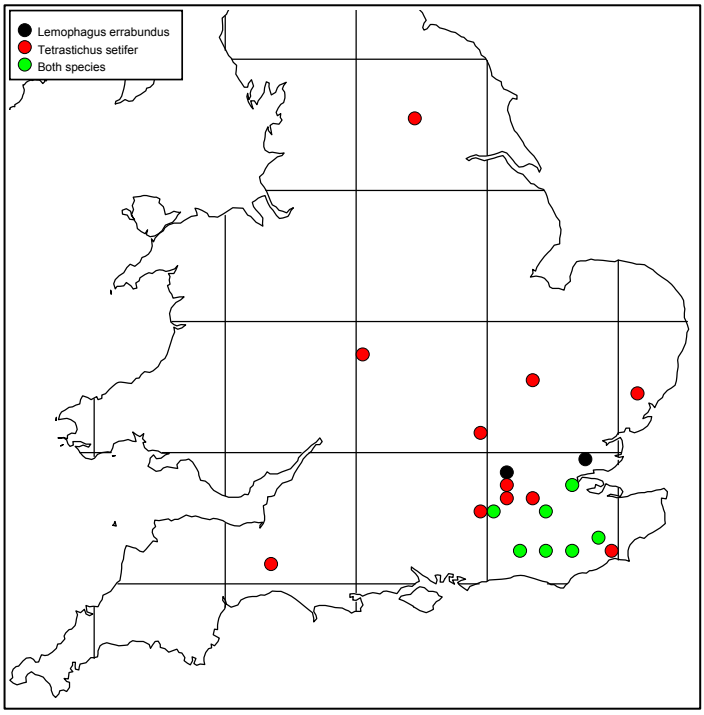


Figure 1.11. Distribution of *Lilioceris lilii* parasitoids in England (at October 2007). Produced using DMAP©.

Table 1.5. Characteristics of larval parasitoids and hyperparasitoid of *Lilioceris lilii* (after Haye and Kenis 2000, Gold et al. 2001, Kenis et al. 2001, Kenis et al. 2002a, Gold 2003, Haye and Kenis 2004).

*Times of larval infestation **Under laboratory conditions

Taxonomy	European distribution	Life cycle*	Specificity**
Hymenoptera: Ichneumonidae			
Lemophagus errabundus (Grav.)	Widespread, UK	Univoltine, Solitary. May - June.	*Lilioceris* spp.
Lemophagus pulcher (Szepligeti)	Widespread (not UK)	Multivoltine. July - August	Criocerinae
Diaparsis jucundus (Holmgren)	Widespread (not UK)	Univoltine. July.	*Lilioceris* spp.
Mesochorus lilioceriphilus Schwenke	Widespread, UK	Solitary	*Lemophagus* spp.
Eulophidae			
Tetrastichus setifer Thomson	Widespread, UK	Univoltine. May-August. Gregarious	*Lilioceris* spp.
Mymaridae			
Anaphes sp. (undescribed)	France, Switzerland	Egg parasitoid. Multivoltine, gregarious	Unknown alternate hosts
Diptera: Tachinidae			
Meigenia species	Widespread (three species in UK)		Generalists

1.8. DEFENCE

Both adults and larvae of *L. lilii* contain carotenoid pigments sequestered and modified from host plants (Mummery and Valadon 1974) and the red colour of the adults is assumed to be aposematic (Jolivet and Verma 2002). Adult *L. lilii* produce phenylanine derivatives as defensive compounds from glands on their pronotum and elytra (Pasteels et al. 1994). Like most adult Criocerinae, *L. lilii* stridulates by contracting and extending its abdomen, causing a 'file and scraper' located on the abdomen and elytra respectively to move against one another (Emmel 1936); this can produce 200 chirps/min with amplitude maxima of 1-1.3k Hz or 6k Hz (Schmitt and Traude 1990). It

has been suggested that this stridulation is a defensive behaviour (Emmel 1936, Schmitt and Traude 1990). However, it is also possible that *L. lilii* uses sound as communication between conspecifics as may be the case with the Orchid beetle *Stethopachys formosa* Balay (Criocerinae), which will stridulate without any noticeable disturbing stimulus (Schmitt 1994). Adult beetles show a feigned death (thanatosis) defence when disturbed, falling to the ground and remaining motionless, often landing on their dorsal side leaving their black ventral side exposed increasing the effectiveness of the defence (Livingston 1996).

It has been suggested that the excrement cover of *L. lilii* larvae provides thermal protection and predator avoidance (Reinecke 1910, Nolte 1939) and the faecal shield reduces predation by the earwig *Forficula auricularia* L. (Schaffner and Kenis 1999). It may be the case that such coverings are multifunctional, acting as thermoregulation and reduction of desiccation (reviewed in Olmstead 1994). Whilst defensive secretions and coverings can provide protection against generalist predators, specialists can exploit them. Schaffner and Müller (2001) investigated the foraging behaviour of the *L. lilii* parasitoid *Lemophagus pulcher*. In static four-chamber olfactometer and contact bioassays, *L. pulcher* adults moved towards larvae with or without faecal shields, to faecal shields alone, to lily leaves with larval damage and to the defensive secretion produced by larvae. In contact bioassays, *L. pulcher* females showed ovipositor probing of shields, (in the presence or absence of larvae), and dummies coated with shield extract, suggesting that the shield plays a primary role in short-range host location and host acceptance, and that the stimulus is chemical. Initial work with *L. pulcher, D. jucunda* and *T. setifer* indicate that these species have similar responses and that in *T. setifer* volatiles emitted by *L. lilii* larvae, shields and damaged plants have a synergistic effect (Scarborough 2002).

1.9. PEST STATUS

Lilioceris lilii is a pest in the UK, North America and the Netherlands where it is a problem for amateur gardeners, as well as in public parks and gardens, but there is also a risk to the native *Lilium* of North America and the native *Fritillaria meleagris* of England (Sutton 2004, Ernst et al. 2007, C. Conjin pers. com. 2000). Gold (2003) considers *L. lilii* to have potential to threaten lily production in the USA, an industry worth $65 million. *Lilioceris lilii* occurs in commercial lily fields in France and Switzerland but rarely causes significant damage in established lily fields, possibly due to high levels of parasitism (Kenis et al. 2001, Casagrande and Gold 2002).

In the UK RHS data indicates the rise of *L. lilii* as a problem to the gardener since 1967 (Figure 1.12). A mean of four *L. lilii* enquiries per year (0.5% of total pest

enquiries) were received during the 1970s; in the 1980s the mean rose to 26.7 (1.8% of total), in the 1990s rising to 76.1 (3.0%); in the new millennium (up to December 2006) the figure is 101 (3.2%). No information is available on the problems the beetle causes the professional horticulturalist in the UK, and an assessment of this risk needed to be carried out (see Chapter 2).

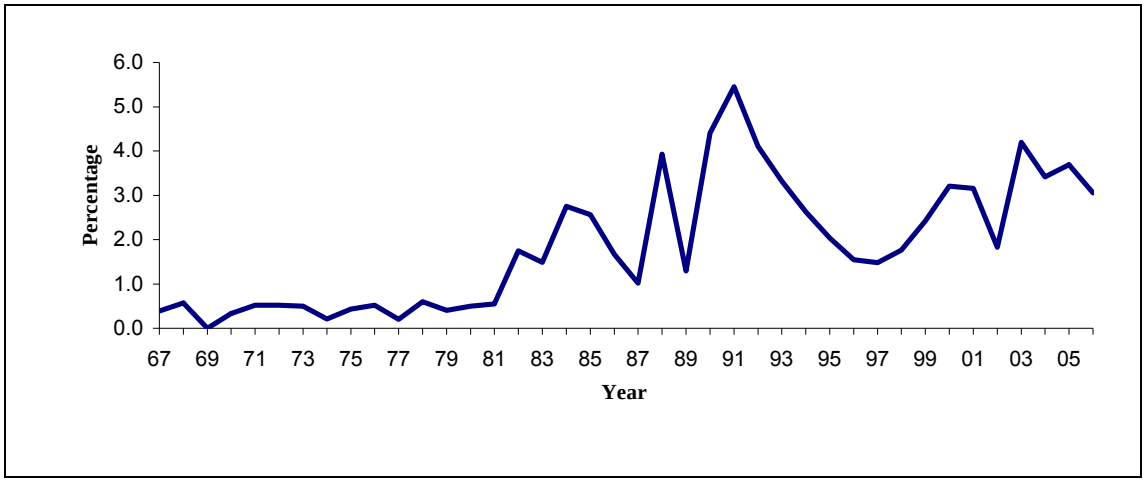


Figure 1.12. *Lilioceris lilii* enquiries as a proportion of all pest enquiries received by the RHS (1967 to 2006).

1.10. MANAGEMENT IN THE UK

Management of *L. lilii* currently relies on hand-picking or the use of pesticides (Alford 1995). These measures often need to be repeated throughout the growing season due to the long period of activity of *L. lilii*. The excrement-covered larvae, and the adult behaviour of dropping to the ground when disturbed, in addition to the time consumed can make hand-picking undesirable (RHS 2007). In 2007, three synthetic insecticide foliar sprays were available to the amateur gardener to control beetle pests on ornamental plants in the UK: bifenthrin and the neonicotinoids thiacloprid and imidacloprid. These active ingredients are broad spectrum and not suitable for use on plants in flower.

Two insecticides tested in the USA (active ingredients imidacloprid and azadirachtin), were found to repel adults but not cause adult mortality (Livingston 1996), adding weight to the conclusion of LeSage (1992) that no insecticide at present can completely eradicate the adults or larvae of *L. lilii*.

1.11. BIOLOGICAL CONTROL

The complex of specific parasitoids of *L. lilii* is similar to that found on other Criocerinae, such as the cereal leaf beetle (*Oulema melanopus* (L.)), an established

alien pest in North America (Casagrande and Kenis 2004). Since introduction, three parasitoids of *O. melanopus* have become widespread in the USA and are important in its control (Barbosa et al. 1994). This success led a team at the University of Rhode Island and CABI Bioscience to instigate a classical biological control programme against *L. lilii* in the USA (Gold et al. 2001, Gold 2003).

Following host testing, a licence was granted to release *T. setifer* in Massachusetts during 1999 to 2003 (Haye and Kenis 2000, Gold 2003, Tewksbury et al. 2005). Three thousand female *T. setifer* were released in trial plots: initially up to 60% parasitism was recorded, but low winter survival was observed, as the bark mulch used on the plots was unsuitable for the overwintering parasitoids (Casagrande and Gold 2002). *Tetrastichus setifer* has now been released in four New England States, is established and beginning to spread from the sites of introduction, parasitism rates of between 37% and 100% had been observed by 2007 and declines in *L. lilii* have been seen as a result (Kenis et al. 2002b, Tewksbury et al. 2005, Casagrande and Tewksbury 2007a). Releases of *L. errabundus* and *D. jucunda* occurred from 2004 to 2006; *L. errabundus* is established at its release sites in Rhode Island and Maine, where parasitism rates of 13% to 90% have been observed and it appears to be spreading from its release sites; *D. jucunda* has not yet become established although additional releases are planned (Casagrande and Tewksbury 2007b). By using a single parasitoid species, early or late host larvae may evade parasitism and so it is believed that the release of these additional species of parasitoid in the USA will result in a high degree of parasitism over the entire season (Casagrande and Gold 2002).

It has been suggested that it is a complex of three or more parasitoid species that reduce *L. lilii* populations to an acceptable level in mainland Europe and that in the UK *L. lilii* may be managed by the introduction of an additional parasitoid (Casagrande and Gold 2002, Kenis et al. 2002a). However, the introduction of non-indigenous species into the UK requires extensive quarantine testing in a registered UK laboratory to satisfy a plethora of government advisory bodies before a release licence can be granted (sections 14 and 16 of the UK Wildlife and Countryside Act 1981; HMSO). Such data collection can take many years, the cost of quarantine facilities can be prohibitive and it is still possible that a licence will not be granted. In the event that a licence is granted, there is no guarantee that the addition of a further parasitoid will control *L. lilii*.

Preliminary laboratory tests have been conducted with a commercial formulation of *Bacillus thuringiensis* var. san diego (Bonide Colorado Potato Beetle Beater), however this gave only 30% larval mortality, in comparison with 100% mortality with conventional insecticides (Livingston 1996).

1.12. CONCLUSIONS

It is clear that there is still much to be learned about *L. lilii*. Inaccuracies from the early literature on *L. lilii* life cycle are still repeated in pest management literature and its phenology is not thoroughly understood. The beetle's host range, in particular the preferences it may have for different *Lilium* or *Fritillaria* has shown potential in the search for resistant varieties but rigorous investigation is lacking. *Lilioceris lilii* has shown considerable range expansion in the UK since its establishment in 1939, and it is likely that the beetle will become more widespread. Whilst there is some knowledge of the extent of the problem for the amateur gardener little is known of the problem faced by the professional horticulturalist and this required further investigation.

Current management options for *L. lilii* are unsatisfactory. In the USA biological control with parasitoids is being attempted, yet despite two parasitoids being present in the UK, *L. lilii* continues to be a problem and the deliberate introduction of further natural enemies to the UK is unlikely. A different pest management approach would be to manipulate *L. lilii* by use of semiochemicals (see review Pickett et al. 1997). Despite the wealth of literature concerning other chrysomelid leaf beetles (see chapter 4), Southgate's (1959) statement that "it is obvious that smell plays a large part in the location of these insects with their food-plant" and the observations of Emmel (1936) that some mating behaviour could be chemically-mediated, very little is known about the chemical ecology of *L. lilii*.

1.13. PROJECT OBJECTIVES AND AIM

Objectives:

1 To investigate the biology and ecology of *L. lilii.*
2 To investigate the chemical ecology (i.e. behaviourally-active insect and plant-produced semiochemicals) of the beetle.
3 In conjunction with survey based data to assess the risk to the lily industry relating to pot plants, cut flowers and outdoor bulb production.

Aim: Based on our understanding of the biology, ecology and chemical ecology (objectives 1 and 2), develop integrated pest management strategies for control of *L. lilii*.

CHAPTER 2. THE LILY BEETLE- A RISK TO UK HORTICULTURE?*

2.1. INTRODUCTION

The lily beetle (*Lilioceris lilii*) is an established pest throughout much of the Northern hemisphere where its host plants (*Lilium*, *Fritillaria* and *Cardiocrinum* – referred to as lilies) are grown (section 1.4). In the UK, North America and the Netherlands *L. lilii* is a problem for the amateur gardener, as well as in public parks and gardens, and there may also be a risk to the native lilies of North America and native snake's head fritillary (*Fritillaria meleagris*) in England (section 1.9, Sutton 2004). Data from the Royal Horticultural Society's (RHS) Members' Advisory Service indicate that *L. lilii* has become more of a problem for the amateur gardener since the 1960s and that gardeners can use hand picking or pesticides to attempt control of the beetle (section 1.10). The extent of *L. lilii* as a problem within professional horticulture in the UK had not been assessed. The report below is a synopsis of an assessment of the risk *L. lilii* poses to UK horticulture and a more detailed report has been produced for the Horticultural Development Council (HDC, Salisbury 2006). In addition to a review of the literature (Chapter 1), the assessment was made by conducting surveys of providers and the end users of the beetle's host plants.

The objectives of the surveys were to:

- Determine the current problems with *L. lilii* in the UK for providers of plants, the amenity horticulturist and amateur gardener.
- Quantify how *L. lilii* host plants are grown in the UK and the extent of the industry.
- Gain an insight into control measures currently used against the beetle.

The surveys had the aim of assessing the likely future effect of *L. lilii* on horticulture in the UK.

* This chapter is adapted from a report sent to participants of the surveys of professional growers of *Lilioceris lilii* host plants. An extended version was produced for the HDC (Salisbury 2006) and summarised in HDC News (Anon 2007)

2.2. RISK ASSESSMENT SURVEY PART 1: EFFECT OF THE LILY BEETLE IN AMATEUR GARDENS

2.2.1. Introduction and methods

The prevalence of a pest such as *L. lilii* may have a deleterious effect on consumer confidence; for example, declines in sales of roses are widely believed to have been due to the rise of black spot disease, *Diplocarpon rosae* (C. Prior, pers. com, 2006). In order to gain some insight into consumer confidence, members of the RHS who enquired about *L. lilii* by phone, e-mail or in person during 2005 and 2006 were asked (after advice was given about *L. lilii*) "If you continue to have a lily beetle problem would you stop growing lilies in the future?"

2.2.2. Results and discussion

Between 13 March 2005 and 9 August 2006, there were 148 responses (Figure 2.1): 26% (39) said they would not grow lilies in the future if *L. lilii* continued to be a problem. This result is likely to be biased towards keen gardeners, those replying being primarily RHS members. However, if this sample is considered representative of the lily-growing public, the results indicate that *L. lilii* problems could cause a decline in the sales of lilies, if it is not already restricting sales.

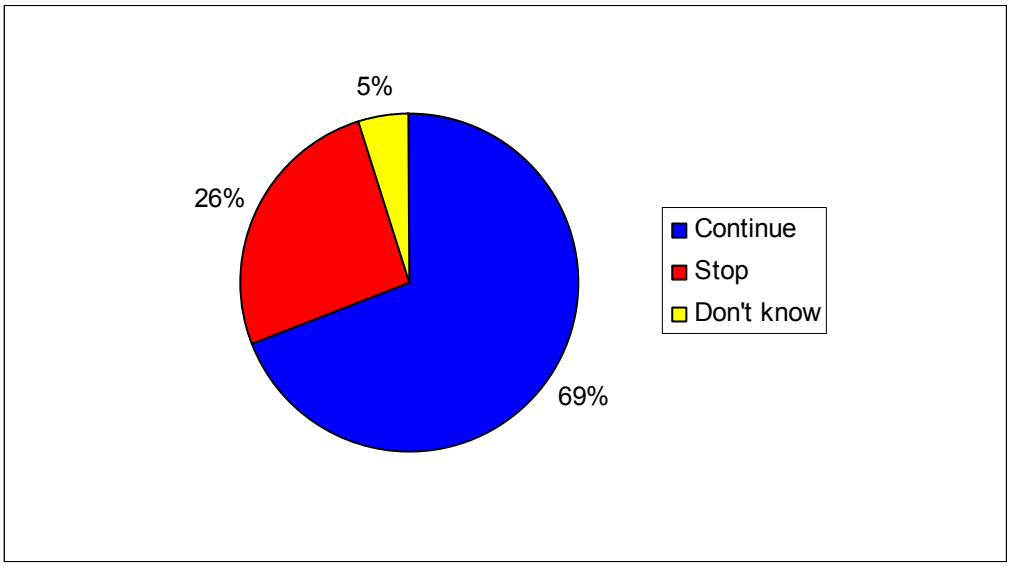


Figure 2.1. Replies from 148 enquirers who were asked "If you continue to have a lily beetle problem would you stop growing lilies in the future?"

2.3. RISK ASSESSMENT SURVEY PART 2: PROVIDERS OF LILIES

2.3.1. Introduction and methods

Reviewing the literature (Chapter 1) provides information on the biology and distribution of *L. lilii*, including published control methods. Enquiries to the RHS (section 1.9) and a question asked of enquirers (section 2.2) show that *L. lilii* is an increasing problem for amateur gardeners. However, this does not provide information on the problems or risk posed by the beetle to professional growers, wholesalers and retailers (the providers) of lilies. A survey of providers of *L. lilii* host plants was therefore instigated. Limited information is publicly available on providers, the only recent data freely available is that 20 million lilies were grown under glass in registered agricultural holdings in England and Wales in 2003 (DEFRA 2004). Thus the survey not only assessed the current problems, control measures and perceived risk of the beetle, but attempted to quantify the lily industry in the UK.

Survey forms were despatched in February 2006: each included a freepost envelope for return and *L. lilii* information sheet (Appendices A.1 and A.2). The survey was sent to 134 lily providers listed in the RHS Plant Finder (RHS 2005) and to 448 HDC members with a return date of 10 March 2006.

Responses have been summarised by the number of providers responding to each question/ category, with numbers who reported a *L. lilii* problem indicated (Q2a). Where appropriate, comparisons between response and the number of lilies produced by the responder, have been made (Q6b). It should be noted that a reported problem with *L. lilii* does not necessarily indicate that the problem is ongoing or that all stock was infested and comparisons presented should be treated only as a guide.

2.3.2. Results and discussion

Response rate. The return rate was 22% (126), 102 of which were from active lily providers. Proportionally more responses were received from providers listed in the Plant Finder (56, 42%, all providing lilies) compared to HDC members (70, 16%, 46 providing lilies). The lower response rate from HDC members may have been partially due to the fact that not all provided lilies (24 returns from this group stated that lilies were not grown). The results below refer to the 102 providers who supply lilies, 81 (79%) of which indicated how many lilies they produced (Q6b) representing 6 700 225 lilies sold in the UK in 2005.

Q2a. *Have you ever had a problem with lily beetle?* Thirty-five providers reported that *L. lilii* had been a problem, ten were from 10 km grid squares where *L. lilii* had not been

previously reported, although these were within the known range of the beetle (see Figure 1.5). Twenty-four providers reporting *L. lilii* specified the number of lilies sold: 593 670 lilies, 9% of the total plants represented by the survey.

Q2b. *What control measures were taken against lily beetle?* Most of the 35 providers (19, 54%) with a *L. lilii* problem relied on manual removal as a control measure, four (11%) relied on chemical control and two stopped growing lilies because of the beetle. However, the majority of lilies were produced under regime where the beetle was controlled by pesticide, 326 520 lilies, 55% of those infested with the beetle.

Q2c. *Have you ever had enquiries or complaints from customers about the lily beetle?* Thirty (29%) providers had received enquiries or complaints about the beetle, 16 of these had not themselves had a problem with *L. lilii*. Thus even if *L. lilii* is not a current problem for providers, they are being made aware of the problem by customers.

Q2d. *To your knowledge, is lily beetle present in other gardens locally (within 5 miles)?* Of the 56 providers stating that the beetle was known locally, 21 (38%) did not have a problem with *L. lilii*. Therefore a provider in an area where *L. lilii* is present does not necessarily have a problem with this pest, although a high proportion (35; 63%) did report a problem.

Q2e. *A number of those who contacted the RHS for advice on lily beetle in 2005 will not purchase any new lilies or fritillaries for their garden due to the beetle problem. In light of this information and the other information provided with this survey, what impact do you think the beetle may have on the lily or fritillary part of your business?* Almost half of providers (48) representing 66% of lilies thought that *L. lilii* would have no effect or even increase sales (Figure 2.2). However, 54 providers expected to see decreased sales, representing 34% of plants in the survey.

Q3. *Please list the* Lilium/ Fritillaria/ Cardiocrinum *that you supply.* Over 300 plant types from all major taxonomic and hybrid groups were represented, thus the survey is representative of lily types provided in the UK. The largest group (38) supplied *Lilium* alone, accounting for 83% of the plants represented.

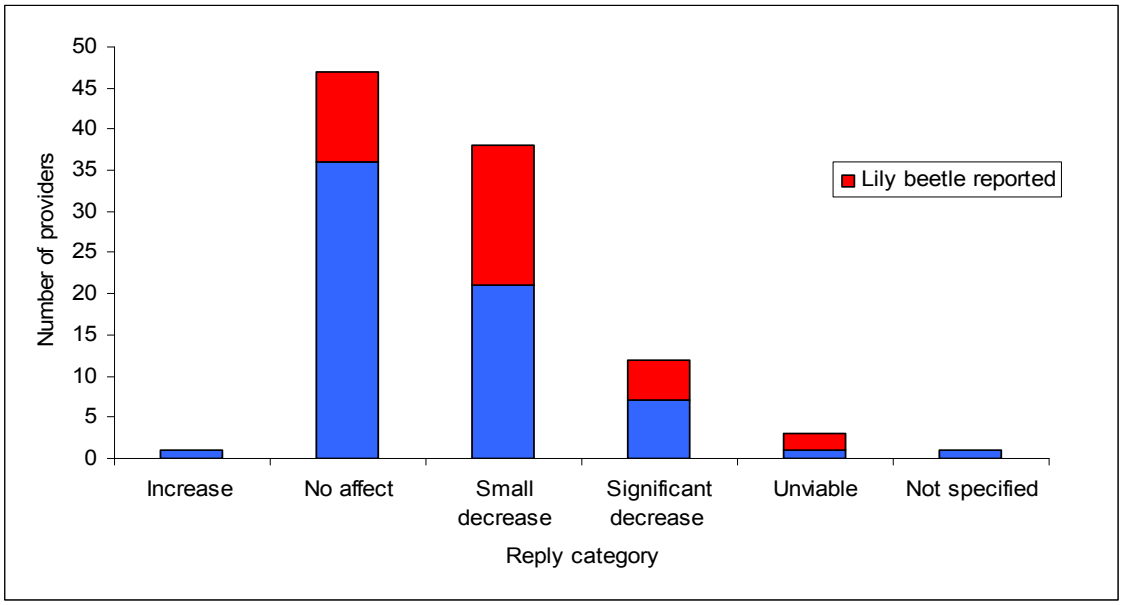


Figure 2.2. Perceived impact of lily beetle on lily sales.

Q4a. *Are you a producer, retailer or wholesaler of* Lilium/ Fritillaria/ Cardiocrinum*?* Most providers grew at least some lilies for retail or wholesale (79, 77%) and 14 (14%) relied entirely on their own production. Most were at least in part retail (74, 73%) and/ or producers (88, 86%). With the exception of wholesalers, *L. lilii* was reported by at least one provider in all categories.

Q4b. *Where do you source new stock (purchased or propagated)?* Most (68, 83%) of the 82 providers responding bought in plants as a partial source of new stock; 36 (44%) exclusively purchased plants from other providers. Some propagation of stock was carried out by 47 (57%) providers, although those propagating alone represent only 1% of the plants in the survey with 75% of lilies represented being purchased externally. Some providers in all categories had experienced a *L. lilii* problem (Figure 2.3).

Q4c. *How do you propagate?* Of the 47 providers propagating lilies, 36 responded. A combination of propagation methods is used in lily production and with the exception of tissue culture, all published methods are used in the UK (see Appendix A.2).

Q4d. *Are you breeding new varieties/ cultivars?* Four providers accounting for 1 515 400 (23%) plants were breeding new varieties of lilies, and three of them reported a problem with *L. lilii*.

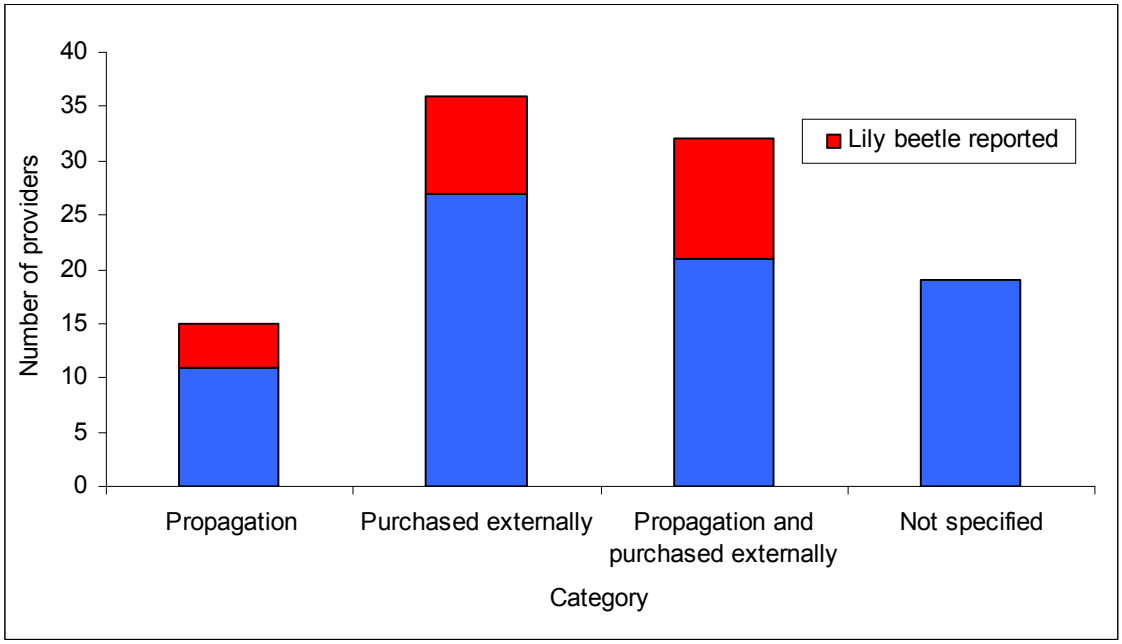


Figure 2.3. Where lily providers source new stock.

Q4e. *What is your current growing regime?* Providers that used polytunnels, bulb frames or glasshouses were categorised as 'grown under protection' (Figure 2.4). The 88 responses indicate that *L. lilii* can be a problem under almost all regimes.

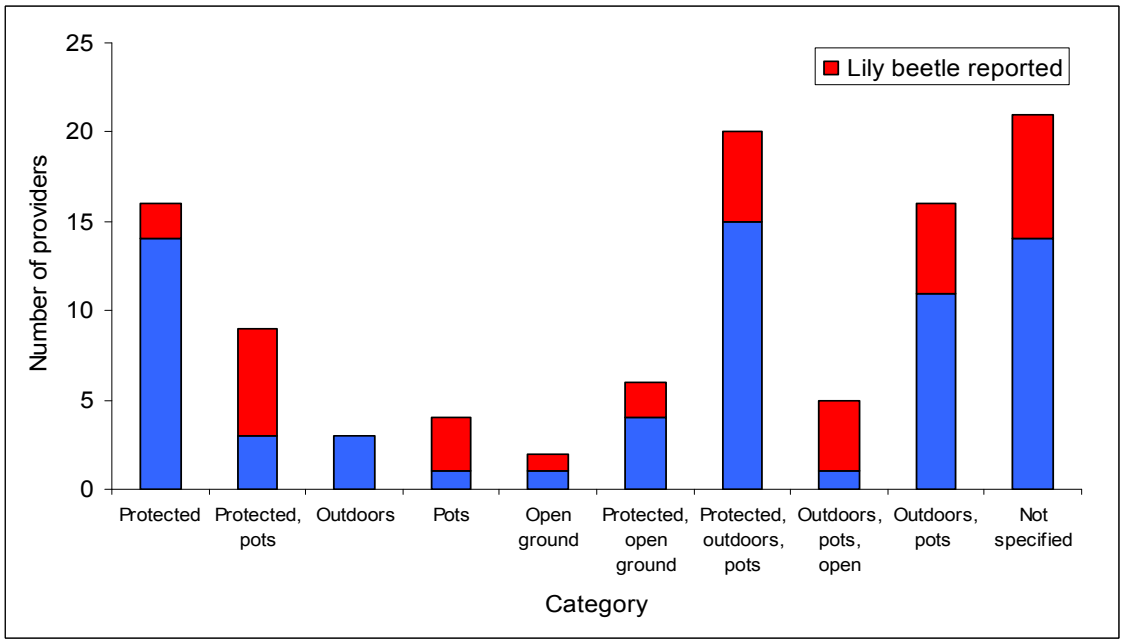


Fig 2.4. Providers' growing regimes for lilies in the UK.

Q5a. *Where do you source (purchase) new stock?* The largest group of providers sourced new stock exclusively from the UK (45), although these providers accounted

for less than 1% of plants. The Netherlands was the biggest source of supply by number of lilies represented (more than 50%).

Q5b. *What stage of plants do you source?* The most likely man-made (anthropogenic) dispersal of lily beetles is by movement of plants in pots, which may contain pupae or adults in the growing medium or adults, eggs or larvae on foliage. There were 94 responses, most (51, 54%) indicated that plants were bought at stages unlikely to contain *L. lilii*. Only five providers sourced plants in pots alone, accounting for 6% of lilies.

Q6a. *At what stage do you sell plants?* There were 98 responses, most (62, 63%) sold plants in pots, accounting for 33% of lilies. Seven providers sold cut flowers alone, yet accounted for 60% of plants. At least one provider in each category reported *L. lilii*. Whilst the risk of spreading the beetle by selling dry bulbs is small, it is possible that any stage of the beetle could be transported in potted plants (see Q5b). In theory eggs, larvae or adults could be transported with foliage in cut flowers; however it is unlikely that eggs or larvae will develop on these cut stems, as these will be disposed of and larvae will have nowhere to pupate (unless disposed of on a compost heap). However, beetles or their damage would result in un-saleable stock.

Q6b. *What is your approximate turnover of plants (number of bulbs, seeds or stems) each year?* A total of 6 700 225 plants are represented by the survey, 81 providers specifying the number of lilies sold. The providers ranged from selling 10 plants to 2 million, with a mean of 82 719. Most (66, 81%) sold less than 10 000 plants per year, but these growers combined supplied less than 1% of lilies represented (Figure 2.5). Two providers produced 52% of plants represented, therefore care should be taken when interpreting results presented by number of plants produced, as the response by these providers will skew results. In all categories at least one grower reported *L. lilii*, with the exception of those providing more than a million plants.

Q6c*. Has this volume been increasing or decreasing over the past five years?* Of the 89 responses, most (70, 79%) providers stated that plant sales had increased or remained stable (Figure 2.6). Nineteen providers had seen sales decrease, corresponding to 6% of plant sales. The responses to this question appear independent of the presence of *L. lilii* and overall it can be concluded that sales of lilies are currently increasing.

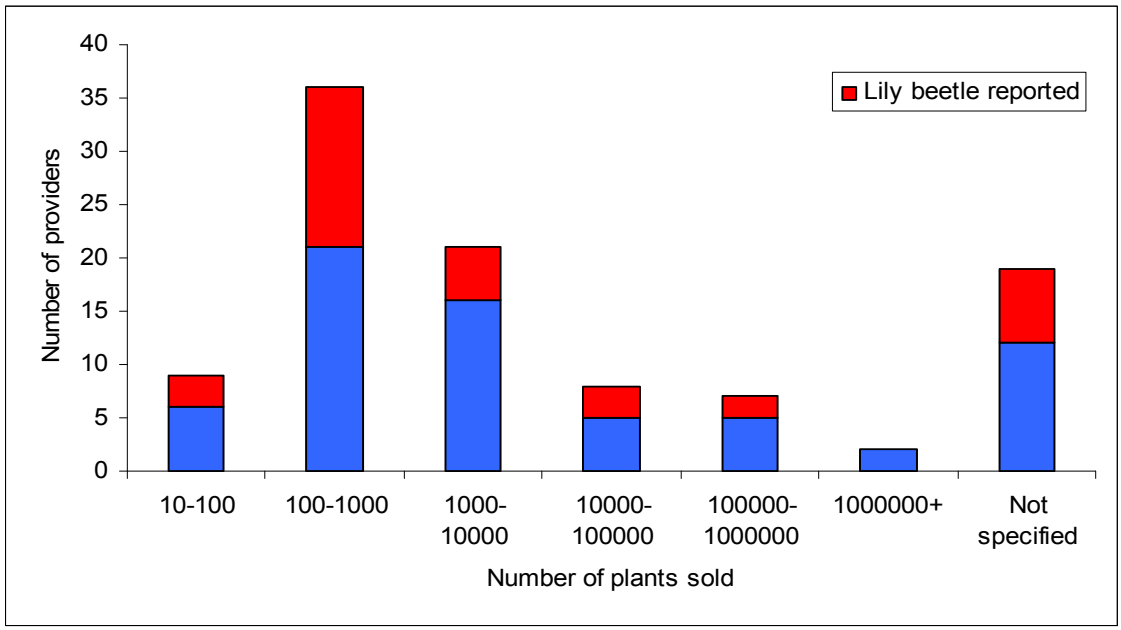


Figure 2.5. Provider size by number of plants sold.

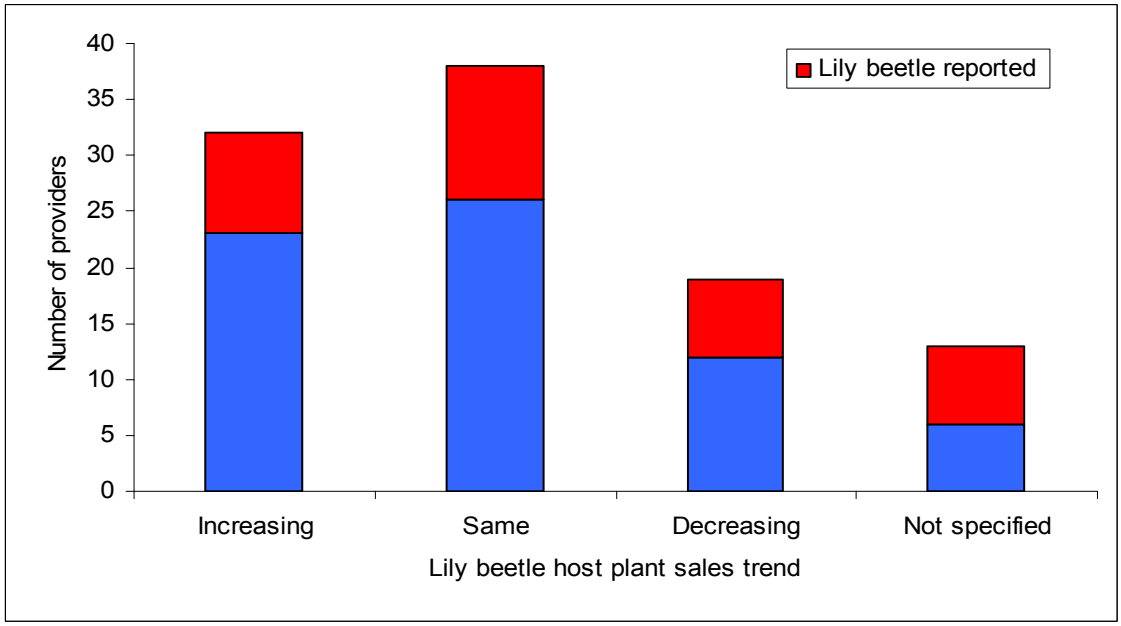


Figure 2.6. Sales trend over the past five years, by number of responses.

Q6d. *Who do you sell plants to?* Of the 100 responses, the largest group sold plants on site usually to private individuals (32 exclusively, 36 at least partly). In most circumstances at least one provider had a problem with *L. lilii* but the two largest providers (supplying large retail outlets and flower pickers) did not report a *L. lilii* problem. This is fortunate as large retailers (e.g. B&Q) have strict quality control and will reject stock and impose other economic penalties if stock is infested with pests.

Q7a *Have you had any pest or disease problems?* **Pests:** Most providers (55, 54%) indicated that pests other than *L. lilii* had been a problem. The most frequent pest by number of providers (45, 44%) and proportion of plants (89%) was aphids. Second in terms of number of providers affected was *L. lilii* (35, 34%), however this only affected providers selling 9% of plants, and was behind thrips (Thysanoptera, 29%), vine weevil (*Otiorhynchus sulcatus* F., 23%) and fungus gnats / shore flies (Diptera: Sciaridae/ Ephydridae, 22%) (Figure 2.7).

Diseases: Most providers (73, 72%) did not respond and it can be concluded that diseases are a lesser problem than pests. Bulb rots were the most frequently encountered diseases by number of plants sold (40%), followed by viruses (38%).

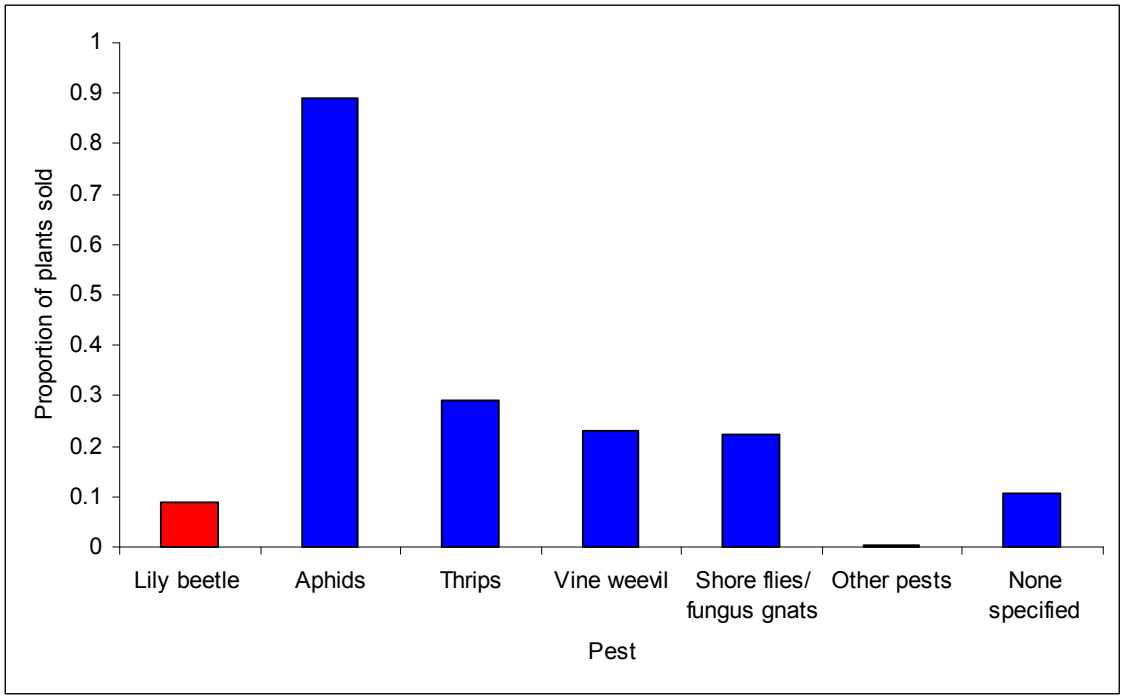


Figure 2.7. Lily provider pest problems, by proportion of lilies sold.

Q7b *What chemicals are used in production / storage?* Most providers (56, 55%) stated that pesticides were used; nine used insecticide, but did not specify pests as being present. The most frequent chemicals used (22 providers) were the neonicotinoid compounds. These broad spectrum insecticides are used to control a variety of insect pests but when considering plants represented by the survey (Figure 2.8), pirimicarb, a selective insecticide for aphids is the most frequently used. Thus if *L. lilii* does become a wider problem it is likely to increase the use of broad spectrum insecticides. More providers used pesticides than fungicides (21, 21%) or fertilizer (47, 46%).

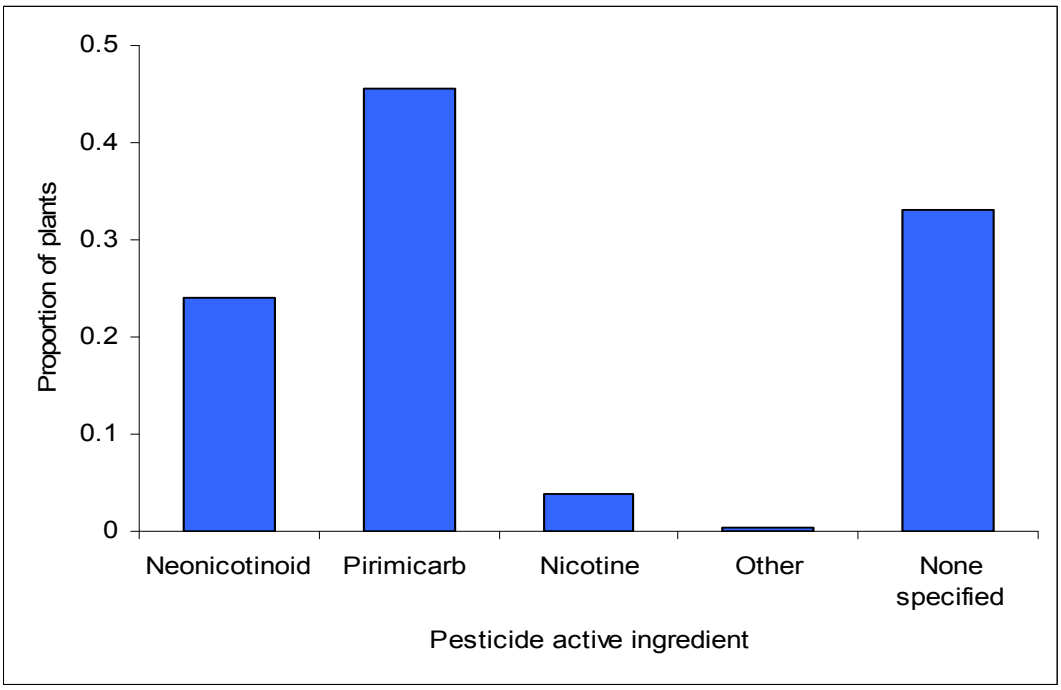


Figure 2.8. Pesticides used by proportion of plants sold (of 6 700 225).

Q7c. *What other pest / disease control strategies are used?* Of the 88 responses, 33 used methods other than chemical to control pests and diseases. The most frequent was manual control (18), but this accounts for less than 1% of plants produced. Biological control was used by ten producers, primarily against vine weevil.

2.4. RISK ASSESSMENT SURVEY PART 3: PROFESSIONAL USERS OF LILIES

2.4.1. Introduction and methods

A survey of providers of lilies has given insights into the status of the beetle within that sector of the industry (section 2.3). Another professional group in the horticulture industry is the amenity horticulturist; consisting of those that maintain gardens open to the public, local authority parks and other amenity plantings. The results of the survey presented below provide an indication of the effects of *L. lilii* provided by a section of these horticulturists.

Survey forms were despatched in February 2006: each included a freepost envelope for return and *L. lilii* information sheet (Appendices A.1 and A.3). Forms were sent to gardens throughout the UK run by the National Trust, RHS partner gardens, English and Scottish Heritage, making 330 gardens in total. The surveys had a return date of 10 March 2006. Responses to the questions have been summarised by number of gardens responding to each question, with those that stated they had had a *L. lilii* problem indicated (Q2b). It should be noted that if a garden reported a beetle problem, there was no indication of the severity of the problem or that the problem was ongoing.

2.4.2. Results and Discussion

Response rate. The response rate was 41% (135 surveys returned).

Q1a. *Are* Lilium/ Fritillaria/ Cardiocrinum *grown in the garden?* Most gardens (115, 85%) grew lilies, confirming that these are popular plants in gardens open to the public. Responses to the questions below relate to the 115 gardens growing lilies.

Q1b. *Approximately how many varieties and bulbs of lilies/ fritillaries are grown*? Most gardens grew only a few lily plants and/or varieties (81% less than 50), and it can be concluded that damage from the beetle will not have a big impact on the appearance of these gardens. However, 16 (14%) gardens grew more than 100 plants and/or varieties, in which case the appearance of large numbers of plants and areas of the gardens could be affected by the beetle. However, as the survey gives no indication of the size of the gardens this conclusion should be considered with care. *Lilioceris lilii* was reported from all categories, indicating that it is able to find hosts no matter how many are grown (Figure 2.9).

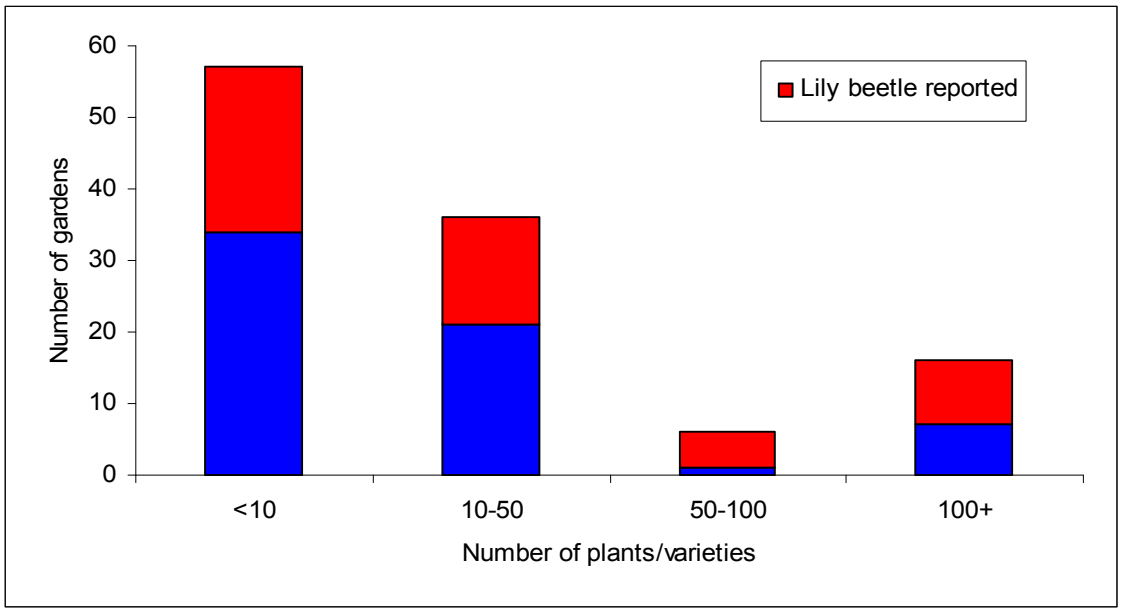


Figure 2.9. Number of lilies grown by gardens in the survey.

Q2a. *Have you ever had a problem with lily beetle?* Fifty-one (43%) gardens reported that *L. lilii* had been a problem. Sixteen (14%) were in 10 km grid squares where *L. lilii* had not previously been reported, but were within the beetle's known range (Figure 1.5).

Q2b. *What control measures were taken against lily beetle?* All 43 (84%) gardens that took control measures against the beetle used manual removal, whilst four (8%) also used insecticides indicating that insecticide use against *L. lilii* in gardens is limited, but staff time is being spent on manual removal.

Q2c. *To your knowledge, is lily beetle present in other gardens locally (within 5 miles)?* Of the 28 (21%) returns stating that the beetle was known locally, two gardens did not have *L. lilii*. Nine gardens that did not know of the beetle locally were in areas where the beetle had been reported (RHS data), suggesting that some are unaware of the beetle's local presence.

Q2d. *If lily beetle becomes a problem (or is already a problem) what effect will this have on lily/ fritillary use in the garden?* The majority (75, 65%) of gardens indicated that they would not change future plans due to the beetle (Figure 2.10). However, 25% would reduce lily planting or no longer grow lilies.

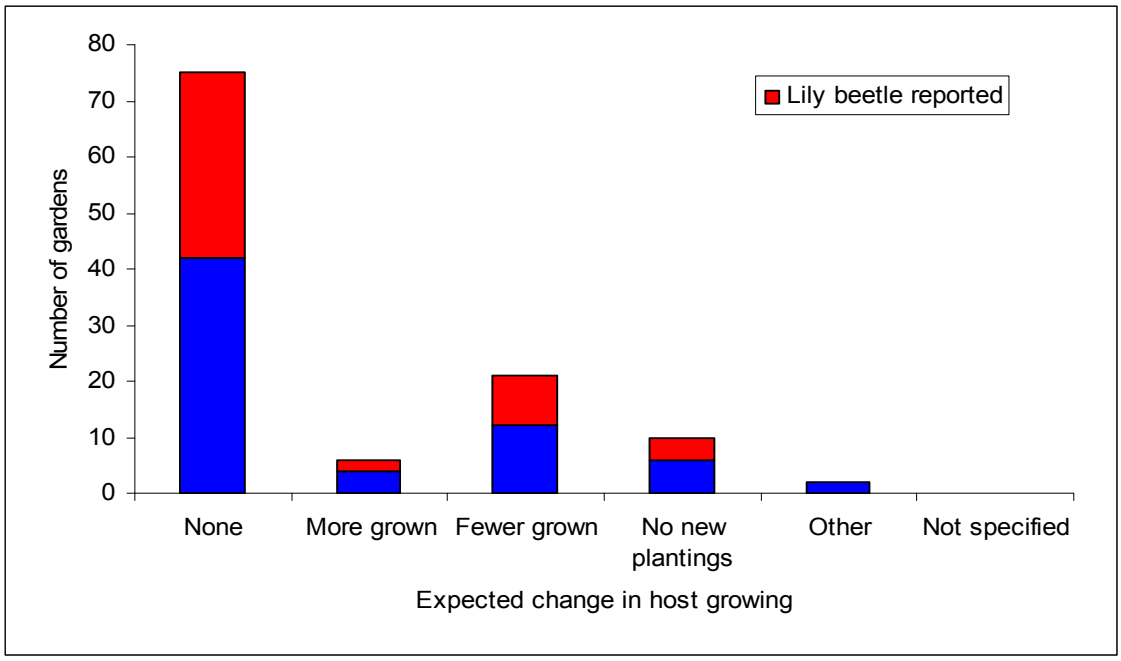


Figure 2.10. Likely effect on lily growing if lily beetle becomes established in gardens open to the public.

Q3a. *Where do you source new stocks of lilies/ fritillaries for the garden?* Responses have been compared with question Q2c. Almost all gardens bought some plants from UK wholesalers or retailers (102, 89%); of this group 28 (23%) indicated that they would reduce or stop growing lilies if *L. lilii* becomes a problem (Figure 2.11), possibly causing a drop in lily sales.

Q3b. *What stage of plants do you source?* Anthropogenic introduction of beetles is most likely with plants in pots. Most (71, 62%) gardens sourced plants as bulbs alone, whilst 23 (20%) gardens sourced plants in pots. Some gardens in all categories had a problem with *L. lilii*, indicating that the beetle does not necessarily arrive with plant purchases.

Q3c. *How do you grow lilies/ fritillaries?* Almost all (113, 98%) gardens grew at least some plants outside. Whether in pots or in the open ground, some gardens had a problem with *L. lilii* only two gardens grew lilies exclusively under glass, one of these had a problem with *L. lilii*.

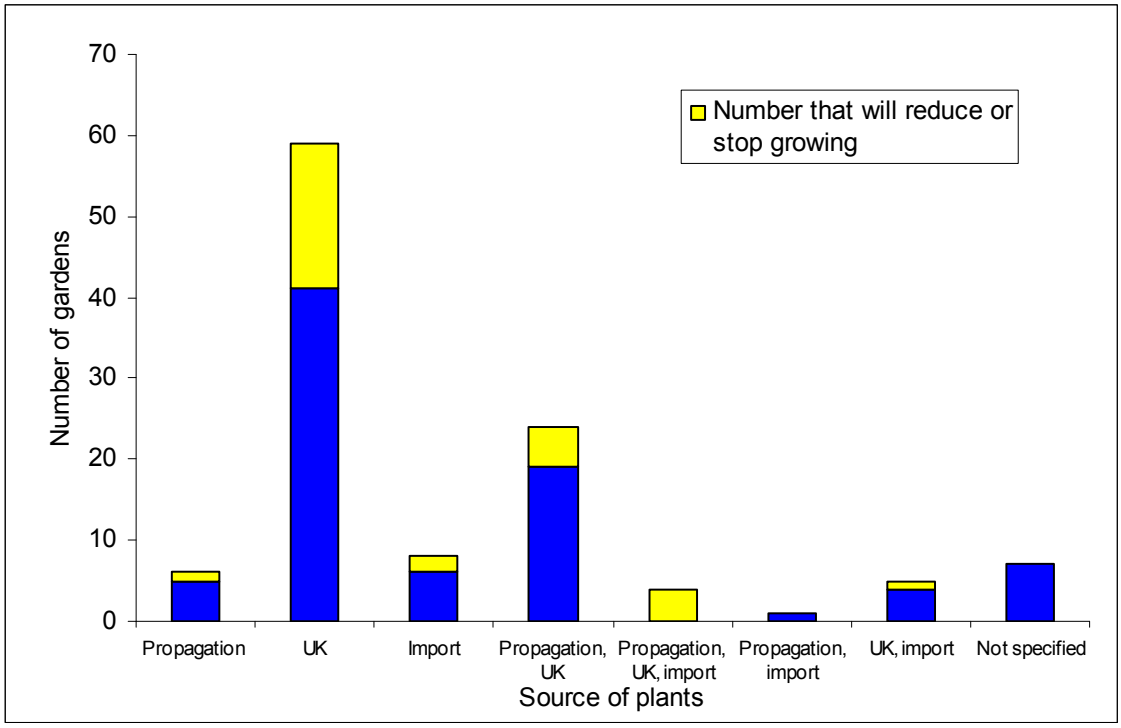


Figure 2.11. Where gardens source lilies and the effect lily beetle may have on plant purchases.

Q4a. *Have you had any pest or disease problems other than lily beetle on lilies/ fritillaries?*

Pests: Combined with those with a *L. lilii* problem (Q2a), 95 gardens reported a pest problem, the most frequent was slugs (54, 47%), second was *L. lilii* (51, 44%; Figure 2.12).

Diseases: Fewer gardens (31; 26%) had problems with lily diseases compared to pests, the biggest problem being bulb rots affecting 23 (20%) gardens. Thus, as with lily providers, (see 2.3.1. Q7a), pests are a greater problem than diseases.

Q4b. *Are there any chemical inputs into lily/ fritillary growing?* Eighty-six (75%) gardens did not use chemical inputs on lilies, 16 (13%) used pesticides, 22 (19%) fertilizer and four (3%) fungicides. Imidacloprid/ thiocloprid were the most popular pesticides (6 gardens), primarily used against vine weevil. It appears that in gardens open to the public, chemical inputs into lily growing are low and, despite *L. lilii*, are likely to remain low (see also Q2b).

Q5a. *Do you have a plant centre on site?* Of the 135 survey forms returned, 129 responded, and 91 (70%) sold plants on site.

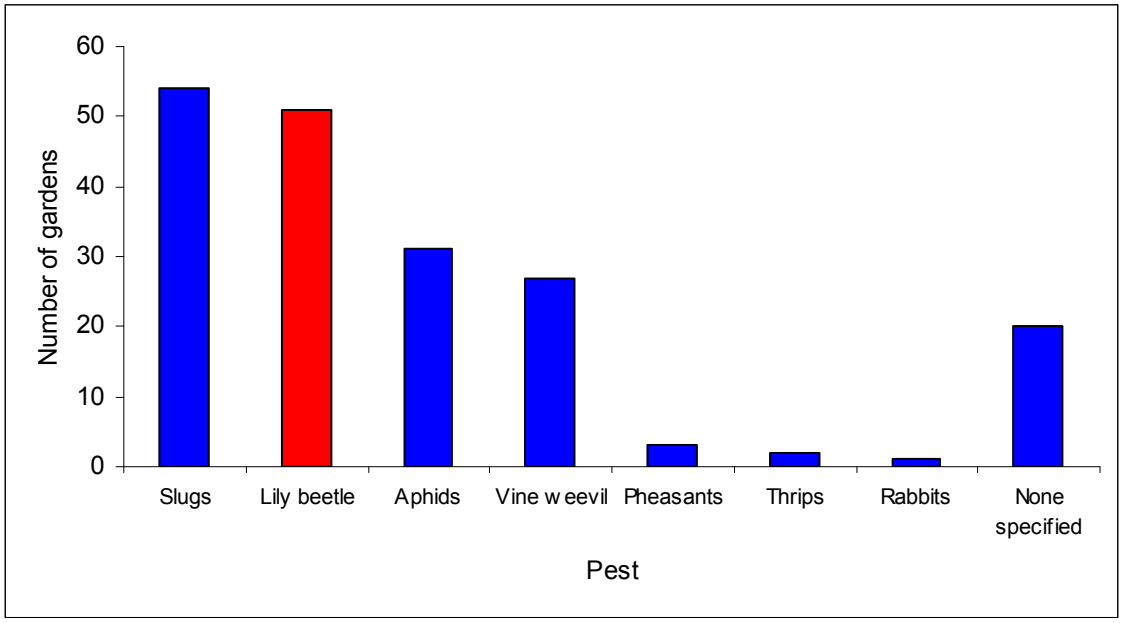


Figure 2.12. Pests experienced on lilies in gardens open to the public.

Q5b. *Does the plant centre sell lilies/ fritillaries?* Forty-five (49%) of the 91 gardens sold lilies, which indicates these plant's popularity in sales. Most (42), of these gardens sold lilies in pots and as stated above (2.3.2, Q5b) this is the stage that presents the biggest risk for artificially spreading the beetle.

Q5c. *Has the volume of sales of lilies/ fritillaries been increasing or decreasing over the past five years?* There were 33 responses, most (28, 73%) stated that sales of lilies were increasing or staying the same. Five gardens stated that lily sales were decreasing; it is not known if this is due to the beetle.

Q5d. *Have plant sales staff received queries about the lily beetle from customers?* Of the 53 responses, 13 had received enquiries on *L. lilii*.

2.5. CONCLUSIONS

It is clear that *L. lilii* is a problem for many amateur lily growers throughout England and Wales. The beetle's recent rapid spread in England and Wales, its survival in Scotland and Northern Ireland and its worldwide distribution indicate that it will eventually become distributed throughout the UK, wherever its host plants are grown, and become a more serious problem for the professional horticulturist. The beetle can be a problem under almost any growing regime, including plants grown under protection, and is currently the second most important pest on lilies for providers and professional gardeners. *Lilioceris lilii* infestations are unlikely to increase chemical inputs into gardens open to the public, although more time is likely to be spent manually removing beetles. For the provider of lilies it is likely to involve greater use of broad-spectrum insecticides and time maintaining the crop, resulting in production cost increases.

The effect of *L. lilii* on lily sales is less clear. Whilst both amateur and professional gardeners have indicated that fewer lilies would be planted, the surveys indicate that sales of lilies have increased over the past five years.

It has often been suggested that the lily industry may spread the beetle throughout the UK through distribution of potted lilies. There is no evidence from these surveys to support or refute this theory. Whilst the beetle was introduced into England in the 1930s, most probably with plants (Barton 1940, Fox Wilson 1942), and the infestations in Scotland and Northern Ireland are likely to have been imported with plant material it is likely that most of its spread in England and Wales has been due to other factors (Chapter 1).

2.6. RECOMMENDATIONS

The surveys and literature review (Chapter 1) have resulted in the following recommendations:

- Lily providers and professional gardeners should remain vigilant and take action against the beetle as necessary. For providers, currently the most successful action is likely to be the use of a broad spectrum foliar insecticide; this may need repeating throughout the natural growing season.
- Providers who supply lilies in pots should take care that outgoing stock is free from the beetle and its damage. This is particularly important when supplying large retail outlets where contamination with beetles or their damage can result in economic penalties and rejected shipments.

- In gardens, mulching the ground around lilies should be avoided where possible as this may reduce the overwintering success of the beetle's parasitoids (see section 1.11).
- Gardeners should remain vigilant and attempt to reduce populations, by either hand picking or pesticide. However, with large collections of lilies it may prove impossible to control the beetle.
- The risk to native populations of *Fritillaria meleagris*, which is a rare UK wildflower, should be assessed.

CHAPTER 3. DO LILIES VARY IN SUSCEPTIBILITY TO THE LILY BEETLE?

3.1. INTRODUCTION

Lilioceris lilii is a serious pest primarily of its *Lilium* hosts in the UK, North America and the Netherlands (Chapters 1 and 2). There are approximately 100 *Lilium* species which are classified into seven species groups (Comber 1949, Table 1.2). This classification is based on the native distribution, morphological and developmental characteristics of *Lilium* species and has remained largely unchanged since conception (Jefferson-Brown 2003). There are more than 10 000 named lily hybrids (K. Donald, International Lily Registrar, pers. com, 2007) currently classified into eight divisions usually based on the group or division of the dominant parent (Leslie 1982, Matthews 2007, Table 1.2). This system of classification has become increasingly arbitrary as breeding barriers between the different divisions have been broken down and a reassessment of the classification of hybrid lilies is long overdue, although this has not yet been undertaken (K. Donald pers. com, 2007). It is the hybrid lilies that are most widely grown as these are easily cultivated in contrast to many species lilies, which have a reputation of being short lived and difficult to propagate (Jefferson-Brown and Howland 1995).

At least one life stage of *L. lilii* has been observed on 30 species and 57 hybrid *Lilium* and all the species groups and most of the hybrid divisions (except division III), are represented (section 1.3). Several studies indicate that different *Lilium* vary in susceptibility to *L. lilii* attack (section 1.3). These studies have only assessed 15 *Lilium* and the conclusions do not give a clear indication or quantification of lilies that are less prone to attack. It is clear that further investigations using experimental designs that enable comparisons of lilies representative of the lily species groups and hybrid divisions are required to ascertain if there are any patterns of resistance of different *Lilium* to *L. lilii.*

Much of what is known about the life cycle and phenology of *L. lilii* is based on observations made during the early part of the 20th century (e.g. Lataste 1932); these published works often provide data that is unsubstantiated and later contradicted (section 1.2). Some early misconceptions on *L. lilii* life cycle are still widely reported in the pest control literature, for example Alford (1995) refers to two generations of *L. lilii* a year; recent research has indicated that the beetle is strictly univoltine (Haye and Kenis 2004). There have been no recent published studies on the beetle in the field and it was clear that additional work would be required to clarify the phenology of *L. lilii* under UK conditions.

A field trial monitored over three years has enabled a comparison of the susceptibility of six lilies to *L. lilii.* The lilies in the trial were representative of those in

commercial production in Europe. In addition, data on the phenology of *L. lilii* was obtained and compared with observations made in a garden situation where the beetle had been established for more than 50 years.

3.2. MATERIALS AND METHODS

3.2.1. Field trial

Choice of lily: A commercial producer and importer of lilies (G.V. Verdegaal Export, Kings Lynn) provided at least 120 bulbs of each of six *Lilium* (Table 3.1). With the exception of *L. regale*, species lilies were not used due to the unavailability of the quantities of bulbs required and that many species lilies tend to be short lived and are not widely grown (Jefferson-Brown and Howland 1995).

Trial design: The trial was laid out over two replicate plots (10 x 10 m), at the Deers Farm trials site (Wisley Village, Surrey). No lilies had been planted at the site for the previous three years and the nearest *Lilium* were approximately 100 m away from plot 1 and 200 m away from plot 2, in a private garden. Each plot was sub-divided into a six by six grid, giving 36 sub-plots. Each sub-plot contained nine equally spaced (3 x 3) lilies (of the same *Lilium*) in 3 L pots. The plants in each sub-plot were numbered from 1 to 9 (top left = 1, bottom right = 9). The lily types were randomly allocated a letter (A-F), reallocated for the second plot, and placed in the plots following a quasi-complete Latin square design (Table 3.1, Figures 3.1, 3.2, 3.3). This design equalised edge and neighbour effects, as each *Lilium* was on the edge three times and neighbours with the other lilies on both the horizontal and vertical twice.

Table 3.1. *Lilium* used in the field trial and allocation of positions in plots.

Lilium	**Species group/ hybrid division***	**Position****	
		Plot 1	**Plot 2**
regale	6	A	B
'Eleganza'	I	E	F
'Golden Joy'	I	D	D
'Brindisi'	V	F	E
'Tiber'	VII	C	C
'Conca d'Or'	VIII	B	A

*See Table 1.2. ** See Figure 3.1.

C	E	A	B	D	F
F	D	B	A	E	C
A	F	E	D	C	B
E	B	F	C	A	D
B	C	D	E	F	A
D	A	C	F	B	E

Figure 3.1. The 6 x 6 Latin square design, used in the field trial.

Treatment of plants: The lilies were grown in 3 L plastic pots in Hardy Ornamental Compost (Sinclair Horticulture, Lincoln). Bulbs were potted up and placed in the plots in January 2005, with the lower two-thirds of the pots buried in the soil to increase stability. Each pot was irrigated individually from April to October, using an automatic system. Plants that were not growing by mid May of each season were replaced from spare stock, which was grown under fine netting to protect them from *L. lilii* infestation (Figure 3.3). Both the pots and the surrounding soil were hand weeded as necessary. In December of each year dead stems were removed to reduce the potential for fungal infection, mirroring common garden practice. Compost was also topped up in the pots, replacing that which had been removed during weeding. In March of each year the plants were fed with a slow-release fertilizer (Vitax Q4, Vitax Ltd. Coalville, Leicestershire) at approximately 5 mg per pot, to maintain plant vigour.

Data collection: Measurements (Table 3.2) were made each week from when the first lily shoots appeared until all plants had senesced (March to October). On each sampling date, the plant for inspection was designated by randomly choosing a number from 1-9; all the plants in that position in a plot were inspected. The random number was recalculated for the second plot. If a plant was missing (e.g. due to death or senescence) measurements were treated as missing values.

Figure 3.2. Plot 1 of the field trial (July 2005).

Figure 3.3. Plot 2 and spare lilies (under fine netting) of the field trial (July 2005).

Table 3.2. Data gathered weekly in the *Lilium* field trial and in the Wild Garden.

Measurement	Notes
Beetle damage score	0: No damage
	1: Adult damage only (leaf edges notched)
	2: Adult and larval damage (<50% defoliation)
	3: Serious damage (50-90% defoliation)
	4: Plant stripped (90%+ defoliation)
Presence of eggs	Number in batch and position
Presence of larvae	Number in each group*, position and an estimate of instar**
Presence of adults	Number, position and notes on behaviour

* A group of larvae was defined as larvae together on a leaf or within 5 mm of each other on a stem/ flower or flower bud.

** Instar estimates were based on a visual estimate of length. First-instar <2 mm, second/ third-instar 2 to 5 mm, fourth-instar >5 mm.

3.2.2. Observations in the Wild Garden

To allow some comparisons of phenology between the field trial and an established population of *L. lilii*, observations were made in the Wild Garden, Wisley Garden (Surrey), where the beetle has been present for at least 50 years (RHS data). During summer 2004 14 patches of *Lilium* were chosen at random for weekly observation. The patches ranged in size from one to over 50 stems. Approximately 10% of the stems were chosen for observations per patch (i.e. patch size <10 stems, 1 stem; 10-20 stems, 2 stems…50+ stems, 5 stems). The method of stem 'choice' was dependent on patch size. In patches with less than 10 stems the n^{th} stem along from the left hand side of the lily patch was chosen randomly. For larger patches random numbers were used to give a 'grid location' for a plant in a patch. In total 24 stems (Table 3.3) were visited weekly on the same dates the field trial and measurements (Table 3.2) were completed.

3.2.3. Data analysis

Field trial: For each sub-plot within each plot a damage index was calculated by totalling the damage score from each visit for each year, and dividing by the number of visits in the year. When a plant was missing the visit for that week was not included in the calculation, taking some account of missing values. Scoring visits were those from the first recording visit in May until the first visit in August, 14 visits each year. This encompassed the period when most plants had emerged until all plants had flowered

and the majority of plants had begun to senesce. A repeated measures analysis of variance was carried out on the resulting index using Genstat 9.1.0 (Lawes Agricultural Trust). Indexes of adult, larval and egg presence (mean numbers/ plant/ visit) were calculated and analysed similarly.

Analysis of the data from each visit to enable comparisons between *Lilium* and visits was attempted. In this case however the data structure was often inappropriate for analysis by ANOVA and provided limited additional information to analysis of the indexes.

Phenology observations and comparisons between the field trial and the Wild Garden: As the field trial was a designed experiment and observations in the Wild Garden were made on lilies planted for aesthetic purposes it was not possible to compare measurements statistically. Nevertheless observations on mean plant damage, times of occurrence, positions on the plant and groupings of each beetle life stage provided some useful observations and casual comparisons. Summary statistics were obtained using Genstat 9.1.0.

Table 3.3. *Lilium* in the Wild Garden, Wisley Garden on which weekly observations were made.

Lilium	**Species group/ hybrid division***	**Stems in patch (number visited)**
L. hansonii	1	30 to 40 (4)
L. henryi	5	5 (1)
L. lancifolium	5	7 (1)
L. lancifolium var. *splendens* (patch A)	5	50+ (5)
L. lancifolium var. *splendens* (patch B)	5	4 (1)
L. formosanum var. *pricei*	6	4 (1)
L. leucanthum	6	17 (2)
L. regale	6	17 (2)
L. sargentiae	6	6 (1)
L. 'Prins Constatjn'	I	10 (1)
L. x *hollandicum*	I	7 (1)
L. 'San Gabrial'	IV	2 (1)
L. x centigale	VI	19 (2)
L. 'Everest'	VII	1 (1)

* See Table 1.2.

3.3. RESULTS

3.3.1. Do different lilies vary in susceptibility?

Plant damage: Analysis of the plant damage index indicates that the *Lilium* in the field trial differed in susceptibility to *L. lilii* (Figure 3.4). In the first year of the trial no significant differences in the damage index between the lilies were apparent. In the following two years the *Lilium* can be divided in to those that had a higher damage index ('Brindisi', 'Conca d'Or', 'Eleganza', 'Golden Joy') and one with a significantly lower damage index (*L. regale*). Results are less clear with 'Tiber', which in the second year of the trial had a comparatively low damage index, however in year three its index was closer to the Lilies with a higher index, and was the only *Lilium* to show an increase in damage index compared to the previous year. It is apparent that in most cases damage index was lower in year three than in year two and the results indicate that differences between year are important factors in how lilies are affected.

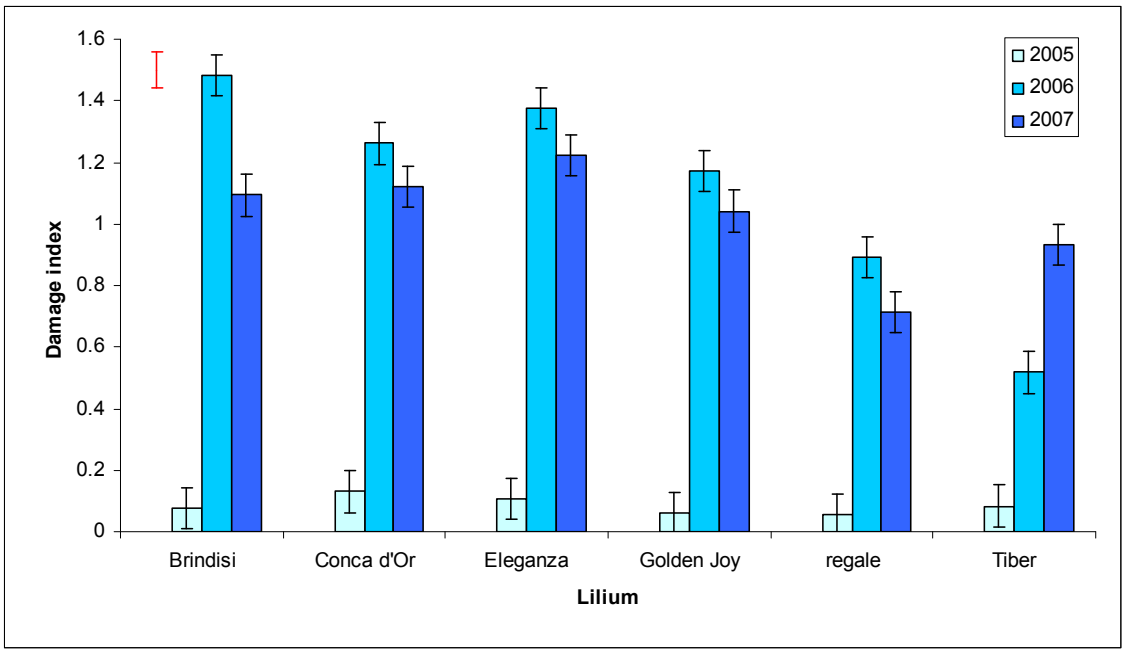


Figure 3.4. *Lilioceris lilii* damage index for the field trial. Results obtained using repeated measures analysis (ANOVA, Genstat 9.1.0). Where *Lilium* differences have F5, 45 = 23.17, p <0.001. Year differences F 1.39, 91.77 = 214.92, p < 0.001. Year x *Lilium* interaction F 6.95, 91.77 = 4.02 p < 0.001. SED (black) = 0.13, d.f. 91.77 is for comparing year within *Lilium*. SED (red) = 0.12, d.f. 122.86 is for comparing year across *Lilium*.

Adult occurrence: As with the damage index the adult index in the first year gave a low value for all *Lilium* in the trial (Figure 3.5). For the next two years of the trial the lilies

can be divided into those which had a consistently lower index (*L. regale* and 'Tiber') and those for which the index varied from year to year ('Brindisi', 'Conca d'Or, 'Eleganza' and 'Golden Joy'). In the second year of the trial 'Brindisi' 'Conca d'Or', 'Eleganza' and 'Golden Joy' can be grouped with lilies with a higher adult index. In the third year the results are less clear, 'Golden Joy' had a low adult index whilst 'Eleganza' and 'Conca d'Or' have a relatively high index, however there is considerable overlap with 'Brindisi', which had a similar index to *L. regale*.

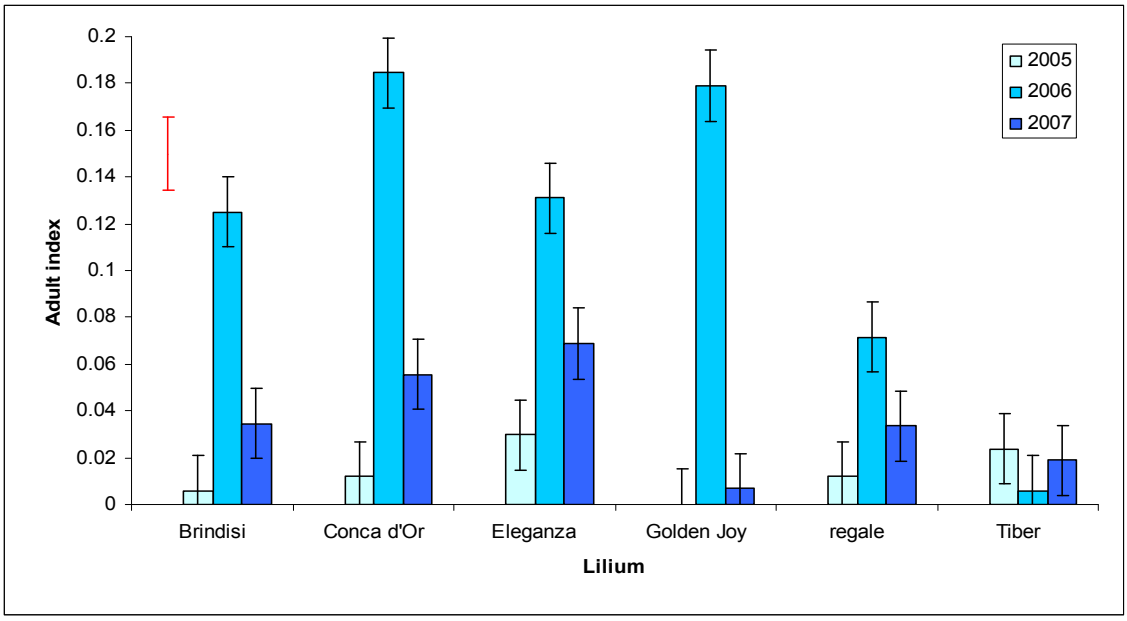


Figure. 3.5. Mean number of adult *Lilioceris lilii* per scoring visit in the field trial. Results obtained using repeated measures analysis (ANOVA, Genstat 9.1.0). Where *Lilium* differences have F 5, 45 = 5.43, p < 0.001. Year differences F 1.79, 118.43 = 19.93, p < 0.001. Year x *Lilium* interaction F 8.97, 156.25 = 2.93, p = 0.004. SED (black) = 0.03, d.f.118.43 is for comparing year means within *Lilium*. SED (red) = 0.03, d.f. 156.25 is for comparing year means across *Lilium*.

Larval occurrence: Lily beetle larvae were observed on all *Lilium* in the trial. In a similar pattern to the damage and adult indexes in the first year of the trial on all *Lilium*, the larval index was low compared to subsequent years (Figure 3.6). However, unlike the other indexes four of the lilies (Conca d'Or', Eleganza', 'Golden Joy' and 'Tiber') had a higher index in year three compared to year two. *Lilium regale* had consistently low larval indexes and 'Conca d'Or' consistently high indexes. 'Eleganza' also had a high larval index compared to *L. regale* in these years, however in year three there is considerable overlap in the larval indexes of 'Brindisi', 'Eleganza', 'Golden Joy' and 'Tiber'.

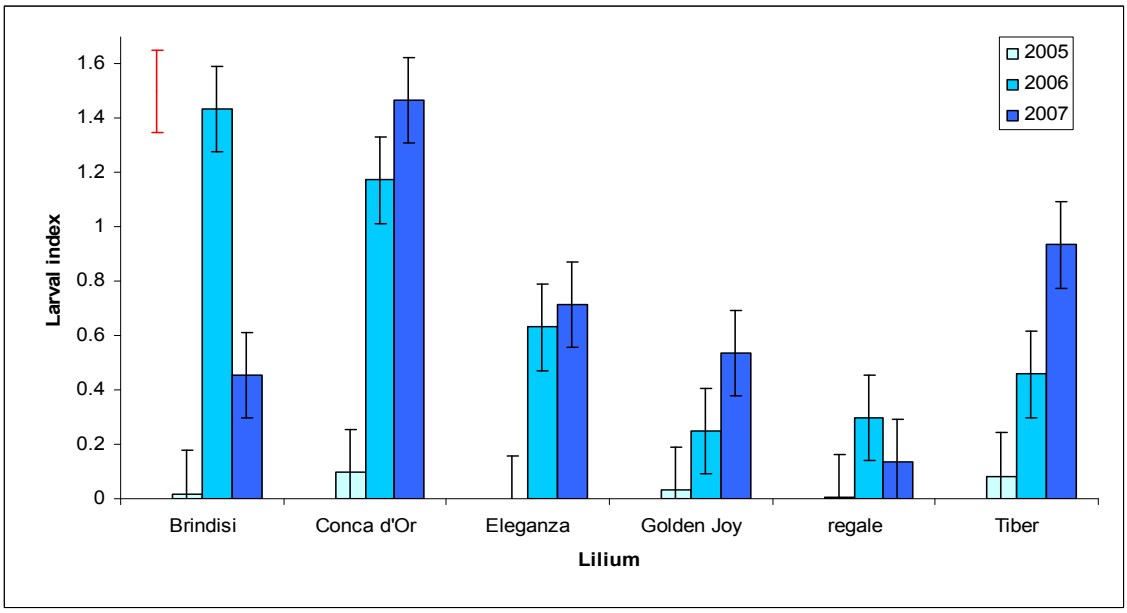


Figure 3.6. Mean number of *Lilioceris lilii* larvae per scoring visit in the field trial. Results obtained using repeated measures analysis (ANOVA, Genstat 9.1.0). Where *Lilium* differences have F 5, 45 = 5.61, p < 0.001. Year differences F 1.65, 109.04 = 17.83, p < 0.001. Year x *Lilium* interaction F 8.26, 153.95 = 2.29, p= 0.025. SED (black) =0.32, d.f.109.04 is for comparing year means within *Lilium*. SED (red) = 0.30, d.f. 153.95 is for comparing year means across *Lilium*.

Egg occurrence: Due to the irregularity of egg observations analyses of the egg index did not provide meaningful results.

3.3.2. Phenology and other observations

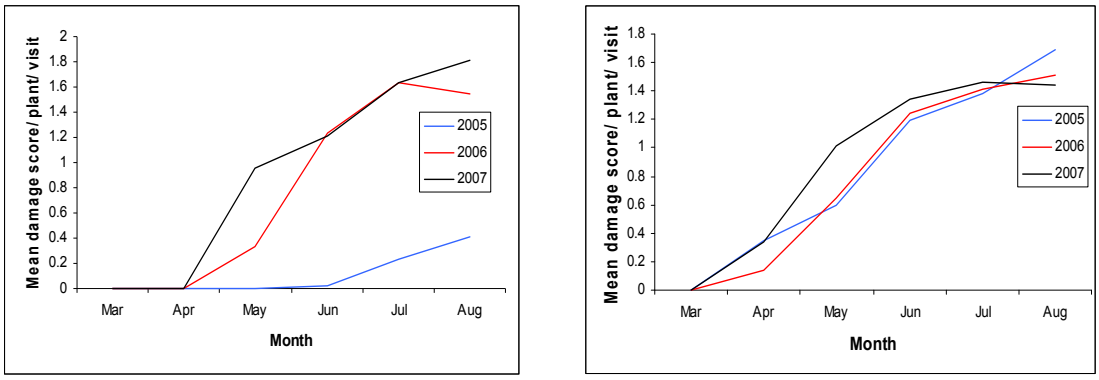
Additional host plants: Adults and larvae were observed on all lilies in the field trial, observations on these *Lilium* hybrids had not been made before, thus the beetle is now known from 62 hybrid *Lilium* (see section 3.1). The beetle had previously been reported on all the *Lilium* observed in the Wild Garden (section 1.3), although adults had not been reported on *L.* 'Everest' (Table 3.4).

Damage score: The mean damage score has only been assessed until the end of August each year, as by this time many plants had senesced, having the effect that the mean damage score appeared to drop. As can be expected damage increases throughout the season, beginning in April or May (Figure 3.7). After the first year of the trial mean damage scores at the sites are similar.

Table 3.4. Summary of observations of *Lilioceris lilii* in the Wild Garden, Wisley.

✓ indicates life stage observed on at least one site visit between 2005 and 2007

Lilium	Adults	Larvae / eggs
L. hansonii	✓	✓
L. henryi	✓	
L. lancifolium	✓	✓
L. lancifolium var. *splendens* (patch A)	✓	✓
L. lancifolium var. *splendens* (patch B)	✓	✓
Lilium formosanum var *pricei*	✓	✓
L. leucanthum	✓	✓
L. regale	✓	✓
Lilium sargentiae	✓	✓
L. 'Prins Constatjn'	✓	✓
L. x *hollandicum*	✓	✓
L. 'San Gabrial'	✓	✓
L. x *centigale*	✓	✓
L. 'Everest'	✓	



Field trial Wild Garden

Figure 3.7. The mean *Lilioceris lilii* damage score on *Lilium* in the Wild Garden and Field Trial at Wisley, over three years.

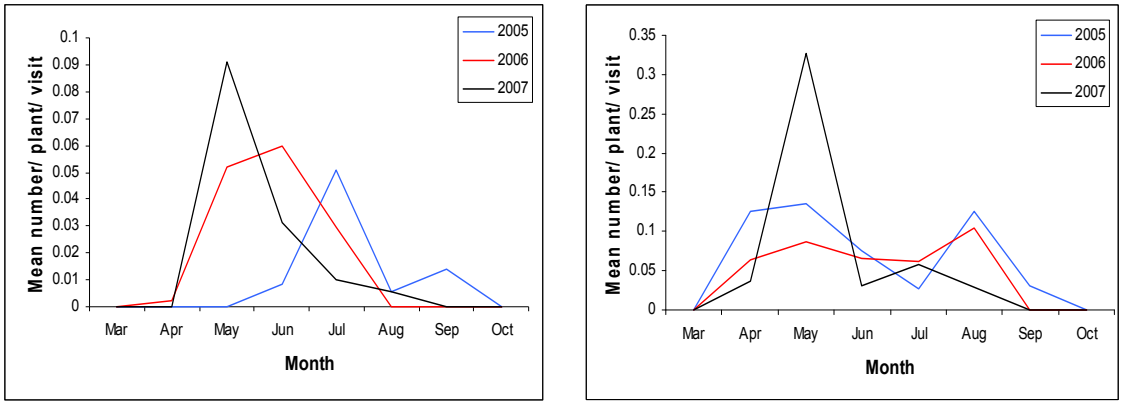
Adults: In the first year of the trial *L. lilii* adults were observed in the Wild Garden two months before they were observed in the field trial (Table 3.5). In the subsequent year the difference was less than two weeks and in the third year of the trial adults were first observed at both sites on the same day. Over the three years adults were observed on plants from early April until mid September and copulating pairs were observed from mid April until July.

Table 3.5. Summary of first, final and mating observations of adult *Lilioceris lilii* at Wisley, Surrey, over three years.

Year	Site	First observation	Final observation	Copulating pairs (first / final observation)
2005	Field trial	8 June	15 September	16 June*
	Wild Garden	6 April	31 August	13, April / 15 June
2006	Field trial	25 April	26 July	10 May / 12 July
	Wild Garden	13 April	9 August	13 April*
2007	Field trial	11 April	8 August	13 June*
	Wild Garden	11 April	8 August	24 April/ 13 June

*Only one observation of copulating pairs at these sites in these years.

Considering the mean number of adults seen per plant per visit for each month (Figure 3.8), the two sites show some differences in the patterns of occurrence. In all years the mean number of adults per plant was up to three times greater in the Wild Garden than in the field trial. Data from the Wild Garden shows two peaks of adult occurrence, one in the early season (May in all years) and one later in the season (August) in the first two years. The field trial data gave two peaks that lag a month behind those of the Wild Garden in the first year. In subsequent years of the trial there is only one peak, in the second year lagging behind the first peak in the Wild Garden by a month. In the third year the first peak of occurrence of the beetle was in May as it was in the Wild Garden. In the final year of the trial the first peak of adults in the Wild Garden was noticeably higher than the second and that of previous years.



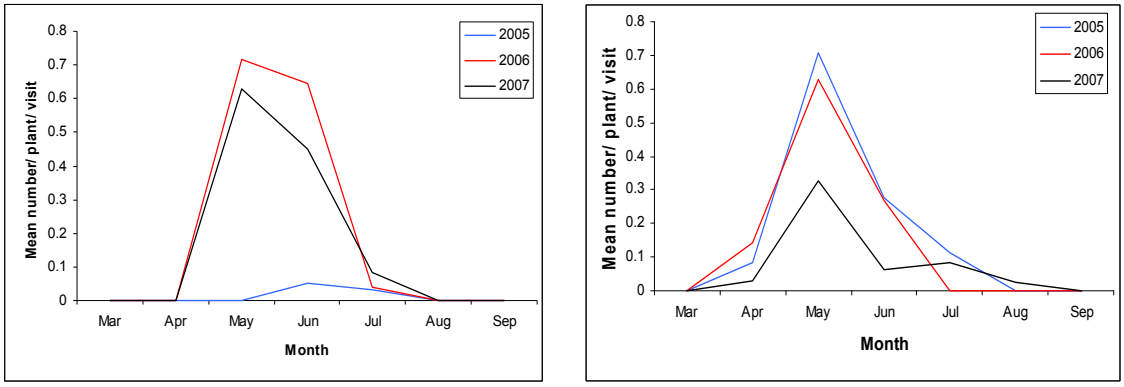
Field trial Wild Garden

Figure 3.8. The occurrence (mean number) of adult *Lilioceris lilii* observed in the Wild Garden and Field Trial, Wisley, over three years.

Eggs: The first eggs were observed in April in all three years and final observations made in late July or early August (Table 3.6). With the exception of the field trial in the first year peak egg occurrence occurred in May at both sites (Figure 3.9). The peak in mean number of eggs observed per plant was similar in the Wild Garden and field trial.

Table 3.6. Summary of first and final observations of *Lilioceris lilii* eggs at Wisley, Surrey, over three years.

Year	Site	First observation	Final observation
2005	Field trial	8 June	20 July
	Wild Garden	6 April	20 July
2006	Field trial	24 May	1 August
	Wild Garden	19 April	7 June
2007	Field trial	11 April	3 July
	Wild Garden	25 April	1 August



Field trial Wild garden

Figure 3.9. The occurrence (mean number) of *Lilioceris lilii* eggs observed in the Wild Garden and Field Trial at Wisley, over three years.

In total 125 egg batches (646 individual eggs) were observed over the three years at both sites, all were on the ventral side of leaves in linear rows. The number of eggs in a batch ranged from 1 to 15, with a median of 6 and a mean of 5.6 (SD 2.27, Figure 3.10).

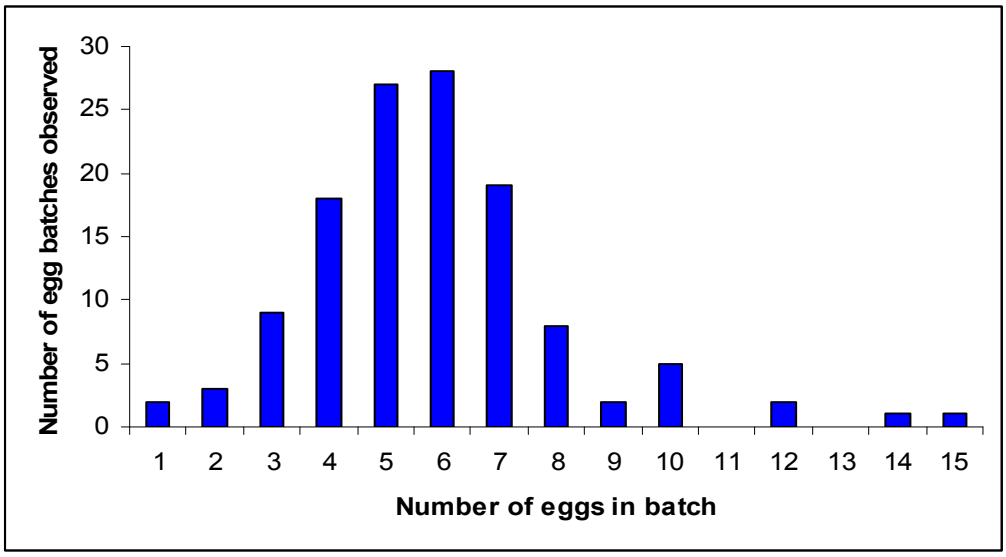


Figure 3.10. Frequency of *Lilioceris lilii* egg batch size observed at Wisley. Data combined from all egg observations over three years in the field trial and Wild Garden, Wisley.

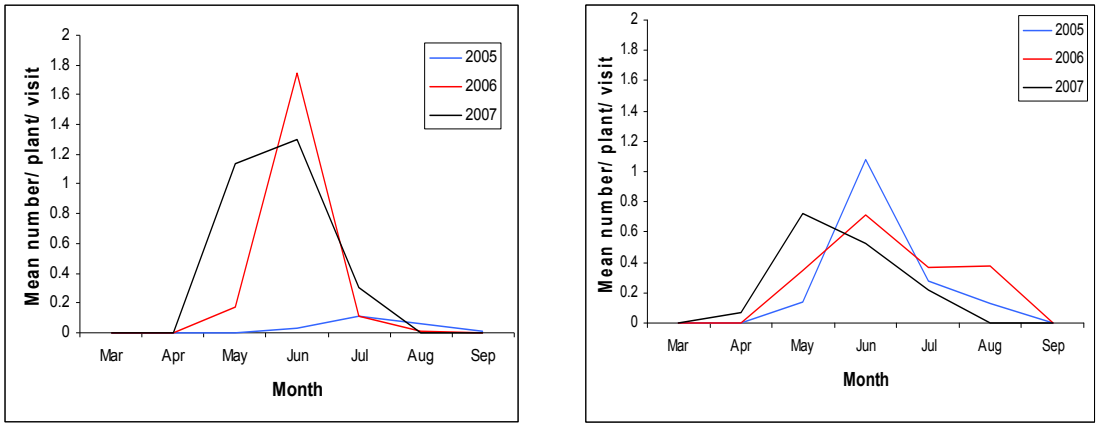
Larvae: Larvae were observed from early May to mid August (Table 3.7). With the exception of the final year in the Wild Garden and the first year in the field trial, larval numbers peaked in June at both sites. In the final year in the Wild Garden larval numbers peaked in May (Figure 3.11).

Table 3.7. Summary of first and final observations of *Lilioceris lilii* larvae at Wisley, Surrey, over three years.

Year	Site	First observation	Final observation
2005	Field trial	21 June	10 August
	Wild Garden	18 May	10 August
2006	Field trial	16 May	1 August
	Wild Garden	10 May	9 August
2007	Field trial	5 May	12 July
	Wild Garden	5 May	12 July

Combining the data from both sites for all three years 1784 larvae in 725 groups were observed (Figure 3.12). All 655 first instar larvae observed were found on the ventral side of leaves. These were found in groups with a mean size of 4.82 (SD 2.76, median 5). Second/ third instar larvae accounted for 853 larvae and were found in groups with a mean size of 2.26 (SD 1.96, median 2), almost all were observed on the

ventral side of the leaf, six observed on the dorsal side and one on a flower bud. Fourth instar larvae accounted for 276 of the larvae observed and were found in groups with a mean size of 1.3 (SD 1.66, median 1), with the exception of four observed on the dorsal side of the leaf and two on a lily stem all were observed on the ventral side of leaves.



Field trial Wild Garden

Figure 3.11. The occurrence (mean number) of *Lilioceris lilii* larvae observed in the Wild Garden and Field Trial at Wisley, over three years.

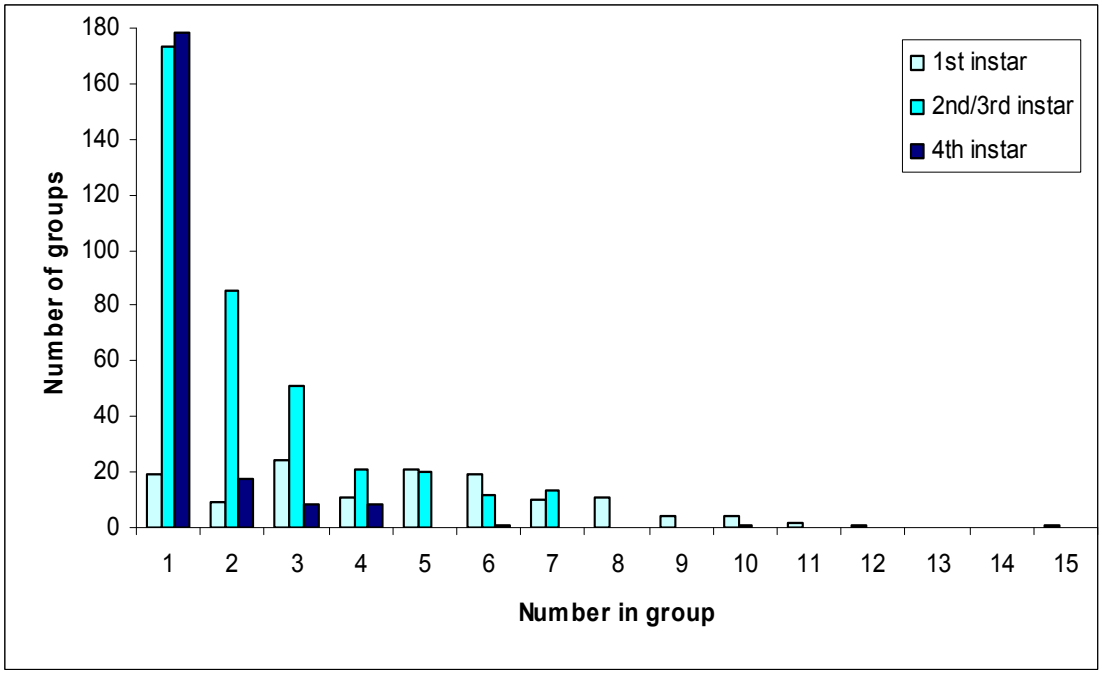


Figure. 3.12. *Lilioceris lilii* larval group size. Data combined from all larval observations over three years in the field trial and Wild Garden, Wisley.

3.4. DISCUSSION

3.4.1. Do different lilies vary in susceptibility?

It is clear that all the *Lilium* in the trial can act as hosts for the beetle, as larvae were observed on all lilies, a similar result to observations in other susceptibility trials with *Lilium* (Conjin pers. com, 2000). However, the damage, adult and larval indexes indicate that there are differences between the susceptibility of different *Lilium*, although interpretation of the results requires some care. In year one of the trial all three indexes showed no differences in the susceptibility of the lilies. This result may have been due to the fact that the beetle did not occur in the trial until June of that year, whereas in subsequent years and in the Wild Garden, the beetle was observed from late April or early May. Thus the low indexes and limited differences between the lilies in the first year are likely to be due to this being the year when the beetle was becoming established in the plots and therefore present at low densities. This supposition is supported by comparisons with infestation levels at a site where the beetle has been established for more than 50 years (section 3.2.2). Data from all the indexes in years two and three indicate that, of the *Lilium* tested in the trial, *L. regale* was least susceptible to *L. lilii* damage whilst damage and larval indexes indicate that *L.* 'Conca d'Or' was most susceptible to damage. The damage index indicates that *L.* 'Eleganza', 'Brindisi' and 'Golden Joy' were also more susceptible than the others tested, however this result is only partially supported by the two indexes of beetle occurrence where differences between year are more apparent. The lack of consistently clear results with the beetle occurrence indexes may have been due to the ephemeral nature of the presence of the beetle. Adult *L. lilii* are mobile and often drop off plants when approached (RHS 2007), they are also adept at 'hiding' at the base of leaves or on warm days flying from the plant from which measurements are being taken (pers. obs). Eggs, although orange and immobile are small, found on the underside of leaves and transient, each batch is only present for 4 to 10 days (Haye and Kenis 2004). Every leaf needs to be examined to find all egg batches (pers. obs), this proved impossible in the field, probably leading to under-recording of egg numbers and the lack of dependable results from the egg index. Larval occurrence should be more reliable, as larvae are relatively immobile and the damage caused is usually apparent on the dorsal surface of the leaf leading to detection of the larvae on the underside, however each larva is only present for up to 24 days (Haye and Kenis 2004). Plant damage once caused remains evident for the rest of the season, and may be considered a more reliable indication of relative susceptibility to the beetle. However, the measurements were subjective, and reliant on consistency of recording; all measurements were made a single observer, generally considered the best way of

maintaining consistency in recording (Martin and Bateson 2007). Taking these factors into account it is possible to categorise the lilies in the trial into those which are most susceptible to damage and those which are least susceptible (Table 3.8). The variety 'Tiber' appeared to be attacked more heavily as the trial progressed and for this reason has been tentatively placed as a more susceptible *Lilium*.

Table 3.8. Summary of knowledge of *Lilium* susceptibility to *Lilioceris lilii.*

[1] = This trial, [2] = Livingston (1996), [3] = Conjin (pers. com, 2000) [4] = Casagrande and Tewksbury (2007a).

Least susceptible	Group*	Most susceptible	Group*
henryi[2,3]	5	*auratum*[4]	4
speciosum[2]	4	*lancifolium*[2]	4
regale[1]	6	'Eleganza'[1]	I
'Lollypop'[4]	I	'Golden Joy'[1]	I
'Reinesse'[3]	I	'Grand Cru'[3]	I
'Black Beauty' [3,4]	VII	'Brindisi'[1]	V
'Donau'[3]	VII	'African Queen'[3]	VI
		Oriental hybrids[2]	VI
		'Acapulco'[4]	VII
		'Berlin'[3]	VII
		'Casa Blanca'[4]	VII
		'Oriental Pink'[4]	VII
		'Tiber'[1**]	VII
		'Conca d'Or'[1]	VIII

*see Table 1.2.

** In the field trial 'Tiber' gave results that varied from year to year and has tentatively been placed as a more susceptible *Lilium*.

This trial used different methodologies to other studies, and care should be taken with comparisons of results (Table 3.8). Livingston (1996) and Casagrande and Tewksbury (2007b) used laboratory 'choice' tests and measure numbers of the different life stages present and surviving on plants. Conjin (pers. com. 2000) assessed plants for percentage damage in the field, therefore if future tests of the susceptibility of *L. lilii* host plants to the beetle are to be carried out the lilies need to be compared with a standard. This should be a lily that is widely available, has shown some consistency in trial results and is a known host for the beetle. A suitable candidate would be *L.*

regale as it is known that the beetle can complete its life cycle on this species as it is used for laboratory culture (chapter 4). Despite the differences in methodologies if it is assumed that the conclusions of other authors regarding the least and most susceptible lilies are valid it is apparent that susceptibility to lily beetle varies within a hybrid division or species group. *Lilium* belonging to species group 4 and hybrid divisions I and VII are represented in both the most susceptible and least susceptible categories. This may indicate that susceptibility of *Lilium* to the beetle is not related to what is currently considered the taxonomic relationships of *Lilium*. However, no species from groups 1, 2, 3 or 7 have been tested, nor hybrids from divisions II, III, IV, and no observations of *L. lilii* on plants in division III have been published (section 1.3). In addition the lilies investigated to date represent a small proportion of the 100 species and 10 000 hybrids (section 3.1) and further trials are required before the conclusions presented can be established. It is perhaps more likely that individual *Lilium* hybrids and species vary in susceptibility due to phenology or morphology.

Livingston (1996) suggests that more resistant plants may be late blooming, however *L.* 'Reinesse' and *L.* 'Donau' are early flowering and considered less susceptible (Table 3.8). It is possible that different *Lilium* have different arrays of secondary chemicals, some may be volatile and affect adult *L. lilii* host selection behaviour and it is possible that biochemical changes following beetle attack could also influence beetle host choice. Odour-mediated host location in this beetle has now been demonstrated (Chapter 4). Other possibilities include nutritional factors, the presence of secondary structures on leaves, and other morphological characteristics, such as leaf colour and structure of the plant (see Fernandez and Hilker 2007). Further work is required to determine the causes of differences in susceptibility. Additionally *L. lilii* is able to feed and develop on *Cardiocrinum* and *Fritillaria*, this author can find no comparative work between the three host genera and this is another potential avenue for investigation.

Variations in the susceptibility of *Lilium* may be utilised in management of *L.* lilii. Breeding lilies to maximise resistance is unlikely as hybridising lilies for resistance to the lily beetle is considered a low priority for growers (Livingston 1996). As all *Lilium* tested appear to be suitable hosts for the beetle, planting of lilies less susceptible to damage alone is unlikely to have an effect on beetle numbers. However, differences in susceptibility enables the possibility that more susceptible lilies could be used as trap crops which can be use to draw the beetle away from less susceptible lilies, to an area where they can be treated. Additional trials are required to determine the feasibility of this type of management.

This trial ran for three years, however it is possible that the lily beetle population and the patterns of damage caused to plants will continue to change with time. The differences between year were shown to be a significant factor in the trial, therefore longer term trials may provide clearer information on the susceptibility of the lilies tested.

3.4.2. Phenology and other observations

It should be noted that phenology data have only been collected for three years and as has been indicated from the field trial index data, between-year differences can be greater than those between different *Lilium*. Thus whilst some inferences can be drawn from the data, observations should continue before firm conclusions are made. It should also be noted that in the field trial and Wild Garden no observations were made after host plants had senesced (late September/ early October). Some *Lilium* have green leaves into November and adult beetles have been observed until 1 November at Wisley Garden (pers. obs).

Occurrence data from the Wild Garden and field trial has added further to the list of *Lilium* on which *L. lilii* has been observed. Larvae were found on all but two plants (*L. henryi* and *L.* 'Everest'). The lack of larvae on *L. henryi* corresponds with the trial of Livingstone (1996) who concluded that this species shows some resistance to the beetle, although larvae have been observed by other authors on this *Lilium* (Table 1.3).

As the field trial was a site where no lilies had been grown for at least the previous three years it is clear that *L. lilii* can travel, possibly by flight, at least 100 m, as this is the distance from the closest source of infestation. As eggs were noted at the same time as the first adults in the field trial (8th June 2005) it can be assumed that some post-diapause beetles in a reproductive state disperse. In the first year of the trial adult beetles were observed two months later than in the Wild Garden, by the third year of the trial the first adult beetles were observed both in the Wild Garden and field trial sites in early April. This may be evidence that at least some beetles overwinter near to where they were feeding the previous year and re-colonise plants in the same area.

The mean damage score per plant per visit shows some similarities between the two sites, and demonstrates that on average the damage score for plants in the field trial are similar, if a little higher than those observed in the Wild Garden. The dates of occurrence of adult, egg and larval observations correspond with recent literature, as do the observations made on the presence, position, groupings and behaviour of eggs and larvae (Livingston 1996, section 1.2). However, several additional observations

have been made. First instar larvae feed in groups on the underside of leaves in numbers which roughly correspond to the size of egg batches. As the larvae develop, the groups disperse and fourth instar larvae are usually found singly. It is not known if the spatially aggregated groups of first instar larvae are due to chance, as the eggs are laid in groups, or if their behaviour is truly gregarious.

The two peaks of adult occurrence that occurred in the Wild Garden in two of the three years may correspond with the beetle's univoltine life cycle (section 1.2). The first peak is possibly due to adults emerging from overwintering sites, mating and then numbers declining. The second peak, occurring in July or August corresponds to known dates for the emergence of 'new' adults which were larvae in the current year (Haye and Kenis 2004). With the exception of the first year there was no second peak in the field trial, however the mean number of adults observed per visit per plant were lower than that in the Wild Garden and it may be that such a pattern has yet to emerge. This may also indicate that the population of beetles in the field trial has not yet fully developed. Whilst mean adult numbers per plant per visit remained lower in the field trial the numbers of larvae and eggs and damage score seen per plant were similar to that in the Wild Garden.

CHAPTER 4. ODOUR-MEDIATED BEHAVIOUR OF THE LILY BEETLE (*Lilioceris lilii*)

4.1. INTRODUCTION

In the insects, volatile and non-volatile chemicals (semiochemicals) are important in mediating behaviour, and the leaf beetles (Coleoptera: Chrysomelidae) are no exception. A variety of studies have provided insights into the chemically-mediated responses of this family and the substances involved (section 4.1.1). Several chrysomelids are pests and the potential of utilising knowledge of odour-mediated behaviour in pest management strategies has been demonstrated (section 4.1.1.3). However, most species studied have been agricultural pests and little is known about the odour-mediated behaviour of chrysomelid pests affecting amenity and amateur horticulture. One such species, the lily beetle (*Lilioceris* lilii), has become a serious pest of lilies (*Lilium*) and fritillaries (*Fritillaria*) in the UK and North America (Chapters 1 and 2).

The behavioural responses of *L. lilii* to volatile odours from conspecifics and host plants have not been thoroughly studied. This is despite Southgate (1959) stating "it is obvious that smell plays a large part in the location of these insects with their food-plant" and the behavioural observations of Emmel (1936) suggesting the presence of a pheromone. Following preliminary bioassays at Rothamsted Research, the linear-track olfactometer (LTO) (Sakuma and Fukami 1985, Cook et al. 2002) was used to investigate behavioural responses of adult *L. lilii* to volatile odours produced by combinations of conspecifics and host plants and to determine if responses differ with the beetles physiological state and sex. Adult *L. lilii* have two discernable physiological states; those that have overwintered (diapaused) and are in a reproductive state and those which have not diapaused (pre-diapause) and are not in a reproductive state (Chapter 1, Haye and Kenis 2004). Laboratory culture used to obtain beetles of known physiological state provided some data on the beetle's development. A brief review of the literature concerning chemical-mediated host and conspecific location in the Chrysomelidae is presented to provide a wider context for the observed responses of *L. lilii.*

4.1.1. Chemical-mediated behaviour of the Chrysomelidae

This review briefly outlines current knowledge of chemical-mediated host and conspecific location by chrysomelids, and then discusses current application and future prospects for management of pest species aided by the use of semiochemicals.

4.1.1.1. Host location and acceptance

The Chrysomelidae is a large family of beetles, many of which are specialist herbivores. Typically host location in this group appears efficient, the mechanisms of which were recently reviewed (Fernandez and Hilker 2007) and only a brief summary is presented here. Host location and acceptance in the Chrysomelidae is at least in part odour-mediated, the substances involved (kairomones) can be divided into those that are volatile, acting over a long distance (> 0.5 m) and those which mediate a response at close range, either upon contact or once feeding has been initiated (Mitchell 1988).

Plant odour may contain volatile compounds that are plant-specific or specificity may be achieved by a particular ratio of volatiles, the constituent compounds of which may be produced by many plant species (e.g. the green leaf volatiles (GLV), Visser 1986). Long distance host perception was first demonstrated in olfactometer bioassays with the Colorado beetle, *Leptinotarsa decemlineata* (Say), which moved into odour streams emanating from potato and some other related plants (McIndoo 1926). This response is due to GLV ratio rather than any single volatile component (Ma and Visser 1972, Visser 1979). The rootworms (*Diabrotica* species) feeding on maize (*Zea mays* L.) behave similarly, with a combination of host volatiles having a synergistic effect (Lampman and Metcalf 1987, Lampman et al. 1987). This type of response to a ratio of volatile components is better quantified in the willow leaf beetles *Phratora vulgatissima* (L.), *P. vitellinae* (L.) and *Galerucella lineola* (F.), where host preferences are in part due to the relative amounts of *cis*-3-hexnyl acetate to other GLV produced by different *Salix* varieties (Peacock et al. 2001b). Other chrysomelids respond to volatiles that are more specific to the host; the flea beetles *Phyllotreta striolata* (F.), *P. cruciferae* (Goeze) and *Psylliodes chrysocephala* L. respond to volatile mustard oils (isothiocyanates) that are relatively specific to brassicas (Feeny et al. 1970, Blight et al. 1989). In all the above cases the 'correct' odour blend may emanate from hosts and some non-hosts, however many sympatric plants are excluded and the process of host recognition continues when the beetle arrives in the vicinity of a potential host (Mitchell 1988).

Several chrysomelids aggregate on hosts in response to volatiles induced by herbivore damage; *L. decemlineata* shows a preference for odour-streams from conspecific-damaged potato plants over intact or mechanically damaged hosts (Bolter et al. 1997, Dickens 2000) and *Diorhabda elongata* Brullé responds to blends of four volatiles released from its host (saltcedar, *Tamarix* spp.) when infested by conspecifics (Cossé et al. 2006). Possible explanations for these responses include: defoliation before the hosts' defences can accumulate and affect the herbivore (Schütz et al. 1997); to promote the use of plants suitable for feeding and larval development

(Kalberer et al. 2001, Peacock et al. 2001b); to provide collective defence (Sillén-Tullberg and Leimar 1988) and to facilitate mate location (see section 4.1.1.2). It may, of course be a combination of the above that results in the observed behaviours.

In addition to aggregation, volatile-induced density-dependent repellent effects have been observed. *Xanthogaleruca luteola* (Müller) is repelled by the odour of elm twigs heavily infested with conspecific eggs and/or suffering feeding damage. However, there is a preference for twigs with low levels of infestation over uninfested twigs as oviposition sites. This type of response may reduce both conspecific competition and parasitism (Meiners et al. 2005).

Differences in host-odour induced behaviour have been observed between the sexes and physiological states of chrysomelid beetles. Male *Diabrotica virgifera virgifera* LeConte are more responsive to host (maize) volatiles in late July and late September; females are more responsive during August and early September. This is possibly due to males being more responsive to female pheromones during the breeding season and more responsive to host odour at other times (section 4.1.1.2, Anderson and Metcalf 1986). Male *L. decemlineata* react more strongly to volatiles from intact hosts than do females, behaviour which is consistent with the presence of a male-produced aggregation pheromone in this species (Oliver et al. 2002, section 4.1.1.2). This species has also shown a diminished response to host plant odour 24 h after contact (with the opposite sex) or copulation, which is restored 72 h later (Dickens 2007). This is postulated to be a mechanism that reduces dispersal from plants where potential mates are located and the restoration of sensitivity increasing the likelihood of re-mating following oviposition. Stronger female responses have been observed; both sexes of *P. vulgatissima* aggregate on *Salix* spp. in the spring in response to host odour, and the response is synergistic with the addition of female beetles or beetle damage (Peacock et al. 2001a). Diurnal patterns of response also occur; male *D. v. virgifera* and *D. barberi* Smith & Lawrence are more responsive to host volatiles during the day and to sex pheromones at night (Lance 1990).

Within close proximity to and contact with a potential host, phytophagous insects continue to receive volatile stimuli but also respond to tactile stimuli and compounds with low or no volatility in leaf surface waxes (Städler 1986). In addition, chemical stimuli (including nutrients) are received from within the leaf upon damage to the epidermis (Mitchell 1988). Host acceptance in the chrysomelids studied is probably due to chemical cues rather than tactile stimuli; the composition of glucosides rather than leaf structure determines host acceptance in several willow-feeding chrysomelids (Kolehmainen et al. 1995), and two non-polar contact stimuli are sufficient for host identification by *Cassida canaliculata* Laich without the addition of physical cues from

the plant (Heisswolf et al. 2007). Additional evidence for the role of chemical components in host acceptance is that several chrysomelids will feed on artificial foods or non-hosts if given appropriate feeding stimulants; *G. lineola*, *Lochmaea capreae* L., *P. vitellinae* and *Phaedon cochleariae* F. will feed on artificial diets provided they contain appropriate glucosides (Tanton 1965, Kolehmainen et al. 1995).

Some of the compounds that induce feeding in the chrysomelids are secondary plant chemicals that are antifeedants for other, generalist herbivores, the presence of such chemicals being utilized by the more specialist chrysomelids; cucurbitacins produced by the Cucurbitaceae are potent anitfeedants for generalist herbivores but are feeding stimulants and arrestants for *Diabrotica* species (Metcalf et al. 1980). Some chrysomelids sequester and utilise these secondary compounds in defensive secretions (see Kuhn et al. 2007 and references therein). Feeding stimulants may become antifeedant (allomones) when concentrations change: high concentrations of α-tocopherylquinone, a feeding stimulant for *Chrysomela scripta* F. result in reduced feeding by the beetle (Lin et al. 1998). Chrysomelids are also in contact with primary plant compounds (amino acids, sugars and organic acids), some of these ubiquitous compounds act as feeding stimulants and these should be considered part of the host acceptance process (Mitchell 1988).

Chemical cues are not the only stimuli received and utilized by chrysomelids searching for hosts; *D. v. virgifera* locate curcubit blossoms not only by odour, but by their yellow colour (Anderson and Metcalf 1987), *Oreina cacaliae* (Schrank) respond more readily in olfactometer experiments with the addition of visual cues (Kalberer et al. 2001) and only visual cues have been identified as stimuli in the host location of *Altica engstroemi* Sahlberg (Stenberg and Ericson 2007). Within the Arthropoda other factors can include vegetation density, plant genotype, phenotype, size, leaf age, leaf morphology, the presence of phytopathogens, predators and parasites, and abiotic conditions (see Fernandez and Hilker 2007 and references therein).

4.1.1.2. Conspecific location

Male-produced aggregation pheromones that affect the behaviour of both sexes over long distances (> 0.5 m) have been identified from four chrysomelid subfamilies (Table 4.1). It has been suggested that ‘pioneer males’ initiate colonisation, locating host plants using odour and then produce an aggregation pheromone to which a majority of colonising beetles of both sexes respond (Landolt 1997 and references therein). The primary function of aggregation pheromones produced by one sex is thought to be mate finding and choice. In the Chrysomelidae it is possible that male respondents are opportunists seeking access to females, as male-produced aggregation pheromones

have been found only to elicit a strong response in sexually responsive individuals of both sexes (Landolt and Phillips 1997, Smyth and Hoffmann 2003). In addition, male-produced aggregation pheromones are thought to have evolved with patchy or ephemeral host resources, enhancing the potential to make nutritional and ovipositional resources more accessible to females (Landolt 1997, Landolt and Phillips 1997). Aggregation of aposematic beetles such as *Acalymma vittatum* F. and *L. decemlineata* should also confer density-dependent defence against generalist predators (Sillén-Tullberg and Leimar 1988, Smyth and Hoffmann 2003). These theories are supported by the apparent synergism between host-plant odour and male-produced aggregation pheromone in *A. vittatum*, *D. elongata* and *P. cruciferae* (Smyth and Hoffmann 2003, Soroka et al. 2005, Cossé et al. 2006). The aggregation pheromones may also have density-dependent repellent effects; male *Aphthona nigriscuitis* Foudras produce an unidentified chemical cue which at high densities has a repellent effect to which males are more responsive (Tansey et al. 2005). These authors suggest that this may reduce the likelihood that host-plant resources are consumed before larvae can complete development.

Table 4.1. Male-produced aggregation pheromones in the Chrysomelidae.

Subfamily/ *species*	**Pheromone**	**Reference**
Chrysomelinae		
Leptinotarsa decemlineata	(S)-3,7-dimethyl-2-oxo-oct-6-ene-1,3-diol	Oliver et al. (2002)
Criocerinae		
Oulema melanopus	(*E*)-8-hydroxy-6-methyl-6-octen-3-one	Cossé et al. (2002)
Galerucinae		
Acalymma vittatum	Vittatalactone	Morris et al. (2005)
Diorhabda elongata	(*E*,*Z*)-2,4-heptadienal' (*E*,*Z*)-2,4-heptan-1-ol	Cossé et al. (2005)
Galerucella calmariensis & *G. pusilla*	Dimethylfuran lactone	Bartelt et al. (2006)
Halticinae		
Epitrix fuscula	(2*E*,4*E*,6*Z*)-2,4,6-nonatrienal; (2 *E*,4*E*, 6*E*)-2,4,6-nonatrienal.	Zilkowski et al. (2006)

Short-range, male-produced pheromones may exist in the Chrysomelidae. Male *P. chrysocephala* have antennal glands typical of those used in pheromone production, which females do not possess (Bartlet et al. 1994). It was hypothesised that the glands produce a volatile pheromone which has an arrestant or aphrodisiac effect during copulation.

Female-produced sex pheromones have been characterised for 11 *Diabrotica* species (Table 4.2). Unlike the aggregation pheromones, sex pheromones elicit a response from the opposite sex only, acting purely as a mate-finding mechanism (Anderson and Metcalf 1986). The presence of female-produced pheromones in other chrysomelids is not clear. McIndoo (1926) concluded that *L. decemlineata* establish another's sex by smell and additional bioassays have indicated the presence of a female-produced sex pheromone (De Wilde et al. 1969, Edwards and Seabrook 1997). However, in bioassays involving caged *L. decemlineata*, males showed no sign of detecting females or female-extract, only combinations of dummy beetles and female-extract elicited a response (Levinson et al. 1979, Jermy and Butt 1991). Thus, although contact and close range chemoreception is involved in *L. decemlineata* sex recognition (Dubis et al. 1987), there is some debate over the existence of a female-produced pheromone in this species.

Table 4.2. Female-produced sex pheromones of *Diabrotica* species. (Guss et al. 1982, Guss et al. 1983a, Guss et al. 1983b, Guss et al. 1985, Chuman et al. 1987).

MDP **=** 8-methyl-10-decanol propanoatae

***fucata* subgroup**	*Pheromone*
Diabrotica balteata	(R,R) 6,12-dimethylpentadecan-2-one
D. undecimpunctata undecimpunctata	10-methyltridecan-2-one
D. u. duodecimnotata	10-methyltridecan-2-one
D. u. howardi	10-methyltridecan-2-one
***virgifera* subgroup**	
D. cristata	8-methyl-10-decanol acetatae (2S, 8R)
D. longicornis	MDP (2S, 8R)
D. longicollis barberi	MDP (2R, 8R)
D. lemniscata	MDP (2S, 8R)
D. porracea	MDP (2S, 8R)
D. virgifera virgifera	MDP (2R, 8R)
D. v. zeae	MDP (2R, 8R)

4.1.1.3. Application of Chrysomelid semiochemistry

Semiochemicals are regularly used for the prediction and monitoring of some agricultural pests, either by monitoring pest density in relation to economic threshold or detection of outlying infestations, thus reducing pesticide use by improved targeting (Metcalf 1994b). Within the Chrysomelidae, trapping has been most widely used to monitor corn rootworms (*Diabrotica* spp). Kairomonal traps for several *Diabrotica*

species are commercially available, and these can provide good predictions of future outbreaks (Jackson et al. 2005). Sex pheromone-baited traps can help predict the best time to spray against *D. u. howardi* in peanut fields (Herbert et al. 1996). However, catches of other male *Diabrotica* species in sex pheromone-baited sticky traps often correlate poorly with levels of infestation (Brandenburg et al. 1992). Sex pheromone traps have been used to monitor the invasion by *D. v. virgifera* of Europe where this species has recently become established (Anon 2005).

Suggested management strategies involving the manipulation of pest species using semiochemicals include mass removal trapping, attract-annihilate, behavioural disruption, the use of antifeedants and the attraction of specialist natural enemies (see Foster and Harris 1997). Kairomones derived from cucurbits and maize or similar synthetic compounds have been used in attempted attract-annihilate strategies for corn rootworms using baits and/or traps laced with insecticide (Metcalf et al. 1987, Lance and Sutter 1992, Metcalf et al. 1993). Temporal and spatial placement of baits and traps is critical and dependent on the species concerned, as is colour of the lures: adult *Diabrotica* spp. are more attracted to yellow traps than to other colour (Weissling and Meinke 1991, Hoffmann et al. 1996, Jackson et al. 2005). Good initial control has been achieved in small experimental plots and large field trials but over time the effect subsides and no long term control is gained (Lance and Elliott 1991, Lance and Sutter 1992). More success has been achieved with *D. u. howardi* using bait containing insecticide, a cucurbitacin feeding stimulant and several floral volatiles in fields of cantaloupe melon, which gave comparable yield increases to pesticide use alone, with the additional benefits of increasing the rate of pollination and reducing bacterial infection in the crop (Brust and Foster 1995). The use of an attract and kill product, based on host kairomones and a pyrethroid insecticide has shown the potential to reduce the commercial application rate by 92% (by active ingredient) against *L. decemlineata* larvae (Martel et al. 2007).

The use of trap crops (plant stands grown to attract pests away from the main crop where management can then be carried out more economically, Hokkanen 1991) can be effective against *D. v. virgifera* and *D. barberi* (Hill and Mayo 1974), and this can be enhanced by the application of non-pheromonal attractants. However, such a strategy is limited to the management of preovipositional females and likely to have little effect on future infestation levels (Lance 1993, Hammack 2003). The addition of synthetic kairomones to solanaceous trap crops was found to increase the numbers of adults and larvae of *L. decemlineata* in the trap crop and reduce the number in the main crop, which led to increased yield and decreased use of insecticides (Martel et al. 2005). The potential use of the male-produced aggregation pheromone to draw beetles

to a point source where insecticides may be used more efficiently has also been demonstrated for this species (Kuhar et al. 2006).

4.1.1.4 Conclusions

The odour-mediated behaviour of the Chrysomelidae can appear complex, with many identified semiochemicals having multiple functions, for example aggregation pheromones that induce dispersal at high densities. This type of multifunctional (and often paradoxical) role appears to be common in the Arthropoda (Blum 1996). Although for convenience this review has categorised the function of a subsection of the semiochemicals into host and conspecific cues (kairomones and pheromones), it is clear that in nature the categories are not mutually exclusive, as is the case with many semiochemical interactions (Nordlund and Lewis 1976).

More than 35,000 species of Chrysomelidae have been described, all of which are phytophagous (Metcalf 1994a) some, such as *L. decemlineata* and *Diabrotica* spp., are serious pests of agriculture, and most research has been directed towards these species. Studies on these species highlight the need for detailed knowledge of the behaviour and ecology of a pest and its host(s) if semiochemical-based management strategies are to be successful. These factors emphasize that semiochemical-based pest monitoring and management within the Chrysomelidae is more likely to be successful if combined with other approaches. Such a strategy could involve a push-pull approach (see Pickett et al. 1997), in which semiochemicals make the main crop less attractive whilst at the same time other semiochemicals make an adjacent trap crop (or trap) more attractive and pathogens or pesticides can be deployed to the latter. Additional considerations include economic viability, type of dispensing mechanism, toxicity of attractant to man, simplicity of use, influence of atmospheric conditions and effects on non-target organisms (Hammack and Petroski 2004, Martel et al. 2007). However, the potential for enhanced management of chrysomelid pests by exploiting behavioural responses to semiochemicals has been demonstrated.

A series of laboratory experiments were carried to elucidate the behavioural responses of the lily beetle *L. lilii* to semiochemical information.

4.2. MATERIALS AND METHODS

4.2.1. Plant material

Young and mature *Lilium regale* plants were maintained for experiments and as food plants for laboratory cultures. Mature plants for laboratory culture were obtained as bulbs in winter 2004/5 and treated as for the field trial (Chapter 3) but kept under fine netting to protect them from L. lilii infestation. Young *L. regale* (stem length 15-30 cm) were grown from small bulbs (< 4 cm diameter) in 7.5 cm diameter pots in hardy ornamental (HO) compost (Sinclair Horticulture, Lincoln). These, together, with some mature plants were kept in a glasshouse, some in a compartment which was heated (minimum 15 °C) from mid February, in order to obtain plant material earlier in the season and so allowing an earlier start to experimentation.

4.2.2. Insects

Methods of *L. lilii* collection and culture were modified from those developed at the Royal Horticultural Society (RHS), and CABI, Switzerland (pers. obs, M. Kenis pers. com, 2005). From March to October incubator temperatures were maintained at 20 °C (± 1 °C) with a 16:8 hour light-dark cycle. Laboratory conditions during insect rearing were temperatures of 24 °C ± 5 °C, and a natural daylight cycle.

Lilioceris lilii adults were collected from Surrey and surrounding areas of adjacent counties (Wisley Garden and donations by RHS staff). These were kept in polypropylene cages (Bugdorm-1, 30 x 34 x 34 cm, Megaview Science Education Services, Taiwan) in an incubator at a maximum of 75 per cage. *Lilium regale* was provided as cut-stems in water as a food source. *Lilioceris lilii* adults collected before mid June were assumed to be diapaused individuals. After mid June it is possible that adults would have developed from larvae in the current year, and therefore not be in a reproductive state (see section 1.2). The cages were checked five times a week, food replaced as necessary, detritus removed and eggs (attached to leaf sections) transferred to 9 cm Petri dishes lined with damp filter paper. Eggs were kept in an incubator and monitored five days (Monday-Friday) a week for ecdysis.

Larval rearing method-1: Hatchling larvae were reared individually, in ventilated glass tubes (7.5 x 2.5 cm), filled to 2.5 cm with HO compost and kept in an incubator. Leaves of *L. regale* were provided as food, replenished as necessary. Each tube was inspected five days a week. When a larva entered the compost to pupate surplus leaves were removed and observations continued until an adult emerged. If no adult emerged after eight weeks the tube was emptied and inspected for beetle remains. A

maximum of 100 larvae were reared using method-1 at any time, whilst additional larvae were reared using method-2.

*Larval rearing method-2***:** Larvae were reared in mini cages (230 mm x 125 mm diameter, Watkins and Doncaster, Kent) kept in the laboratory. The base of each cage was filled with damp fine vermiculite (1 to 3 mm grain size). Up to 75 larvae were reared in each cage and cut stems of *L. regale* were provided as a food source, replaced as necessary. The cages were inspected five times a week and 'new' adult beetles removed as they emerged.

'New' adults: 'New' pre-diapause adults emerging from larval cultures were kept in polypropylene cages (Bugdorm-1), up to 75 adults in each cage. Cages were maintained as for wild collected adults. New adults were kept for at least a seven days before use in linear-track olfactometer (LTO) tests.

4.2.3. Linear-track olfactometer tests

Laboratory conditions: All olfactometer experiments were carried out in a laboratory at room temperature 22 $^{\circ}$C (± 4 $^{\circ}$C); relative humidity 30-70%; atmospheric pressure 996-1025 mb.

The linear-track olfactometer and general experimental design: Perspex and glass linear-track olfactometers (LTO) of the design used by Cook et al. (2002) and illustrated in Figures 4.1 and 4.2, were used to examine the responses of *L. lilii* to odours from host plants and conspecifics. Air was drawn through a charcoal filter and into the apparatus by a diaphragm pump (Capex L2 230V STD). Airflow through the equipment was monitored by flow meters (Gapmeter NG/LG), the flow from each side being equalised at 0.8 to 0.9 L m^{-1} (always exactly equal in the two arms). The entire apparatus was screened to exclude visual distractions (by placing the LTO in a cardboard box lined with white paper). Test materials were placed in 5 L glass vessels out of view of the olfactometer, to which they were connected using Teflon® tubing (1.6 mm bore x 0.8 mm wall, Fisher).

For each replicate, five *L. lilii* were introduced to the apparatus via a holding pot (25 mm diameter plastic bottle cap with the centre punched out). Beetles left the pot by climbing a vertical wire to meet the horizontal wire at the T-junction. At this point they chose a left or right turn into one of two airstreams that had passed over the test materials. The first *L. lilii* to walk out of the holding cap up the central wire and to move 60 mm left or right (measured to head) within 15 min was taken as the responder. This

beetle was then removed from the apparatus. If no beetle responded after 15 min a note was made and the replicate was repeated.

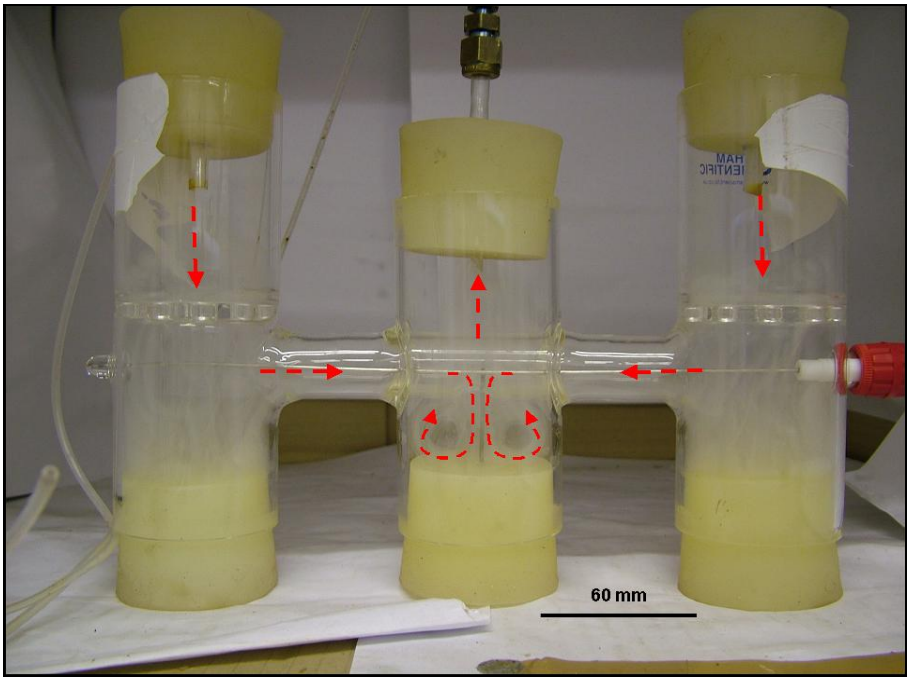


Figure 4.1. The glass linear-track olfactometer, running a smoke test. Red arrows indicate direction of airflow.

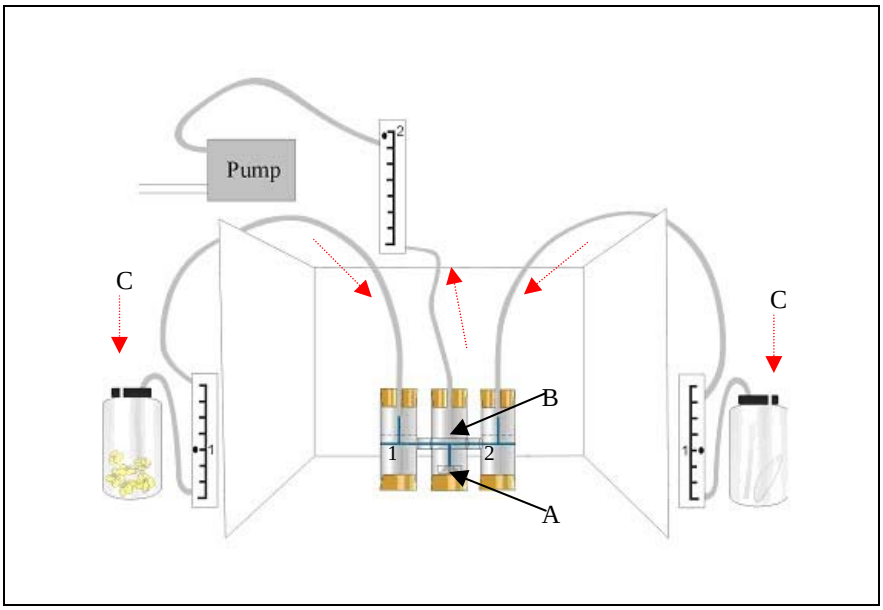


Figure 4.2. Schematic diagram of the linear-track olfactometer (LTO) experimental set up (After Cook et al. 2002). Air is drawn by a pump through two sources and into the LTO. A = holding pot where beetles are introduced; B = T-junction where two air streams meet and the point where beetles make their choice. C = incoming air, filtered through charcoal. Dotted red arrows show air movement through the apparatus. 1 / 2 = chamber number.

For each test two LTOs were used alternately, and airstreams (odours) were introduced into each chamber of the LTO (chamber 1 & 2) in each direction (left and right) on an equal number of occasions to reduce bias. Air was drawn through the equipment from the sources for 5 min before the start of each test. After a maximum of six replicates the LTOs were soaked in 5% Decon 75 solution for 15 min, rinsed with water and dried in an oven at 50 °C to remove residual odours. The central wire of the apparatus was replaced with a clean wire after each replicate, to reduce the risk of an effect from the presence of theoretically possible trail pheromone. To remove residual odours before each test the equipment was dismantled and soaked in 5% Decon 75 for at least 12 h and dried in an oven at 50 °C.

Due to the lack of reliable external sexual characters of *L. lilii* (M. Cox pers. com, 1999), it was not possible to separate the sexes before use in experiments and all beetles were dissected after the tests to determine gender. Therefore, with the exception of bias checks, the number of replicates used for each test was usually 96, to enable a satisfactory number of responding male and female beetles for analysis. In some tests additional replicates were required to clarify results, this occurring when the initial 96 replicates gave a close to significant result (i.e. $0.1 > p > 0.05$).

Some initial tests were carried out in Perspex LTOs, whilst glass equipment was being manufactured.

Equipment checks: Two methods were used to check the equipment, a bias check and a smoke test to demonstrate airflow. For the bias check the airstream was drawn over one young *L. regale* plant and split so that it was drawn into both chambers of the LTO. Forty replicates were conducted on two occasions per season, once with diapaused and once with pre-diapause beetles. In all cases Chi squared tests (Genstat 9.1.0) indicated that there was no significant movement of the beetles to either the left or right chambers and it was assumed that the experimental design showed little or no bias.

A smoke test was carried out to demonstrate the airflow in one LTO, set up as for the bias check, but without beetles, plant or replication. The vessel was filled with smoke by lighting an incense stick. The pump was then started and the smoke observed travelling through the apparatus (Figure 4.1).

LTO experiments

Lilium regale *vs. plant pot with compost*: A young *L. regale* plant in a 7.5 cm diameter pot with HO compost was placed in one glass vessel and a 7.5 cm diameter pot containing HO compost placed in the other. Beetles were starved for 24 h prior to the test. With diapaused beetles 136 replicates were carried out over three days, 40 in

Perspex and 96 in glass LTOs. With pre-diapause beetles 128 replicates were conducted over three days, 80 in Perspex and 48 in glass LTOs.

Five L. lilii *vs. clean air control:* Five adult *L. lilii* (of the same physiological state as the test beetles) were placed in one vessel and the other vessel left empty. With both diapaused and pre-diapause beetles 96 replicates were carried out over two days, in glass LTOs. The stimulus *L. lilii* were replaced every 24 replicates (2-3 h).

Ten L. lilii *vs. clean air control*: Carried out as with five *L. lilii* but with ten adults as a stimulus; 96 replicates were carried out with diapaused beetles in Perspex LTOs over two days.

Lilium regale *with five adult* L. lilii *vs.* L. regale *alone*
Provisional method: One *L. regale* was placed in each glass vessel and five *L. lilii* were added to the plant in one of the vessels 10 min before the tests began. The test plants and beetles were replaced every ten replicates (1-2 h); 40 replicates with diapaused beetles were conducted, in Perspex LTOs.

Main method: Due to the need for a greater number of replicates the above test was repeated, however the availability of beetles and plant material necessitated some alteration to methodology. A young *L. regale* was placed in each glass vessel and five *L. lilii* were added to one vessel 10 min before the tests began. Test beetles and plants were replaced every 24 replicates (2-3 h). The test was run with diapaused beetles and 192 replicates were carried out in glass LTOs over four days. This test was also carried out with pre-diapause beetles; 144 replicates carried out over three days, in glass LTOs.

Lilium regale *with five adult* L. lilii *separated from the plant vs.* L. regale *alone:* One small *L. regale* was placed in each glass vessel and in one vessel five *L. lilii* confined within a plastic tube (50 mm x 30 mm diameter) capped with fine nylon netting, were added 10 min before the tests began. The test plants and beetles were replaced every twelve replicates (1-2 h); 144 replicates with diapaused beetles in glass LTOs were conducted over three days.

Lilium regale *and adult* L. lilii *vs.* L. regale *with mechanical damage*: Five *L. lilii* were added to a small *L. regale* plant in one vessel; the other vessel contained a plant with mechanical damage consisting of five semicircular holes (one 7 mm diameter hole

made with a hole punch in each of five leaves). Test plants and test beetles were replaced every 24 replicates (2-3 h). This test was repeated on 96 occasions with diapaused beetles over two days, using glass LTOs.

Lilium regale *with* L. lilii *damage (beetles removed) vs.* L. regale *with mechanical damage*: In some cases insect-induced plant volatiles are not produced until several days after plant damage has begun (Powell et al. 1998). Therefore damaged plants consisted of young *L. regale* that had been infested with five *L. lilii* for 72 h whilst kept in a plastic cage (Bugdorm-1). Mechanical damage consisted of nine semicircular (7 mm diameter) holes (made with a hole punch, three holes on each day the plants were infested to mimic beetle damage). Before use plants were cleaned with distilled water, in the case of beetle-damaged plants to remove beetle-associated material such as frass. Plants were replaced every 24 (2-3 h) replicates. Ninety-six replicates were conducted over two days with diapaused beetles in glass LTOs.

Lilium regale *with three lily beetle larvae vs. intact* L. regale*:* A young *L. regale* plant that had been infested with three *L. lilii* larvae (2/3 instar) for 24 h was added to one vessel (larvae remaining attached); the other vessel contained an intact plant. Test plants and larvae were replaced every 24 replicates (2-3 h). This test was repeated 96 times with diapaused beetles over two days, in glass LTOs.

Statistics: Data was organised into 2 x 2 contingency tables and analysis carried out using the Chi squared test or in tests with low replication ($n \leq 40$), Fisher's exact test (GenStat 9.1.0). Statistical tests (Generalised linear models) on the data were conducted to check that it was valid to combine data from different days / material of olfactometer and in all cases data could be successfully combined. Summary statistics for observations made during the laboratory culture of beetles were obtained using Genstat 9.1.0.

4.3. RESULTS

4.3.1. Insects

A total of 654 *L. lilii* larvae were reared from eggs in the laboratory using method-1, 205 (31%) of which emerged as adults. Fifty of the larvae that developed through to adults entered the soil to pupate and emerged as an adult on days when the tubes were inspected, enabling some analysis of development times. At 20 $^{\circ}$C ± 1 $^{\circ}$C

the larval stage lasted a mean of 13.7 days (minimum 9, maximum 23 days, s.d. 2.62) and the subterranean stage 24.7 days (minimum 17, maximum 31, s.d. 3.1).

4.3.2. Linear-track olfactometer tests

Lilium regale *vs. plant pot with compost*

i) *Diapaused beetles:* The results (Figure 4.3) show that overall significantly more beetles ($p < 0.01$) moved into the airstream containing *L. regale* odour. Considering the sexes separately, significantly more female *L. lilii* ($p < 0.05$) moved into the air stream from *L. regale*, more males moved into the host plant airstream but this response was not statistically significant. Two replicates were repeated due to no beetle responding within 15 min.

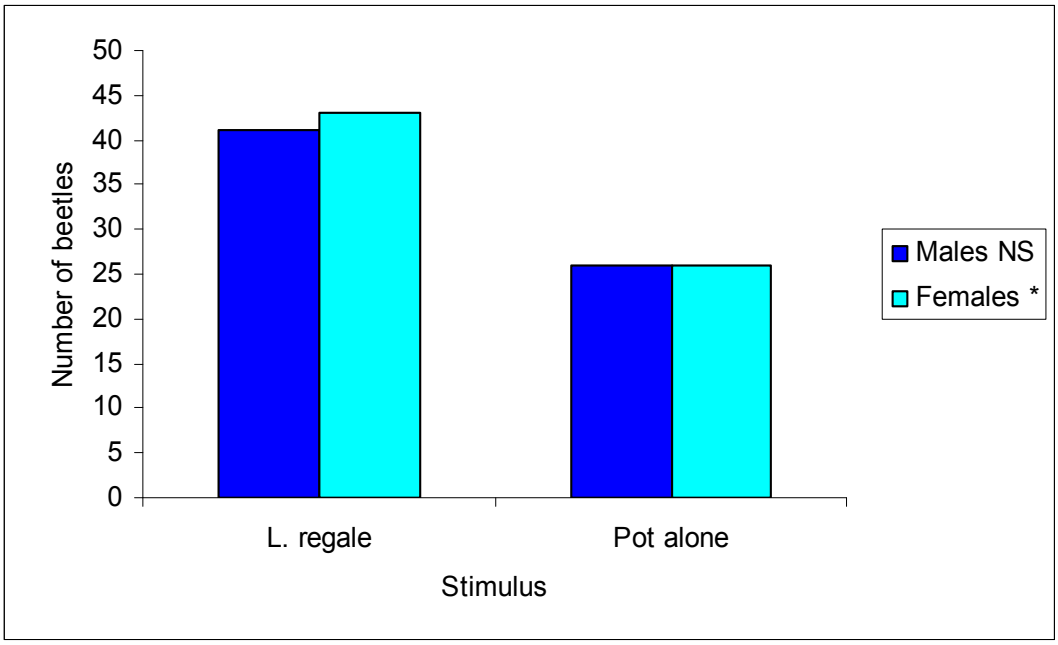


Figure 4.3. Movement of diapaused adult *L. lilii* into the odour streams of *L. regale* and pot with compost in a linear-track olfactometer. Overall (significant $\chi^2 = 7.59$, $p = 0.006$); Females* (significant $\chi^2 = 4.33$; $p = 0.038$); Males NS (not significant, $\chi^2 = 1.47$, $p = 0.071$).**

ii) *Pre-diapause beetles*: Although more *L. lilii* of both sexes moved into the air stream containing *L. regale* odour the responses were not statistically significant (Figure 4.4). Two replicates were repeated due to no beetle responding within 15 minutes.

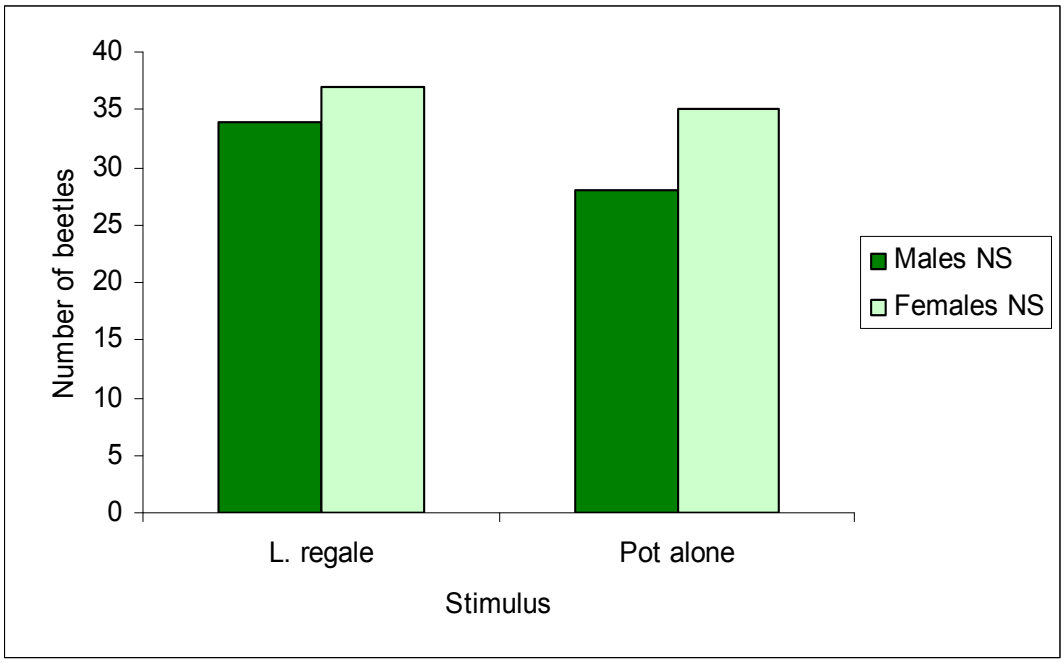


Figure 4.4. Movement of pre-diapause *L. lilii* into the odour streams of *L. regale* and pot with compost in a linear-track olfactometer. NS = Not significant (Overall $\chi^2 = 0.08$, p = 0.793; Males $\chi^2 = 0.63$, p = 0.429; Females $\chi^2 = 0.03$, p = 0.853).

Five adult L. lilii *vs. clean air control*

i) *Diapaused beetles*: In all replicates the beetles used as a stimulus were a mixed sex group (Table 4.3). The results (Figure 4.5) show that overall significantly more individuals ($p < 0.01$) moved into the airstream from the clean air control. Considering the sexes separately, significantly more males ($p < 0.05$) moved into the air stream from the clean air control, whilst more females moved into the airstream of the clean air control this result was not statistically significant.

Table 4.3. Sex of the stimulus beetles in five *L. lilii* vs. clean air control (diapaused beetles).

Replicates	Beetle sex
1-24	1♂ 4♀
25-48	4♂ 1♀
49-72	3♂ 2♀
73-96	3♂ 2♀

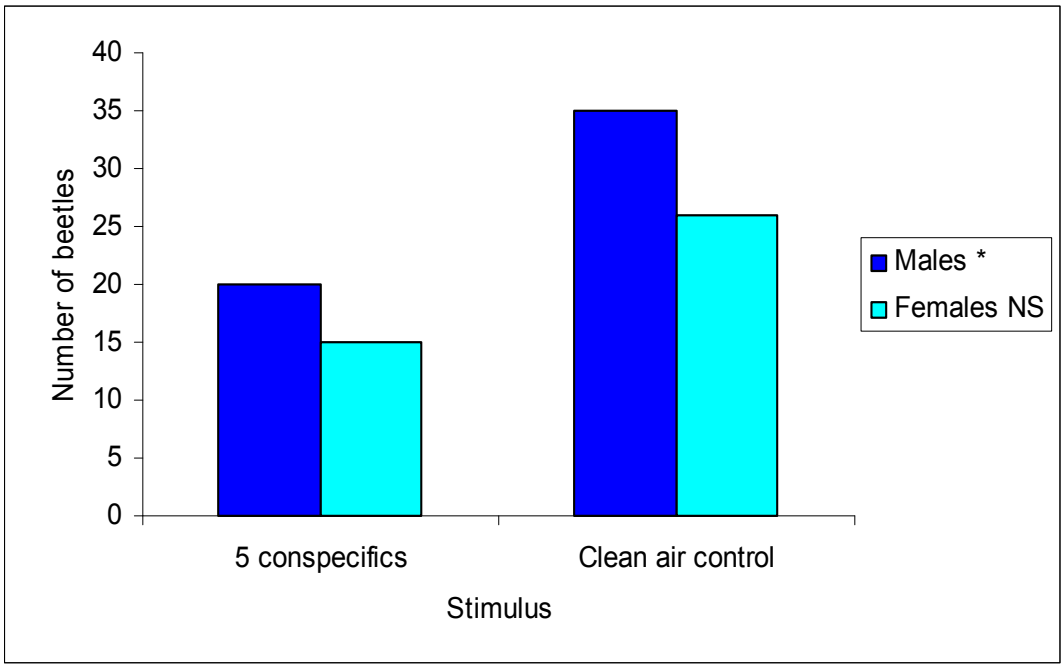


Figure 4.5. Movement of diapaused *L. lilii* to the odour of five conspecifics and clean air control in a linear-track olfactometer. Overall (significant $\chi^2 = 7.04$, $p = 0.008$), Males* (significant $\chi^2 = 4.13$; $p = 0.042$), Females NS (not significant, $\chi^2 = 2.93$; $p = 0.087$).**

ii) *Pre-diapause beetles*: In all replicates the beetles used as a stimulus were a mixed sex group (Table 4.4). Although more females moved into the airstream of the clean air control this result was not statistically significant (Figure 4.6). Four replicates were repeated due to no beetle responding within 15 minutes.

Table 4.4. Sex of the stimulus beetles in five *L. lilii* vs. clean air control (pre-diapause beetles).

Replicates	Beetle sex
1-24	1♂ 4♀
25-48	2♂ 3♀
49-72	2♂ 3♀
73-96	3♂ 2♀

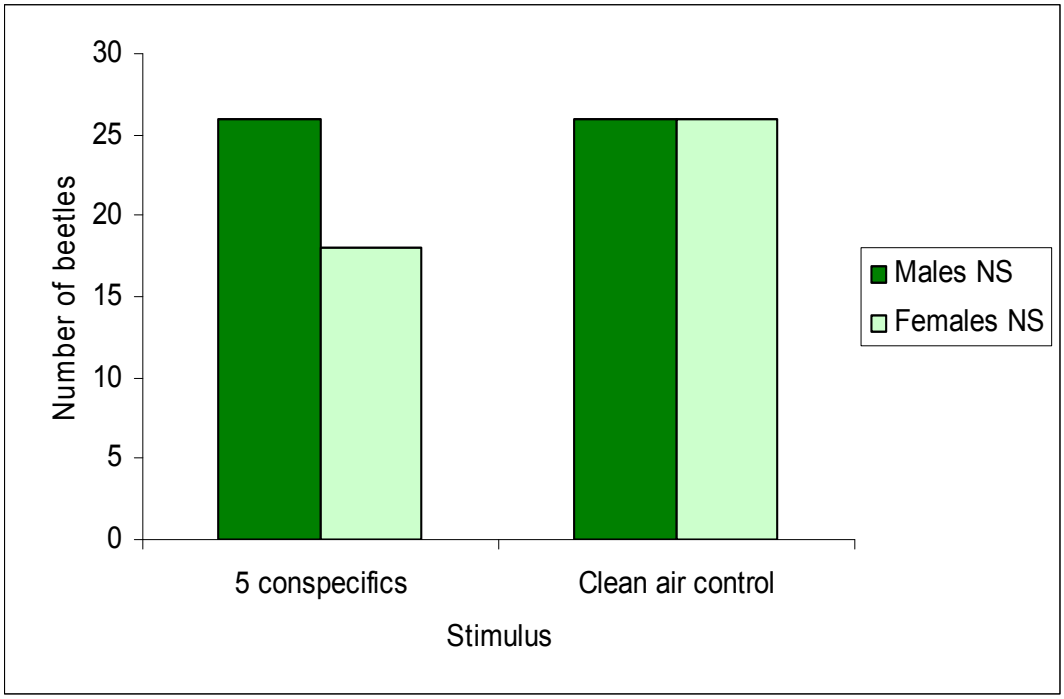


Figure 4.6. Movement of pre-diapause *L. lilii* to the odour of five conspecifics vs. clean air control in a linear-track olfactometer. No significant (NS) movement into either airstream (Overall, $\chi^2 = 0.68$, p = 0.411; Males $\chi^2 = 0.00$, p = 0.991; Females $\chi^2 = 1.89$; p = 0.169).

Ten L. lilii *vs. clean air control*

Diapaused beetles: In all replicates the beetles used as a stimulus were a mixed sex group (Table 4.5). More individuals of both sexes moved into the air stream from ten conspecifics than to the clean air control, however these results were not statistically significant (Figure 4.7).

Table 4.5. Sex of the stimulus beetles in ten *L. lilii* vs. clean air control (diapaused beetles).

Replicates	Beetle sex
1-24	4♂ 6♀
25-48	4♂ 6♀
49-72	4♂ 6♀
73-96	3♂ 7♀

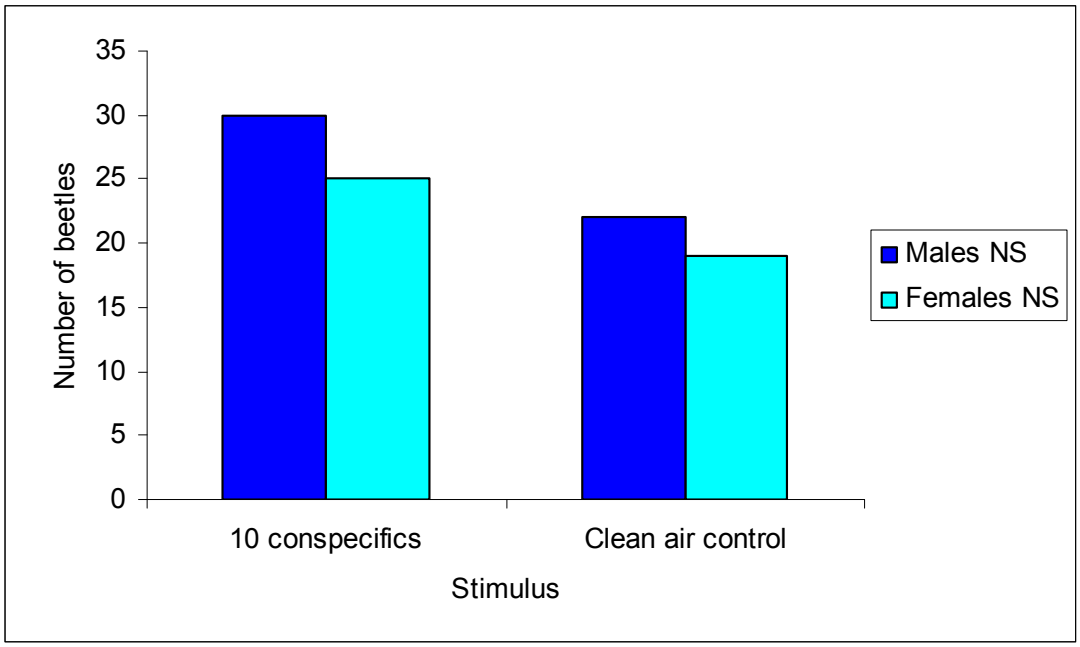


Figure 4.7. Movement of diapaused *L. lilii* to the odour of ten conspecifics vs. clean air control in a linear-track olfactometer. No significant movement (NS) into either airstream (Overall $\chi^2 = 2.05$, $p = 0.152$; Males $\chi^2 = 1.15$, $p = 0.283$; Females $\chi^2 = 0.88$, $p = 0.349$).

Lilium regale *with five adult beetles vs.* L. regale *alone*

i) Diapaused beetles:

Provisional method: In all replicates the beetles used as the stimulus were a mixed sex group (Table 4.6). Results show that significantly more beetles ($p < 0.05$) moved into the air containing *L. regale* odour with beetles than the control (Figure 4.8). When the sexes were analysed separately (Fisher's exact test) neither sex showed a statistically significant movement into the airstream of the infested host.

Table 4.6. Sex of the stimulus beetles in *L. regale* with five adult beetles vs. *L. regale* alone (provisional method, diapaused beetles).

Replicates	Beetle sex
1-10	2♂ 3♀
11-20	2♂ 3♀
21-30	2♂ 3♀
31-40	2♂ 3♀

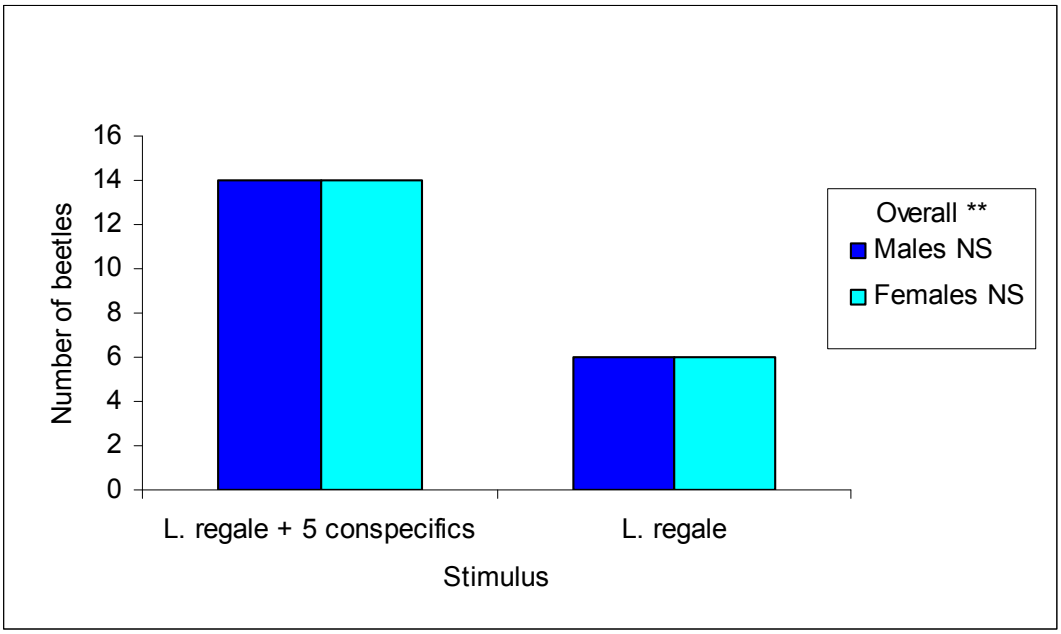


Figure 4.8. Movement of diapaused *L. lilii* into odour streams of *L. regale* with five lily beetles and *L. regale* alone in a linear-track olfactometer (provisional method). (Overall (significant) $\chi^2 = 6.40$; p = 0.011. Fisher's exact test, Females NS (not significant) p = 0.095, Males NS (Not significant) p = 0.095).**

Main method: In all replicates the beetles used as the stimulus were a mixed-sex group (Table 4.7). From the first 96 replicates no significant movement into either airstream was observed (Overall $\chi^2 = 0.17$, p = 0.683; Females $\chi^2 = 0.12$, p = 0.732; Males $\chi^2 = 0.86$, p = 0.535). As the results from the provisional method and the results from additional tests (detailed below), provided significant results a further 96 replicates were carried out. Overall the results of these replicates showed significant movement into the airstream of plant and beetles ($\chi^2 = 5.04$, p = 0.025) and considering the sexes separately females showed significant movement into the airstream of the plant with conspecifics ($\chi^2 = 9.25$, p = 0.002), whereas males showed no significant movement ($\chi^2 = 0.01$, p = 0.934). The significance of the female movement remained when results for all 192 replicates were combined (Figure 4.9). Overall three replicates were repeated due to no beetle responding within 15 minutes.

Table 4.7. Sex of the stimulus beetles in *L. regale* with five adult beetles vs. plant alone (diapaused beetles, main method).

Replicates	Beetle sex
1-24	2♂ 3♀
25-48	3♂ 2♀
49-72	3♂ 2♀
73-96	3♂ 2♀
97-120	2♂ 3♀
121-144	3♂ 2♀
145-168	1♂ 4♀
169-192	2♂ 3♀

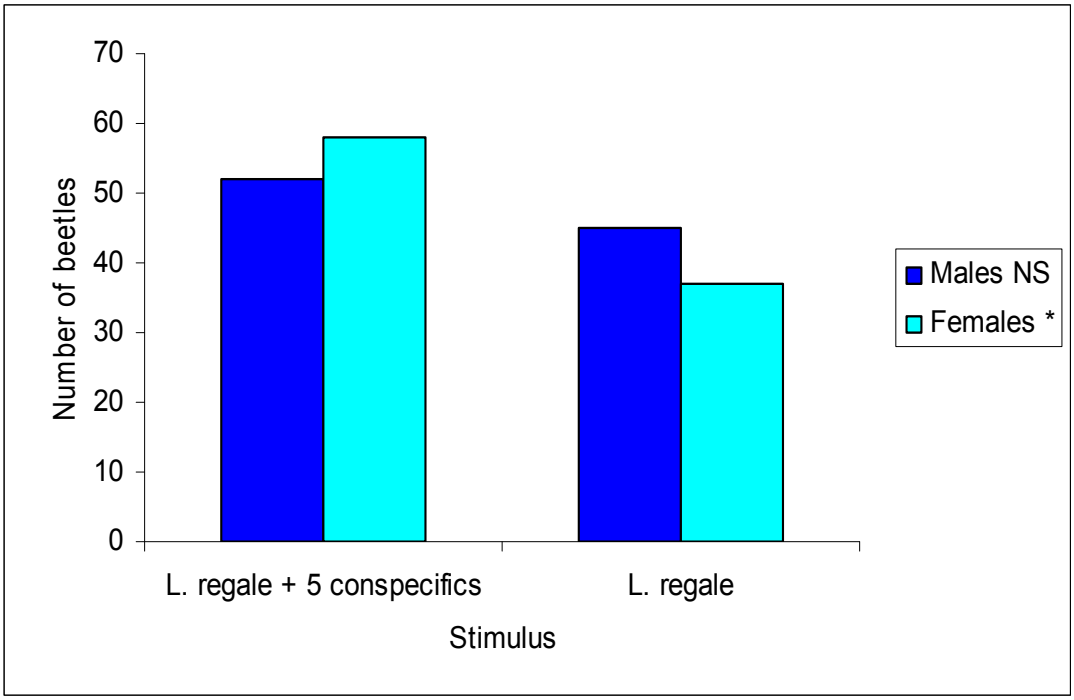


Figure 4.9. Movement of diapaused *L. lilii* responding to the odour of *L. regale* with five lily beetles vs. *L. regale* alone in a linear-track olfactometer (main method). Overall* (significant $\chi^2 = 4.09$, $p = 0.043$); Males NS (not significant $\chi^2 = 0.43$, $p = 0.510$); Females* (Significant $\chi^2 = 4.29$, $p = 0.038$).

ii) *Pre-diapause beetles*: In all replicates the beetles used as a stimulus were a mixed sex group (Table 4.8). Overall significantly more beetles ($p < 0.01$) moved into the airstream of the uninfested *L. regale* (Figure 4.10). Considering the sexes separately, significantly more males ($p < 0.01$) moved into the air stream containing *L. regale* odour alone compared to the odour stream from plants with beetles. More females also

moved into the airstream originating from the *L. regale* alone but this response was not statistically significant. Three replicates were repeated due to no beetle responding within 15 minutes.

Table 4.8. Sex of the stimulus beetles in *L. regale* with five adult beetles vs. *L. regale* alone (pre-diapause beetles).

Replicates	Beetle sex
1-24	2♂ 3♀
25-48	2♂ 3♀
49-72	1♂ 4♀
73-96	2♂ 3♀
97-120	1♂ 4♀
121-144	2♂ 3♀

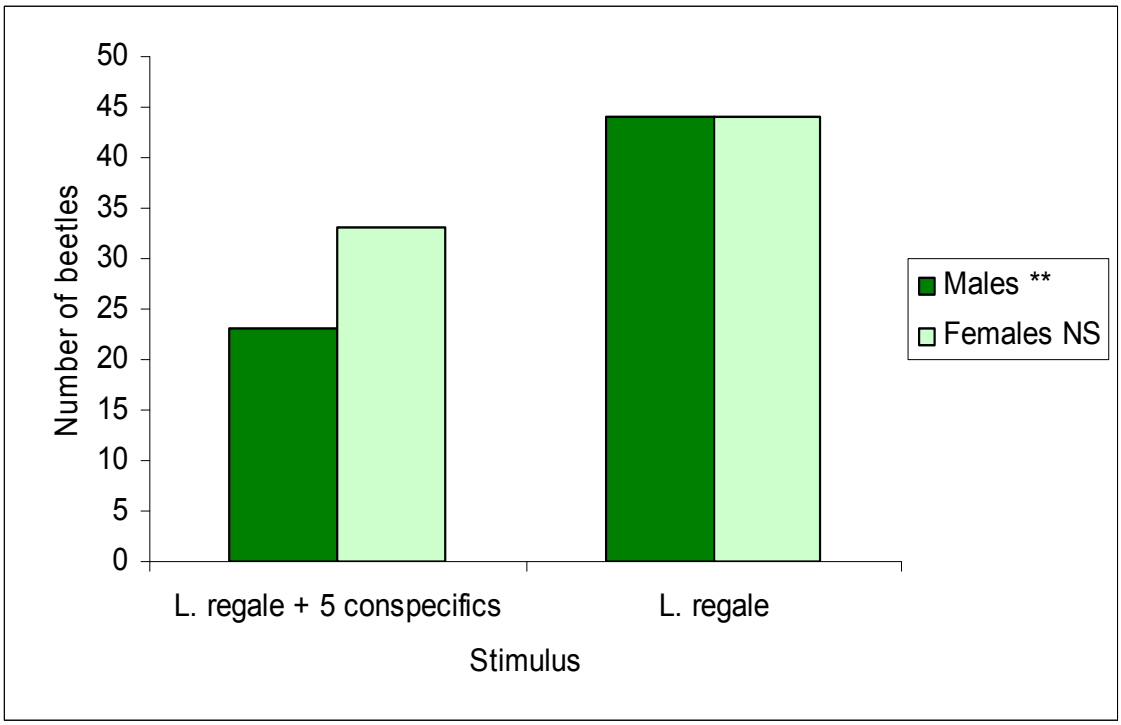


Figure 4.10. Movement of pre-diapause *L. lilii* into the odour streams of *L. regale* with five conspecifics and *L. regale* alone in a linear-track olfactometer. Overall ** (significant χ^2 = 7.20, p = 0.007); Males (significant χ^2 = 6.87, p = 0.009); Females NS (Not Significant χ^2 = 1.46, p = 0.227).**

Lilium regale *with five adult* L. lilii *separated from the plant vs.* L. regale *alone*

Diapaused beetles: In all replicates the beetles used as the stimulus were a mixed sex group (Table 4.9). Overall there was no significant movement into either airstream and although more males moved into the airstream containing the odour of conspecifics and *L. regale* this response was not statistically significant (Figure 4.11). Six replicates were repeated due to no beetle responding within 15 minutes.

Table 4.9. Sex of the stimulus beetles in *L. regale* with five adult beetles (separated from the plant) vs. *L. regale* alone (diapaused beetles).

Replicates	Beetle sex	Replicates	Beetle sex
1-12	2♂ 3♀	73-84	3♂ 2♀
13-24	4♂ 1♀	85-96	2♂ 3♀
25-36	4♂ 1♀	97-108	3♂ 2♀
37-48	2♂ 3♀	108-120	3♂ 2♀
49-60	2♂ 3♀	121-132	3♂ 2♀
61-72	3♂ 2♀	133-144	4♂ 1♀

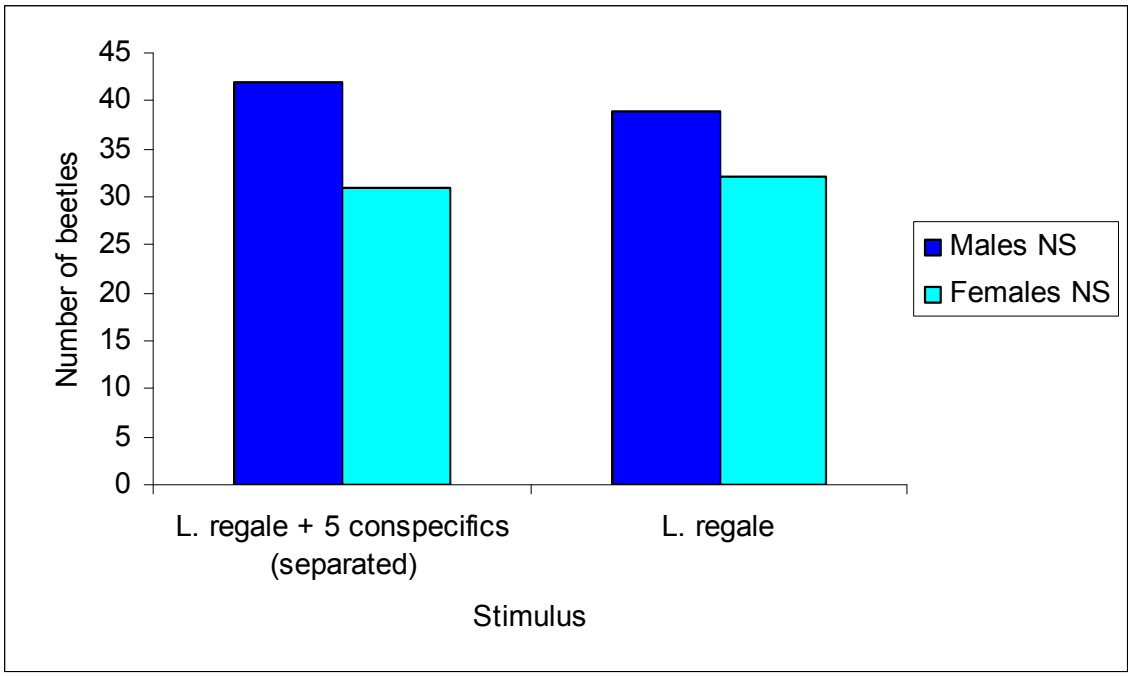


Figure 4.11. Movement of diapaused *L. lilii* responding to the odour of *L. regale* with five conspecifics (separated from the plant) and *L. regale* alone in a linear-track olfactometer. NS = Not significant (Overall $\chi^2 = 0.03$, $p = 0.868$; Males $\chi^2 = 0.14$, $p = 0.712$; Females $\chi^2 = 0.01$, $p = 0.923$).

Lilium regale *and adult* L. lilii *vs.* L. regale *with mechanical damage*

Diapaused beetles: For all replicates the beetles used as a stimulus were a mixed sex group (Table 4.10). Overall no significant movement occurred into either odour stream (Figure 4.12). However, considering the sexes separately, significantly more females ($p < 0.05$) moved into the air stream from a conspecific-infested *L. regale* compared to that of *L. regale* with mechanical damage. More males moved into the airstream of the mechanically damaged *L. regale* but this response was not statistically significant. Two replicates were repeated due to no beetle responding within 15 minutes.

Table 4.10. Sex of the stimulus beetles in *L. regale* with *L. lilii* vs. *L. regale* with mechanical damage (diapaused beetles).

Replicates	Beetle sex
1-24	3♂ 2♀
25-48	2♂ 3♀
49-72	2♂ 3♀
73-96	3♂ 2♀

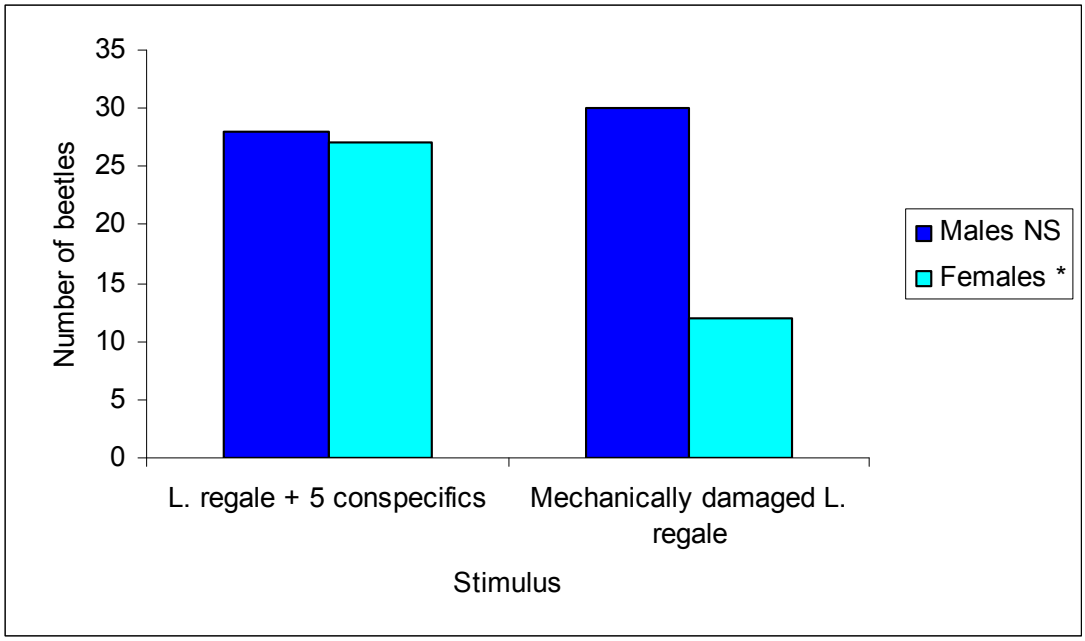


Figure 4.12. Movement of diapaused *L. lilii* responding to the odour streams of *L. regale* with five conspecifics and *L. regale* with mechanical damage in a linear-track olfactometer. Overall (not significant $\chi^2 = 1.51$; $p = 0.219$); Females * (significant $\chi^2 = 5.22$; $p = 0.022$); Males NS (Not significant $\chi^2 = 0.07$; $p = 0.792$).

Lilium regale *with* L. lilii *damage (beetles removed) vs.* L. regale *with mechanical damage*

Diapaused beetles: In all cases the beetles that had fed on the beetle damaged *L. regale* were mixed sex groups (Table 4.11). More *L. lilii* of both sexes moved into the odour streams of *L. regale* with mechanical damage compared to *L. regale* with conspecific damage, but the results were not statistically significant (Figure 4.13). One replicate was repeated due to no beetle responding within 15 minutes.

Table 4.11. Sex of the stimulus beetles in *L. regale* with *L. lilii damage* vs. *L. regale* with mechanical damage (diapaused beetles).

Replicates	Beetle sex
1-24	3♂ 2♀
25-48	2♂ 3♀
49-72	2♂ 3♀
73-96	2♂ 3♀

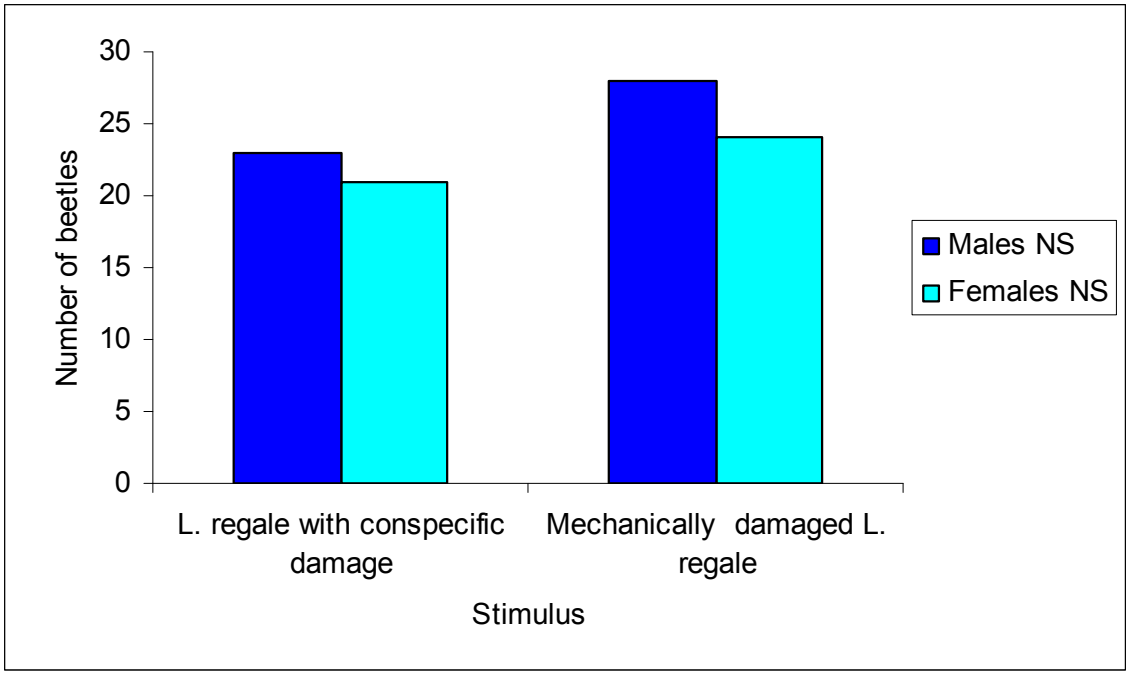


Figure 4.13. Movement of diapaused *L. lilii* responding to the odour of beetle damaged *L. regale* (beetles removed) and a *L. regale* with mechanical damage in a linear-track olfactometer. No significant (NS) movement (Overall $\chi^2 = 0.70$, $p = 0.404$; Males $\chi^2 = 0.41$, $p = 0.524$; Females $\chi^2 = 0.33$, $p = 0.565$).

Lilium regale *with three* L. lilii *larvae vs.* L. regale *alone*

Diapaused beetles: Overall significantly more beetles ($p < 0.05$) moved into the airstream of *L. regale* alone (Figure 4.14). Considering the sexes separately only females showed significant ($p < 0.05$) movement into the airstream of intact *L. regale*. One replicate was repeated due to no beetle responding within 15 minutes.

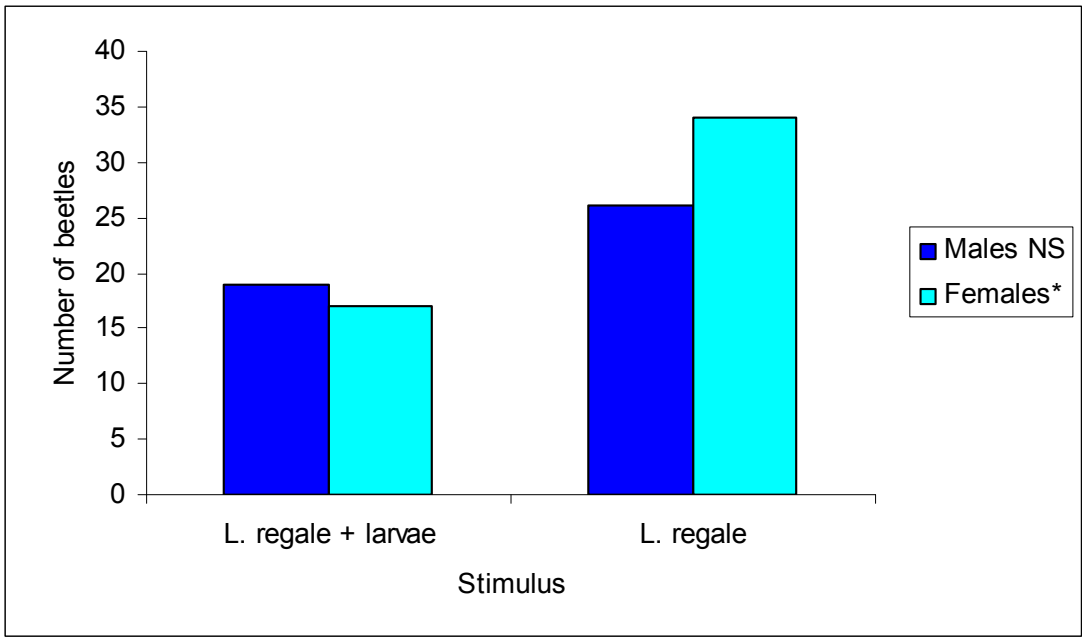


Figure 4.14. Movement of diapaused *L. lilii* responding to the odour of three larvae on *L. regale* and *L. regale* alone in a linear-track olfactometer. Overall* (significant $\chi^2 = 6.01$, $p = 0.014$); Males NS (Not significant $\chi^2 = 1.50$; $p = 0.221$); Females* (significant $\chi^2 = 5.97$, $p = 0.015$).

4.4. DISCUSSION

4.4.1. Insects

The development times of larvae observed in the laboratory are consistent with the modern literature (section 1.2, Haye and Kenis 2004).

4.4.2. Odour-mediated behaviour

It is clear that host and conspecific location behaviour in *L. lilii* is at least in part odour-mediated, and that behavioural responses to odour differ with physiological state and sex. The behaviours observed in these bioassays are comparable with current knowledge of odour-mediated behaviour in the Chrysomelidae (section 4.1.1).

Care should be taken when interpreting the results of these bioassays. The diapaused *L. lilii* used were all wild-caught and kept as mixed-sex cohorts prior to

experimentation, and thus are likely to have mated. *Lilioceris lilii* are polygamous (Nolte 1939) and it is possible that the behavioural effect of odour on individuals changes after contact with the opposite sex. For example the response of *L. decemlineata* to host odour diminishes following contact with the opposite sex (Dickens 2007, section 4.1.1.1) and it is thought that pheromones only elicit a strong response in sexually responsive individuals (Landolt and Phillips 1997). Therefore, some beetles used in the tests may have been less likely than other to respond to the odour streams tested. In one test with diapaused beetles it is possible that such an effect occurred; conspecific infested (mixed sex) *L. regale* vs *L. regale* alone. The provisional method of this test (40 replicates), indicated significant movement into the odour stream of an infested host plant. Replication with the provisional method was too low to enable satisfactory analysis of the responses of each sex individually. The test was repeated with some changes to the methodology (main method), necessitated by the availability of beetles and plant material. The initial 96 replicates using the main method provided no significant results. A further 96 replicates gave significant movement of females into the odour stream of infested host plants, and this significance remained when the results were combined for all 192 replicates. One other test also provided a result inconsistent with other tests; no significant response was observed when a mixed sex group of ten diapaused beetles was used as a stimulus (vs clean air control). However, significantly more diapaused males moved into an airstream from clean air in preference to that of a mixed-sex group of five conspecifics. Another factor that should be taken into account is that with the protocols used in this study the responding beetle was likely to be the most active of the five in the release cap. However, as it is the behaviour of beetles that are responding to odour that is of interest this should not necessarily be considered a drawback of the experimental design. These results highlight that there are many factors that affect the behaviour of an individual beetle and that testing beetle behaviour in an artificial environment can provide results which may not be representative of the insect's innate or learned behaviour. Despite these caveats when significant movement is observed in this type of test it can be concluded that the response is due to the odour stream(s) tested. An additional challenge when working with *L. lilii* is the limitations of working with a beetle that cannot be sexed without dissection. Knowing the sex of individual beetles before the tests would have enabled tests with single sex groups as a stimulus. This would have potentially provided more conclusive evidence of the differences in behaviour of male and female beetles.

Significant movement of diapaused females into odour streams of *L. regale* over a clean air control indicates that host odour is at least in part utilised by *L. lilii* to

locate host plants, to which females are responsive but not males. The significant movement of diapaused females to the odour of a mixed-sex group of conspecifics feeding on a host over that of an intact or mechanically damaged host provides evidence that the odour of hosts with conspecific adults feeding on the plant has a synergistic effect. Although care needs to be taken in interpreting the results of tests with no significant response, the lack of response to a host with conspecific damage (beetles removed) over a mechanically damaged host and to a host with conspecifics (separated) over a host alone provides some additional support to this hypothesis. This type of behavioural response is consistent with that of chrysomelids that produce aggregation pheromones. The aggregation pheromones so far identified in the Chrysomelidae are male-produced and several have been shown to have a synergistic effect with beetle-induced plant damage volatiles (section 4.1.1.2); examples include *A. vittatum* (Smyth and Hoffmann 2003), *P. cruciferae* (Soroka et al. 2005, Tóth et al. 2005), and *D. elongata* (Cossé et al. 2006). However, the behaviours observed are also consistent with a chrysomelid for which an aggregation pheromone probably exists but the sex producing it is currently unknown; both sexes of *P. vulgatissima* aggregate on host plants (*Salix* spp) in the spring, probably due to the combination of host odour and a male or female (or both) produced aggregation pheromone (Peacock et al. 2001a).

The presence of a sex pheromone, acting purely as a mate-finding mechanism and affecting only one sex (section 4.1.1.2) in *L. lilii* cannot be discounted. Only females showed a significant response in the tests with plant material and conspecifics, so it is possible that males produce a sex pheromone. However, in the Chrysomelidae sex pheromones have so far only been identified from female rootworms (*Diabrotica* spp., section 4.1.1.2). Therefore evidence from these bioassays is that a pheromone is produced but it could be either an aggregation pheromone produced by either (or both) sex or a sex pheromone produced by males.

The significant movement of males into the odour stream from a clean air control over that of five conspecifics provides some further evidence for the presence of a pheromone. The behaviour appears analogous to the response of male *A. nigriscuitis* to an unidentified conspecific-produced chemical cue which at high densities of beetles has a repellent effect to which males are more responsive (Tansey et al. 2005). However, it is possible that it was lack of host odour associated with the stimulus beetles in the tests with *L. lilii* that resulted in the observed movement, and the lack of a significant response when ten individuals were used as a stimulus is unclear. As such this test provides not only support for the synergistic effect of odours

emanating from infested host and conspecific odour, but additional evidence of a pheromone.

The movement of diapaused females into the odour stream of an intact host over that of a host with larvae provides evidence of odour-mediated competition avoidance in this species. Within the Chrysomelidae similar behaviour has been observed in *Plagiodera versicolora* (Laicharting) whose larvae repel conspecific females with volatile compounds, which also repel competitors (Raupp et al. 1986, Hilker 1989). In this test it is not possible to determine if the volatiles were produced by larvae (including the fecal shield) or the host. Such a response is likely to induce dispersal of diapaused females to plants that have a low level of larval infestation.

The results of tests with pre-diapause (non-reproductive) individuals gave different behavioural responses to those with diapaused (reproductive) adults. Significantly more pre-diapause males moved into the odour stream containing host odour alone in preference to host with five conspecifics, more females moved into the same airstream but the response was not significant. As with previous tests it is not possible to determine if this behaviour is due to an odour emanating from conspecifics or host damage. If this behaviour is analogous with behaviour in the field it is likely to result in dispersal of pre-diapause males to hosts that have low levels of infestation.

The observed behaviours may relate to the ecology of the beetle in the following ways. Winter diapause of *L. lilii* is necessary before the beetle reaches a reproductive state (Haye and Kenis 2004). It overwinters as adults in 'sheltered positions' but not necessarily adjacent to host bulbs (Fox Wilson 1942). Therefore in spring adult *L. lilii* need to locate host plants and mates. These tests have indicated that spring host and mate location is at least in part mediated by odour and females move towards host plants infested by conspecific adults over intact hosts. The response of males is less clear although there may be some movement towards host odour. During the breeding period the movement of reproductive females can be affected by the odour of hosts infested with larvae, where uninfested plants may be chosen. This is likely to result in oviposition on host plants that have low or zero levels of larval infestation and may result in dispersal; reproductive individuals are known to colonise new areas (Chapter 3). New generation (non-reproductive) adult males have a preference for odours emanating from intact hosts over those infested, a behaviour likely to result in dispersal to plants with low levels of infestation. Once overwintered, possibly close to the plants fed upon in the previous season, it is conceivable that these males colonise plants with the subsequent release of volatile chemicals that draw in other reproductive adults. It is thought that initial host-finding by chrysomelids producing aggregation pheromones is carried out by 'pioneer' individuals, and once a

suitable host is found and feeding initiated an aggregation pheromone is produced to which individuals of both sexes respond (Landolt 1997). However, further evidence is required to determine if the males of *L. lilii* produce an aggregation pheromone.

The plant material in the tests was not producing flower buds and it can be assumed that odours from plants in this vegetative state result in the behaviours observed. Further experimentation is required to understand the effect of the odour of hosts at different stages of development, for example in flower or with seed capsules. In addition, *L. lilii* shows preferences for different *Lilium* (Chapter 3), and the possibility of these preferences being odour-mediated requires further investigation. The host volatiles which elicit the observed responses in *L. lilii* may be specific to *Lilium* or could be a specific ratio of GLV (see section 4.1.1.1). The tests imply that volatiles produced by intact hosts, damaged hosts and conspecifics have behavioural effects on *L. lilii*. Additional work is required to identify the volatiles involved; collection of plant and insect volatiles and their identification using mass spectrometry, gas chromatography and electrophysiological techniques is necessary (see Chapter 5). In addition further investigation into other factors that affect host and conspecific location of *L. lilii,* including short range chemically-mediated host location and acceptance and the many other stimuli such as colour that can affect insect behaviour is required (see section 4.1.1.1).

The behavioural bioassays have provided some useful insights into the odour-mediated behaviour of *L. lilii*. Such information is vital if any management of this species is to be developed using semiochemicals (section 4.1.1.4), further examination of the implications of this work for management of *L. lilii* are discussed in chapter 6.

CHAPTER 5. RESPONSE OF *LILIOCERIS LILII* TO VOLATILES FROM A HOST PLANT

5.1. INTRODUCTION

Some behavioural responses of *Lilioceris lilii* to odour emanating from intact and beetle damaged host plants (*Lilium regale*) have been demonstrated in olfactometer studies (Chapter 4). Whilst the behavioural effects of volatile compounds from various stimuli on insects can be established by the use of behavioural bioassays, such as olfactometers, these methods do not identify the volatiles concerned. The identification and quantification of behaviourally-active compounds is essential if this knowledge is to be applied in pest management (Agelopoulos et al. 1999). Care needs to be taken with the identification of volatiles, from collection through to confirmation of biological activity. In particular in obtaining and handling material (especially plant) before and during collection of volatiles; techniques that result in injury to the plant material allow enzymatic and chemical reactions to take place, altering the volatile composition of the sample (Agelopoulos et al. 1999). Additionally, the volatiles collected can be related to the physical and chemical properties of the trapping agent (Agelopoulos and Pickett 1998). Once collected behaviourally-active compounds often occur at trace quantities accompanied by large amounts of biologically inactive compounds. The use of electrophysiological techniques such as coupled Gas Chromatography-Electroantennography (GC-EAG) can identify compounds that are neurophysiologically-active (see Wadhams 1990). GC-EAG techniques cannot be used in isolation; compounds require further identification using techniques such as Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) and comparing tentatively identified compounds with laboratory standards (GC peak enhancement co-injection, see Pickett 1990). Once identification has been achieved using the above techniques the potential biological activity of a compound or mixture of compounds can be assessed by Electroantennography (EAG), however confirmation of behavioural activity (and the type of behaviour) can only be achieved using bioassays and field studies.

Within the Chrysomelidae electrophysiological techniques have been used to identify the potentially biologically-active isomers of the sex pheromone (8-methyl-2-decyl propanoate (MDP)) for several *Diabrotica* species (corn rootworms, Table 4.2. Wilkin et al. 1986); these compounds have subsequently been used in monitoring programmes (section 4.1.1.3). Similar methodologies have been used to identify 15 potentially active volatiles produced by saltcedar (*Tamarix* spp.) a host of *Diorhabda*

elongata Brullé; following identification work the four most abundant of the volatiles, in combination with the beetle's male-produced aggregation pheromone were found to be attractive to the beetle in the field (Cossé et al. 2006).

Taking the above factors into account, headspace volatiles have been collected from a representative host plant of *L. lilii* (*Lilium regale* L.), both when intact and when infested with beetles, by volatile entrainment using methodologies developed at Rothamsted Research (Agelopoulos et al. 1999, Birkett et al. 2006, Chamberlain et al. 2006). The porous polymer Porapak Q was used to collect volatiles as it was possible to elute the volatiles with a solvent (solvent desorption) and therefore have a liquid sample which could be used a number of times in the process of compound identification. However, this method can have the disadvantages of low sensitivity (only a fraction of the volatiles collected are analysed) and the solvent peak can mask compounds with short retention times in GC analysis (Agelopoulos and Pickett 1998). Volatiles were tentatively identified by GC, using the same headspace volatiles, volatile peaks that were neurophysiologically detected by *L. lilii* were identified using coupled GC-EAG. Further identification was carried out using GC-MS and GC co-injection. Using laboratory standards, eleven compounds were tested using EAG, of which six that provided a significant response were further tested in behavioural bioassays (linear-track olfactometer). In an attempt to isolate sex-specific volatiles (potential pheromone) vacuum distillation and GC/ GC-MS analysis of beetle extract was carried out, a method previously used to identify aphid alarm pheromone components (see Pickett and Griffiths 1980).

5.2. MATERIALS AND METHODS

5.2.1. Collection of plant volatiles (volatile entrainment)

Volatile entrainments (dynamic headspace analysis) were carried out in a laboratory at room temperature (22 ± 4 °C). Adult *L. lilii* were collected from Wisley Garden and *L. regale* plants raised in pots at Deers Farm, Wisley (see sections 4.2.1 and 4.2.2).

The absorbent material and storage of elutions: Volatiles were collected using 8 cm long glass tubes (0.3 cm internal diameter (ID)), packed with Porapak Q polymer (80/100 mesh, 50-60 mg; Supelco, USA) between silanised glass wool plugs. After entrainment, volatiles were eluted with 0.75 ml of redistilled diethyl ether. The elutions (entrainment samples) were sealed in glass ampoules and stored at -20 °C until analysed.

Conditioning of equipment: Before each entrainment, or run of consecutive entrainments, all equipment was conditioned to remove contamination (residual volatiles). Porapak Q tubes were washed through with at least 1 ml of redistilled diethyl ether and baked at 132 °C in a flow of filtered nitrogen for at least two hours. Glassware and aluminium plates were scrubbed with warm water, rinsed with acetone and baked along with the glass wool in an oven at 180 °C for at least two hours. Activated charcoal filters (BDH 10-18 mesh) were conditioned by baking at 180 °C in a filtered nitrogen flow for at least three hours. Once the equipment was conditioned it was handled with cotton gloves to reduce contamination.

Entrainment of headspace volatiles from Lilium regale, *intact or infested with* Lilioceris lilii: Mature *L. regale* plants in 3 litre pots were enclosed within two Pyrex glass vessels clamped together; both vessels measured 30 x 10 cm outside diameter. While the lower vessel was a simple cylinder the upper vessel was closed at one end where there was an air inlet and outlet (Figure 5.1). The base of the chamber was sealed with an aluminium plate (divided in two with a 1 cm diameter central hole for the plant stem), clamped to the base. Gaps between the plant stem and the aluminium plate were packed with glass wool. The entrainments were performed by using a positive pressure system in which clean air purified by passage through a charcoal filter was pumped into the base of the chamber via PTFE tubing at a flow of 1000 ml/ min^{-1}. Air was simultaneously drawn through the absorbent tube inserted in the top of the chamber at 800 ml/ min^{-1}. Flow rates were controlled and measured using flow meters. All pumps were NMP 830 KNDC B, Neuberger, Freiberg.

Lilioceris lilii-infested plants were treated as above but with five beetles added. The beetles were sexed at the end of the entrainment, in all cases being a mixed sex group. Intact and infested plants were run consecutively (using the same plant), volatile collection was initiated as soon as the beetles were added to the chamber. Empty chambers, as blanks, were entrained on several occasions to check for contaminants.

All volatile entrainments ran for a minimum of 24 h and a maximum of 96 h.

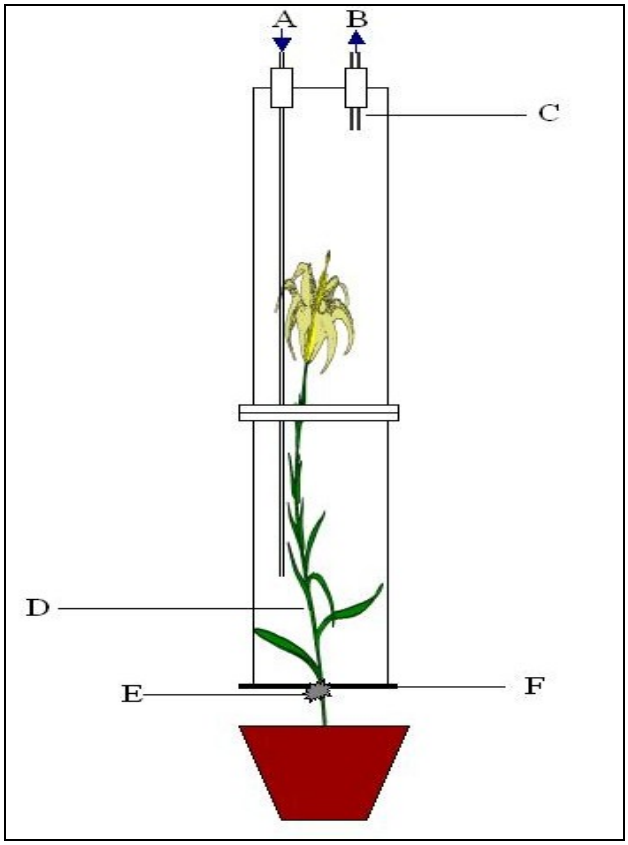


Figure 5.1. Volatile entrainment apparatus used to collect headspace volatiles from *Lilium regale* plants. A = Air input via charcoal filter at 1000 ml/ min^{-1}. B = Air output at 800 ml/ min^{-1}. C = Porapak Q tube. D = Plant material (in pot). E = Glass wool (plugging any gaps). F = Aluminium plates.

5.2.2. Collection of volatiles from adult beetles (Vacuum distillation)

As it was not possible to sex beetles by external morphology (M. Cox pers. com, 1999) adult *L. lilii* were killed by freezing (-20 °C) for 24 h before being dissected to determine sex. Separately 100 males and 100 females were extracted with cold (-20 °C) redistilled hexane (approximately 20 ml). The beetles in hexane were vacuum distilled in apparatus developed at Rothamsted Research (Figure 5.2, Pickett and Griffiths 1980). The ground glass joints of the apparatus were sealed with Grease (Apiezon L). The high vacuum pump was separated from the system by a trap cooled with liquid nitrogen, which arrested any backflow of vapour. The beetles in hexane, in a round-bottomed Pyrex flask, were attached at point A and distilled under vacuum (0.05 mbar) for 24 h. The distillate collected in the upper limit of the U tube where it was cooled by liquid nitrogen (-196 °C). After distillation was complete the taps (T_1 and T_2) were closed and the distillate allowed to thaw and run into the collection vessel. By differential heating (ca. 20 °C / -196 °C) the hexane was allowed to reflux onto the region of the tube marked B, where most of the volatile components had condensed,

ensuring that even the less soluble distillate was washed into the collection vessel. The collection vessel was removed and vacuum distillate transferred to a glass vial which was stored at -20 °C. The process was repeated once with male extract and once with female extract. Vacuum distillation was carried out by Dr Sarah Dewhirst of Rothamsted Research.

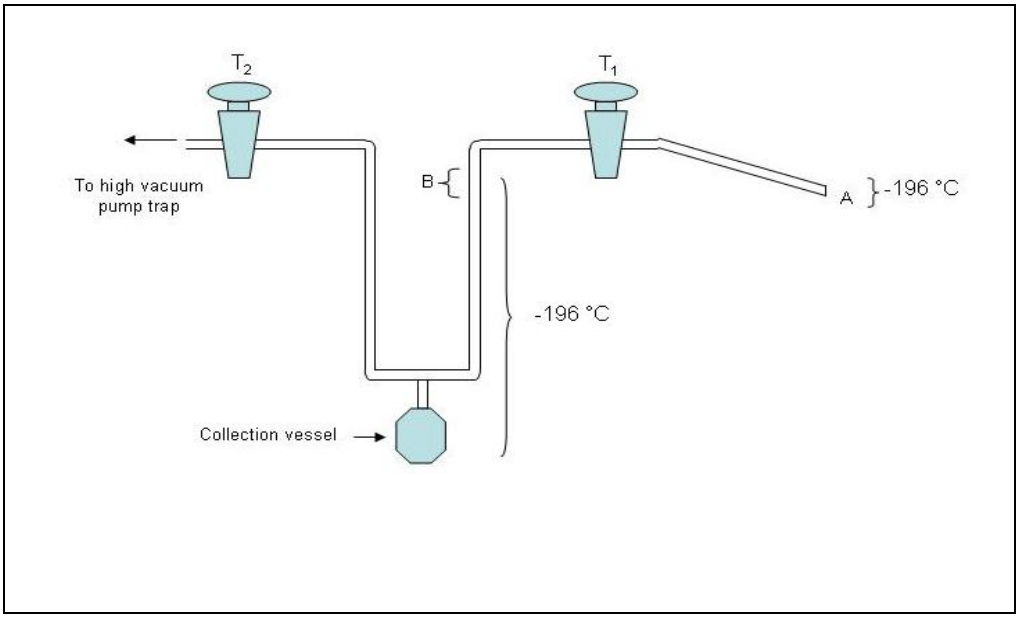


Figure 5.2. Apparatus for vacuum distillation of lily beetle extract (after Pickett and Griffiths 1980). T = tap. A = point at which flask containing lily beetles in hexane was connected. B = area where some volatile constituents condense.

5.2.3. Chromatography and electrophysiology (identification of potentially behaviourally-active volatiles)

Gas Chromatography: Volatiles collected by entrainment onto Porapak Q and eluted in diethyl ether were initially analysed by Gas Chromatography (GC). Analysis was carried out by dual injection using 1 or 4 µl of the eluted solution on both polar (DB-wax, 30 m x 0.32 mm ID x 0.5 µm film thickness) and non-polar (HP-1, 50 m x 0.32 mm ID x 0.5 µm film thickness) capillary columns using a HP6890 GC (Agilent Technologies, UK) fitted with a cool-on-column injector, a deactivated HP-1 pre-column (1 m x 0.53 mm ID) and a flame ionisation detector (FID). The carrier gas was hydrogen and the oven temperature was kept at 30 °C for 0.5 min and then programmed at 5 °C min^{-1} to 150 °C, kept there for 0.1 min and then programmed up to 230 °C at 10 °C min^{-1} for a final 20 min.

Initial analysis of the vacuum distillate from adult beetles was carried out using a HP5890 GC using the same methods as above.

Some initial GC analysis was carried out by Dr Keith Chamberlain of Rothamsted Research.

Tentative identification and quantification of compounds from GC analysis: Peaks of interest were tentatively identified by the use of a Retention Index (RI) (Bartle 1993, Figure 5.3). To enable the calculation of RI a series of known internal reference standards (alkanes, C7-C25 n-hydrocarbons at 1µl of 100 ng/µl diluted in distilled hexane purchased from Sigma Aldrich) were run on the GC columns prior to the analysis of entrainment samples. The RI for a given compound (peak) is a number indicating its retention relative to the adjacent alkanes. The RI was compared to a database of compounds with a known RI (K. Chamberlain pers. com, 2005).

Tentative quantification of the amount (ng) of a compound in the injected sample was calculated by taking the mean amount of the alkanes ((sum of the area of peaks of alkanes/ number of alkanes)/ 100) and multiplying this by the area of each of the peaks. This provided quantifications from which it was possible to determine the approximate amount of compound. This figure was used to compare ratios in peak size before and after peak enhancement co-injection.

$$RI = \left(\frac{100(\log rt(x) - \log rt(z-1))}{(\log rt(z+1) - \log rt(z-1))} \right) + 100(z-1)$$

rt = Retention time

x = Compound of interest

z + 1= alkane after the compound of interest

z - 1 = alkane before the compound of interest

Figure 5.3. Formula used for calculating retention index (RI) (after Bartle 1993).

Coupled Gas Chromatography-Electroantennography (GC-EAG): The coupled GC-EAG system in which effluent from the GC column is simultaneously delivered to an antennal preparation and GC detector has been described by Wadhams (1990, Figure 5.4). Separation of the volatiles was achieved by injecting 1-2 µl of sample onto a non polar capillary column (HP-1, 50 m x 0.32 mm ID x 0.52 µm film thickness) using an HP5890 (Agilent Technologies, UK) equipped with a cool-on-column injector, a deactivated HP-1 pre-column (1 m x 0.53 mm ID) and FID. Oven temperature was

maintained at 30 °C for 2 min and then programmed at 5 °C min^{-1} to 100 °C and then at 10 °C min^{-1} to 250 °C. The carrier gas was hydrogen. These experiments were carried out using elution samples from infested *L. regale*.

The Ag-AgCl electrodes were filled with saline solution (composition as in Maddrell 1969, but without glucose). An antenna was excised and suspended between the two electrodes. The tip of the terminal process of the antenna was removed to ensure a good contact. The preparations were held in a continuous humidified, charcoal filtered air stream (1 L min^{-1}) coming from a glass tube outlet which was positioned 5 mm from the preparation. The signals were passed through a high impedance amplifier (UN-06, Syntech, Hilversum, The Netherlands) and responses from the antennae measured in mV deflections. Analysis was carried out using a customised software package enabling simultaneous records of the EAG and FID responses (GC-EAG 2000 Syntech ®). The procedure was repeated on four occasions. Ms Christine Woodcock of Rothamsted Research assisted with the GC-EAG experiments.

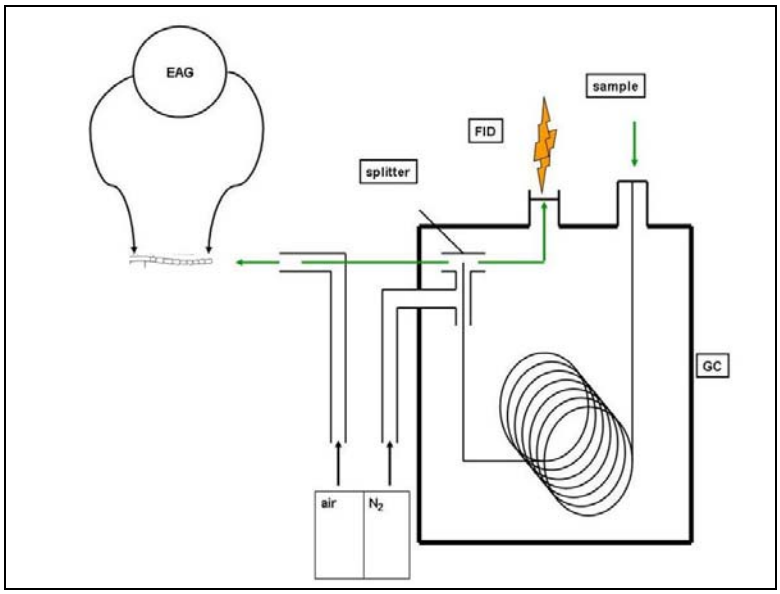


Figure 5.4. Illustration of the Gas Chromatography-Electroantennogram (GC-EAG) apparatus (Rothamsted Research).

Gas Chromatography - Mass Spectrometry (GC-MS): Tentative identification of compounds from entrainment samples focused on peaks that gave a response in GC-EAG. These were carried out using mass spectrometry (MS) as a detector. The sample (1 or 4 µl) was injected into a Trace GC (Agilent Technologies, UK) fitted with a non-polar HP-1 capillary column (50 m, 0.32 mm ID, 0.52 µm film thickness), a cool-on-column injector (Gerstel CIS 3), a deactivated HP-1 pre-column (1 m x 0.53 mm ID)

and FID. The carrier gas was helium. The GC oven temperature was maintained at 30 °C for 5 min and then programmed at 5 °C min^{-1} to 250 °C. The GC was directly coupled to a Thermo-Finnigan MAT95XP Mass spectrometer. Ionisation was by Electron impact, set to 70 electron volts (eV), in positive ionization mode, magnet scan (range was between 40-600 amu). The source temperature was set to 200 °C, the accelerating voltage to 4.6 kV and the filament current in the source set at 1 mA.

Analysis of the vacuum distillate of adult beetles was carried out using similar methods to those above but using a polar column (HP-5, 50 m, 0.32 mm ID, 0.52 µm film thickness) coupled to a MSD benchtop Mass spectrometer.

Dr Mike Birkett and Dr Abdul Mohib of Rothamsted Research conducted the GC-MS experiments.

Peak enhancement co-injection (confirmation of compound identification). Identification of compounds tentatively identified using GC/ GC-EAG and GC-MS, were confirmed (or rejected) by the use of co-injection with a laboratory standard. The amount of standard added to the sample was aimed at doubling the area of the peak for that particular substance, without increasing its width. This was achieved by adding the appropriate amount (concentration) of a solution of the laboratory standard (Table 5.1) to the entrainment sample injected into the GC. This was carried out on polar (DB-Wax) and non-polar (HP-1) columns for increased certainty using the GC methods detailed above. Confirmation of compound identity was achieved by comparing the of peak area with that of other nearby peaks in the sample before and after co-injection.

Electroantennography (EAG). Antennae of *L. lilii* were prepared as for GC-EAG. Individual chemicals were passed over the excised antennae via a filter paper strip (approximately 4 x 60 mm) in a disposable Pasteur pipette cartridge. Using a stimulus controller (model CS-05, Syntech ®), the stimulus (2 sec duration, 100 ml min^{-1}) was delivered via a glass tube (5 mm diameter, 12 cm length) with a hole in the side into which the Pasteur pipette cartridge was introduced. A purified, humidified air stream (900 ml min^{-1} during stimulus delivery, 1 L min^{-1} before and after stimulus delivery) flowed continuously over the antenna. The end of the tube was positioned 5 to10 mm from the antennal preparation. Standard test solutions of ten chemicals (Table 5.1) identified from GC-EAG/ GC-MS and coinjection-GC were used in addition to redistilled hexane (control stimulus), (4a*S*,7*S*,7a*R*)-nepetalactone (used as a standard to which responses to other chemicals were standardised) and *cis*-jasmone (a compound used due to its presence in a wide variety of damaged plant volatile profiles, Bruce et al. 2003). The standard solutions were applied at 10 µl of 1 mg/ ml in redistilled hexane to

the filter paper (i.e. 1g x 10^{-6}). The one dose was used to gain an overall picture of the EAG responses of identified compounds. The solvent (hexane) was allowed to evaporate (30 seconds) before the strip was placed in the cartridge. Each antennal preparation was tested with each of the twelve chemicals, in random order and with at least 1 min between tests. Clean air was used as an equipment check for several of the replicates, as was a control signal (0.1 mV) to provide a scale. Responses were measured in mV deflections, and the signals were passed through a high impedance amplifier (UN-06, Syntech) and analysed with a customised software package (EAG 2000 Syntech®). Ms Christine Woodcock carried out six of the ten successful EAG experiments.

For ease of analysis results were expressed as percentage of the response to the standard application of (4a*S*,7*S*,7a*R*)-nepetalactone. The percentage responses were compared for significant differences using Student's t-test in the software package Genstat 9.1.0.

Table 5.1. Laboratory standard compounds used in EAG recording, GC coinjection and bioassays.

Compound	Source	Purity %
benzaldehyde	Sigma Aldrich	99
α-pinene	Sigma Aldrich	98
6-methyl-5-hepten-2-one	Sigma Aldrich	99
β-pinene	Sigma Aldrich	99
myrcene	Sigma Aldrich	90
1,8-cineole	Sigma Aldrich	99
limonene	Sigma Aldrich	97
nonanal	Sigma Aldrich	95
linalool	Avocado	98
methyl salicylate	Avocado	98
indole	Avocado	99
cis-Jasmone	Alfa Aesar	90
(4aS,7S,7aR)-nepetalactone	Botanix	85%

Chemicals: All laboratory standard chemicals were obtained from Sigma-Aldrich (Gillingham, UK), Avocado Research Chemicals Ltd (Lancashire, UK), Alfa Aesar (Lancashire, UK) and Botanix Ltd (Paddockwood, UK), (Table 5.1). All chemicals were diluted to the appropriate concentration in redistilled hexane prior to use.

5.2.4. Behavioural bioassays

Volatiles identified as having activity in EAG experiments were tested for behavioural activity with diapaused *L. lilii* in linear-track olfactometer (LTO) bioassays. General methods and statistics follow section 4.2. Chemicals were made up to 1 mg/ ml in redistilled hexane. For each replicate 10 µl (i.e. 1g x 10^{-6}) of the stimulus chemical was placed (using a micro-pipette) on a strip of filter paper (approx 60 mm x 4 mm), 10 µm of redistilled hexane was placed on another piece of filter paper and as a control. The strips were charged 1 min before each replicate, to allow the hexane to evaporate. The filter paper strips were placed in the glass vessels and the pump ran for a minimum of two minutes before the introduction of beetles into the olfactometer. Between tests all equipment was thoroughly cleaned (see 4.2.3). Eighty replicates were carried out for each of five chemicals and with one chemical (benzaldehyde) 48 replicates were carried out.

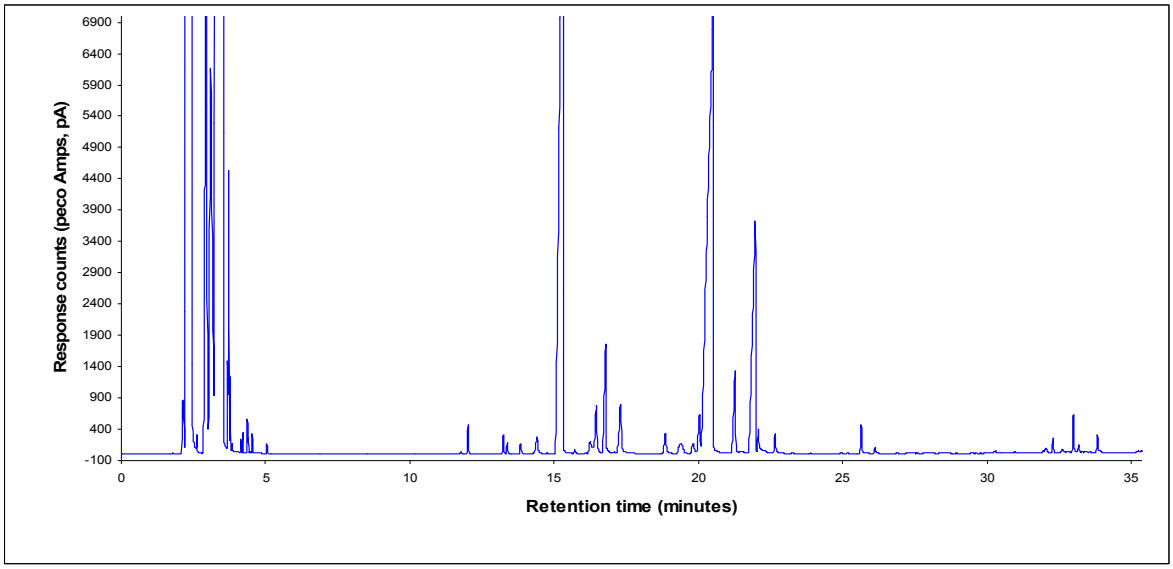
5.3. RESULTS

5.3.1. Identification of potentially behaviourally-active volatiles from beetle damaged plants.

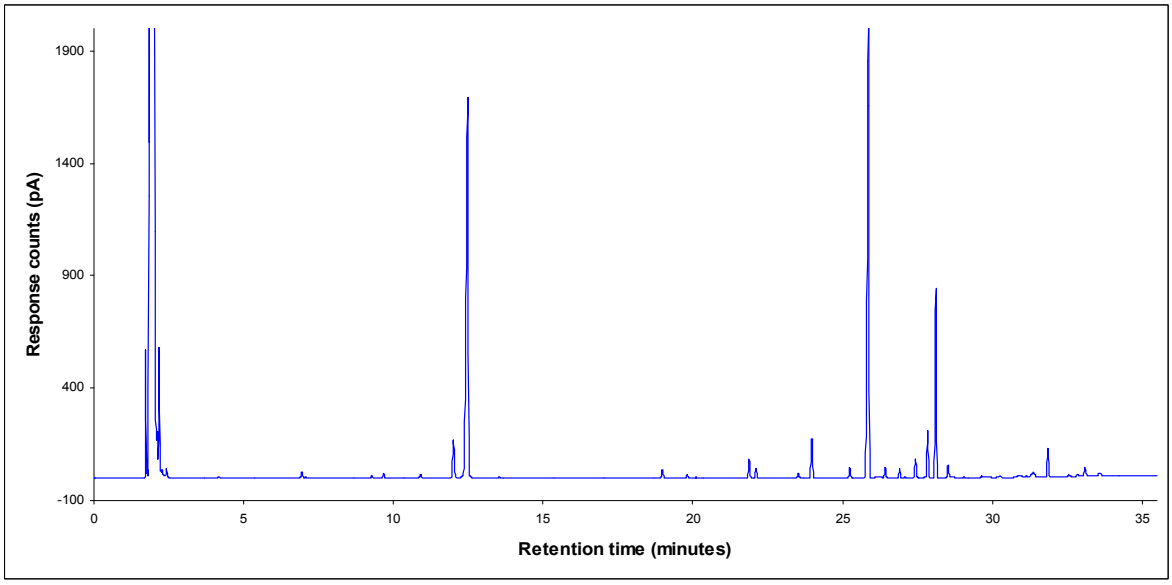
Gas Chromatography (GC): Initial GC analysis of the entrainment samples of plants indicated that the volatiles emitted by *L. regale*, both when intact and when infested with *L. lilii* are a complex mixture of volatile compounds (Figure 5.5). These initial GC analyses did not show clear differences in the volatile composition between infested and intact plants. Therefore investigations focused on electrophysiological techniques to identify potentially behaviourally-active compounds, using elutions from infested *L. regale*.

Coupled Gas Chromatography-Electroantennography (GC-EAG) and compound identification by GC-MS and GC-coinjection: GC-EAG analysis identified twelve EAG-active peaks in the entrainment samples of a *L. lilii* infested *L. regale* (Figure 5.6). GC-mass spectrometry provided tentative identifications of compounds from eleven of the peaks (Table 5.2, Figure 5.7). GC co-injection confirmed the identity of compounds present in the entrainment samples in areas of nine of the peaks (Table 5.2, Figures 5.8, 5.9). It should be noted that for three of the GC-EAG peaks there were two compounds identified as in the entrainment samples. In these cases the compounds gave very similar retention indexes (RI) on a non-polar HP-1 column; it was not possible to determine using the above methods which (if either) of the chemicals

elicited the response in the GC-EAG. Thus twelve compounds which correspond to nine of the peaks highlighted by GC-EAG were confirmed as present in headspace volatiles from an infested *L. regale* (Table 5.2).



A) HP-1



B) DB-Wax

Figure 5.5. Typical GC traces from A) a non-polar column (HP-1) and B) a polar column (DB-wax) of 1 µl of an entrainment sample (elution) of headspace volatiles collected from a *Lilium regale* plant infested with a mixed sex group of *Lilioceris lilii*.

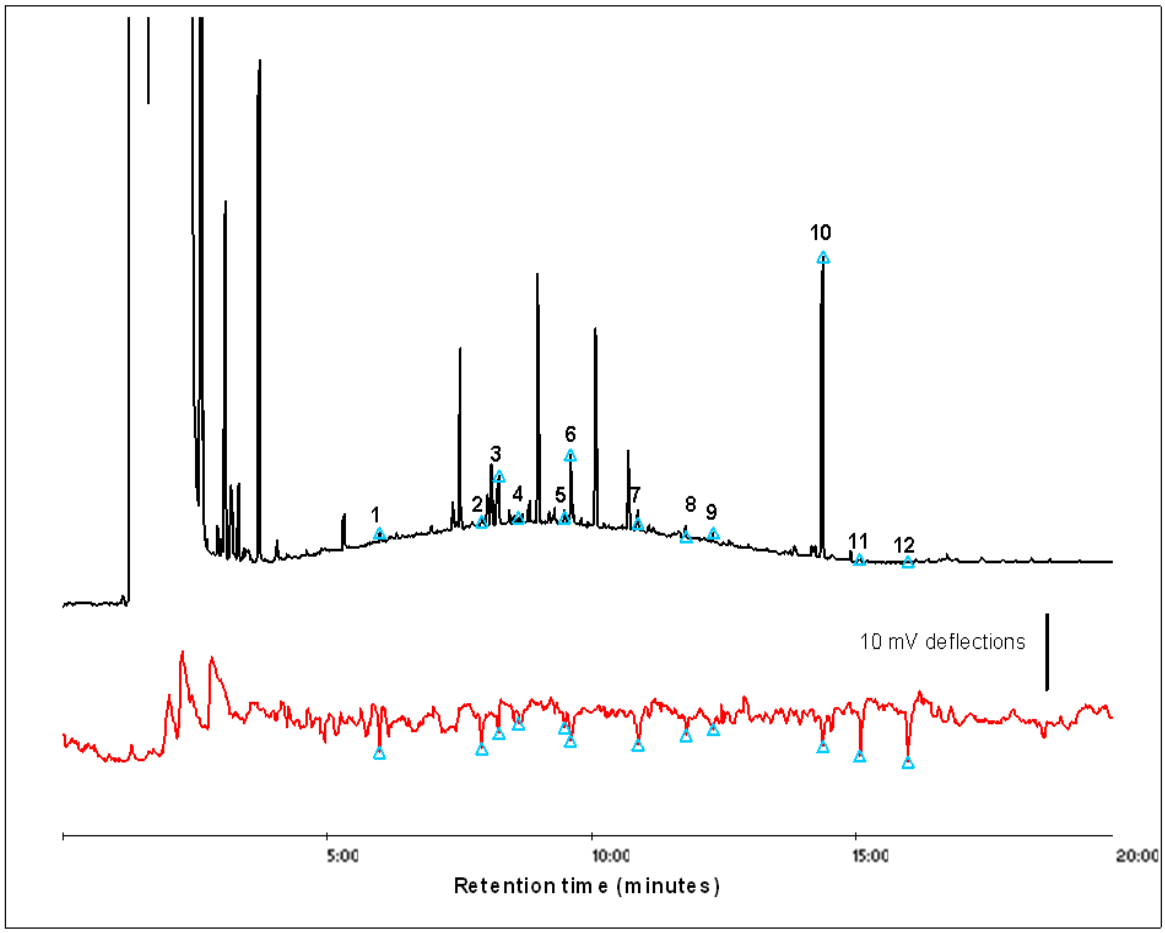
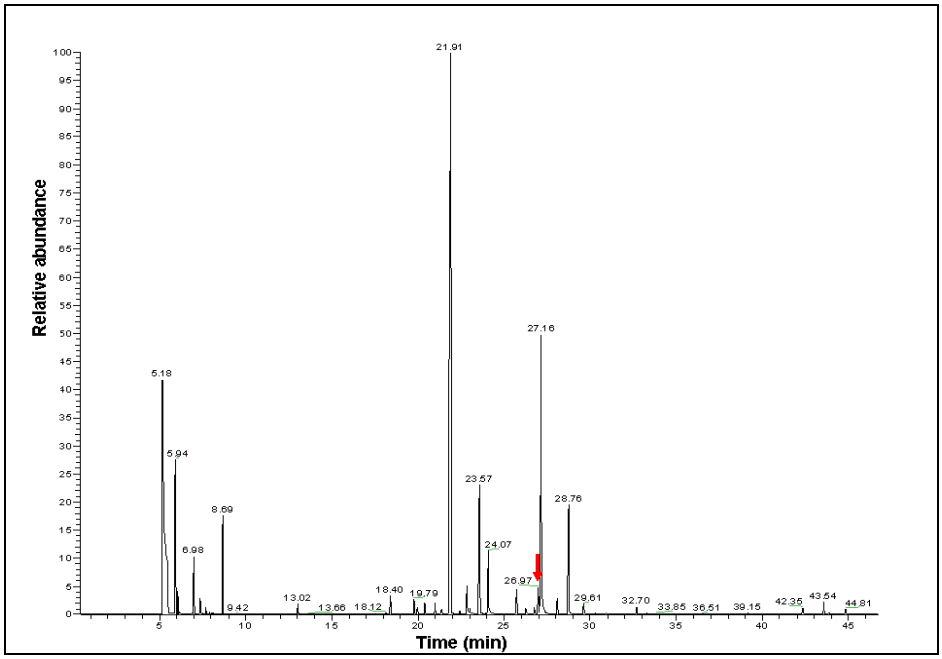
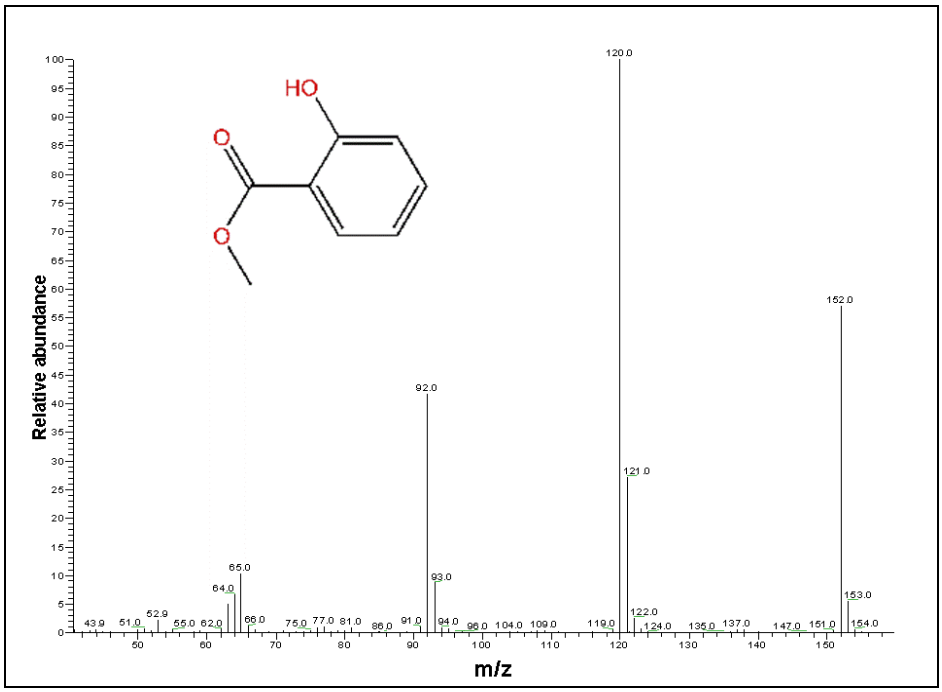


Figure 5.6. Example of a coupled GC-EAG trace of an antennal response of *Lilioceris lilii* to an entrainment sample of headspace volatiles collected from a *Lilium regale* plant infested with a mixed sex group of *L. lilii* adults. Top trace (black) corresponds to the FID detector on the GC. Bottom trace (red) corresponds to the antennal response of the insect preparation. Blue triangles (Δ) indicate antennal responses and corresponding areas of the GC trace of interest. Peak numbers correlate to compounds (listed in Table 5.2), some of which were identified by GC-mass spectrometry (GC-MS) and peak enhancement co-injection with authentic laboratory standards. The GC-EAG experiment was repeated on four occasions.

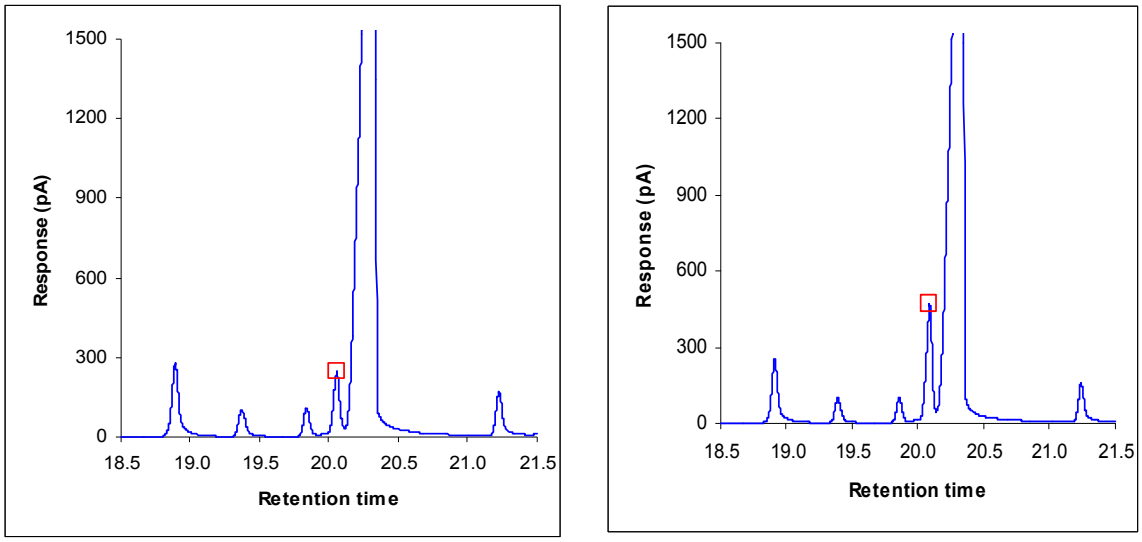


A) GC trace



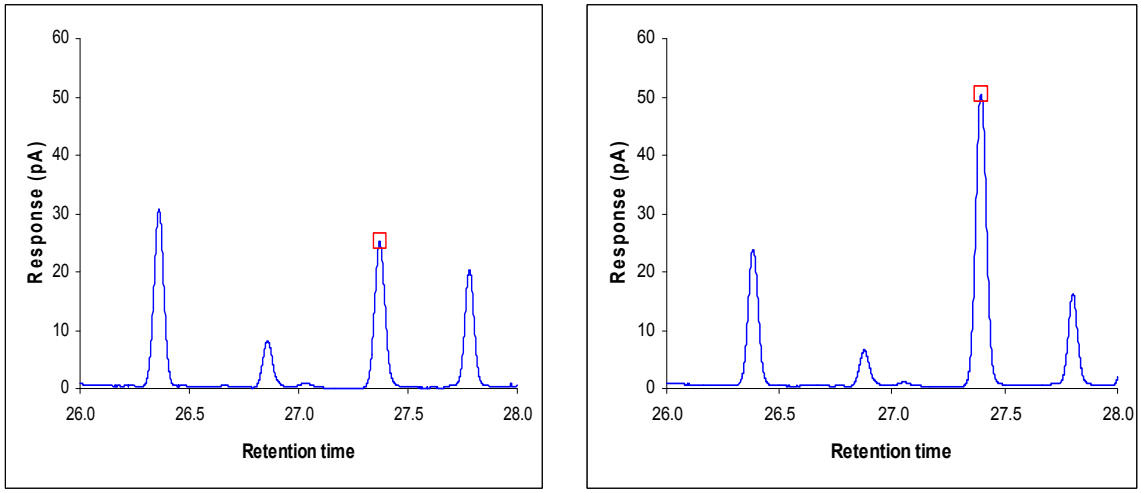
B) Mass spectra

Figure 5.7. Example of A) GC trace and B) Mass spectra of a peak of interest (retention time 26.97 mins) which corresponds to the mass spectra of methyl salicylate in library reference (chemical structure also presented). Original sample from an entrainment of *L. regale* infested with *L. lilii.* m/z = mass to charge ratio. Red arrow (↓) indicates peak in GC trace of interest.



A) **B)**

Figure 5.8. Sections of GC traces from a non-polar column (HP-1) illustrating peak enhancement coinjection with a laboratory standard of methyl salicylate into 1 µl of an entrainment sample from a *Lilium regale* infested with *Lilioceris lilii*. A) Trace before co-injection, B) trace after coinjection. □ Denotes peak of interest.



A) **B)**

Figure 5.9. Sections of GC traces from a polar column (DB-Wax) illustrating peak enhancement coinjection with a laboratory standard of methyl salicylate into 1 µl of an entrainment sample from a *Lilium regale* infested with *Lilioceris lilii*. A) Trace before co-injection, B) trace after coinjection. □ Denotes peak of interest.

Table 5.2. Summary of compounds identified in entrainment samples collected from *Lilium regale* infested with *Lilioceris lilii* adults, corresponding to areas identified as of interest in GC-EAG

Peak	Retention	Retention index		Identity confirmed		Compound
Figure 5.6	time (min)	Hp - 1	DB-Wax	GC-MS	GC co-injection	
1	5.8	772	n/a	✓		hexanal*
2	8.1	933	1535	✓	✓	benzaldehyde
		934	1026	✓	✓	α-pinene
3	8.6	966	1347	✓	✓	6-methyl-5-hepten-2-one
4	8.9	975	1116	✓	✓	β-pinene
		984	1176	✓	✓	myrcene
5	10.0	1022	1221	✓	✓	1,8-cineole
6	10.1	1024	1212	✓	✓	limonene
7	11.3	1084	1401		✓	nonanal
		1086	1555	✓	✓	linalool
8	11.7	1175	1795	✓	✓	methyl salicylate
9	12.2	1260	2481	✓	✓	Indole
10	14.7	1498	1755	✓	✓	(*E*,*E*)-α-farnesene
11	15.2	1570	n/a			Unidentified
12	16.1	1670	n/a	✓		Thujopsene*

*Presence of compound in entrainment sample unconfirmed

Electroantennograms (EAG): Ten of the compounds identified by GC-EAG, GC-MS and GC co-injection in addition to (4a*S*,7*S*,7a*R*)-nepetalactone and *cis*-jasmone were tested by EAG using laboratory standards. Typical antennal response traces to the volatiles are shown in Figure 5.10. Five of the compounds gave a statistically significant response ($p < 0.05$), and five no significant response ($p > 0.05$, Table 5.3). Although not significant ($p = 0.058$) β-pinene was used in additional work as the p

value was deemed close enough to significance considering the number of replicates (n = 10) used. A statistically significant response ($p < 0.001$) was also observed with *cis*-jasmone which was not identified as present in entrainment samples. These experiments therefore provided the possible identities of some compounds that elicited the response peaks in GC-EAG (see Figure 5.6): Peak 1 remains unidentified; peak 2, benzaldehyde; peak 3, 6-methyl-5-hepten-2-one; peak 4 possibly ($p = 0.058$) β-pinene; peaks 5 and 6 are unlikely to be due to 1,8-cineole or limonene and remain unidentified; peak 7 could be due to linalool and / or nonanal; peak 8 methyl salicylate. Compounds eliciting responses at peaks 9-12 remain unconfirmed. The compounds indole and (*E,E*)-α-farnesene, which may relate to peaks 9 and 10, were not tested as they were unavailable.

Table 5.3. Summary of results of ten *Lilioceris lilii* antennae tested against 11 compounds (1mg/ml) in electroantennogram (EAG) experiments. Results expressed as % of the response to a standard application of (4a*S*,7*S*,7a*R*)-nepetalactone at the same level. Control = hexane. NS = Not significant.

Compound	Response (%) ± SEM	Difference from control (P)	Peak GC-EAG (Figure 5.6)
benzaldehyde	38.5 ± 4.1	0.048	2
6-methyl-5-hepten-2-one	39.8 ± 3.7	0.019	3
nonanal	109.3 ± 7.5	< 0.001	7
linalool	67.0 ± 5.1	< 0.001	7
methyl salicylate	124.6 ± 11.1	< 0.001	8
cis-Jasmone	87.5 ± 9.1	< 0.001	n/a
β-pinene	37.5 ± 3.9	0.058 (NS)	4
α-pinene	40.2 ± 7.6	0.159 (NS)	
myrcene	37.0 ± 4.7	0.115 (NS)	
1,8-cineole	37.0 ± 4.9	0.115 (NS)	
limonene	31.4 ± 4.1	0.499 (NS)	
hexane (control)	28.0 ± 2.8	n/a	n/a

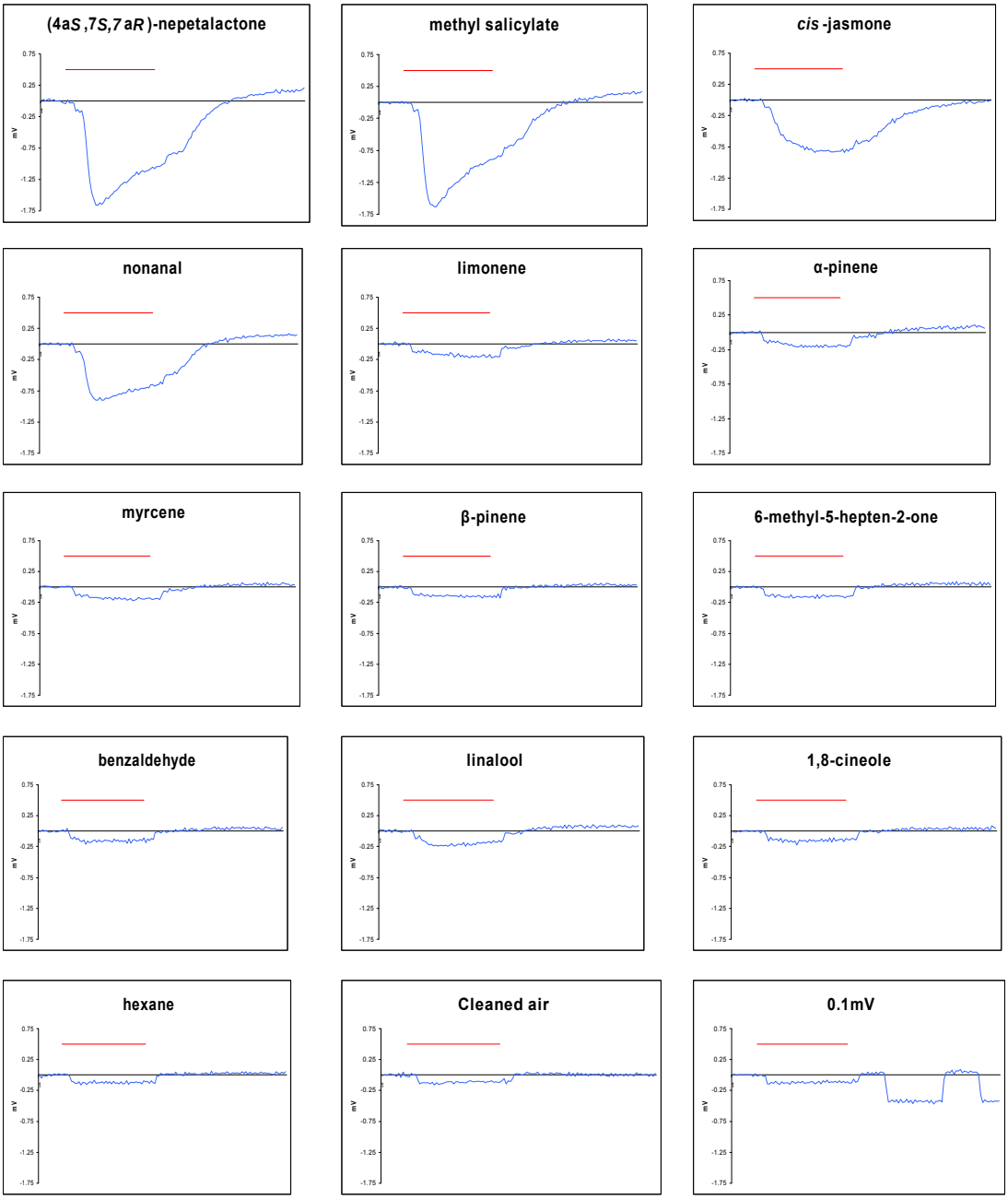
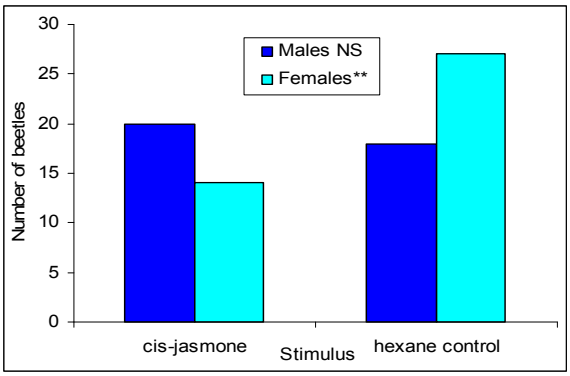


Figure 5.10. Example of EAG responses of a single *Lilioceris lilii* antenna to ten volatiles identified from entrainment sample in addition to hexane (control), *cis*-jasmone and (4aS,7S,7aR)-nepetalactone. A clean air test and 0.1mV control signal as a scale, are also presented. Red line indicates 2 second odour stimulation.

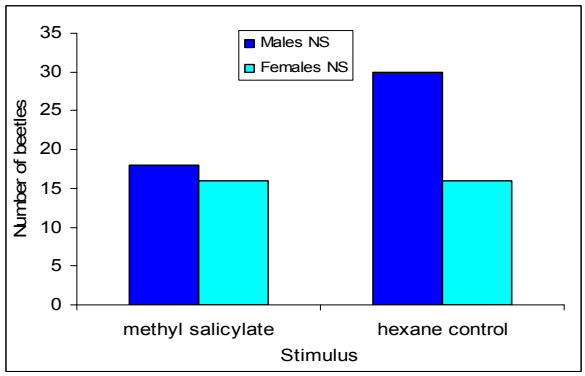
5.3.2. Behavioural bioassays (confirmation of potential behavioural activity of identified compounds)

Eighty replicates of six compounds were tested in the linear-track olfactometer, and one compound tested with 48 replicates (Figure 5.11). At the concentration of compounds tested, one chemical (*cis*-jasmone) gave a significant response, where

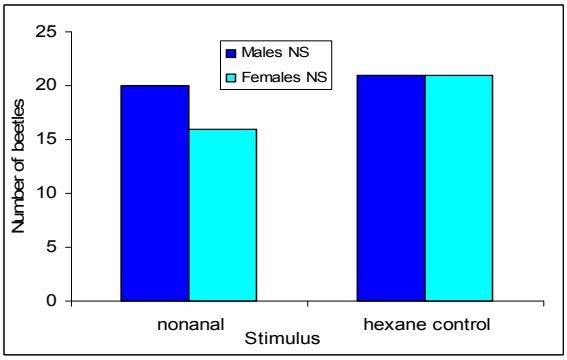
female beetles moved into the airstream emanating from the hexane (clean air) control in preference to this compound. With all other chemicals tested there was no significant response.



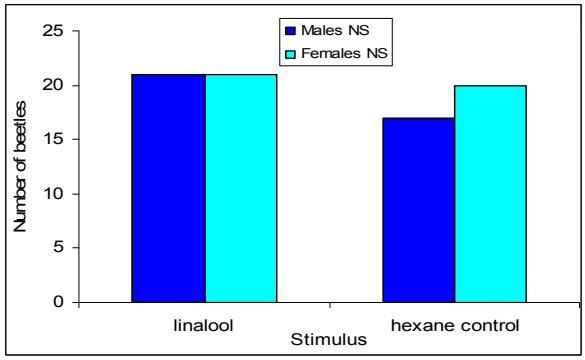
Females** (χ^2 = 6.29; p = 0.01); Males NS (χ^2 = 0.16, p = 0.69).



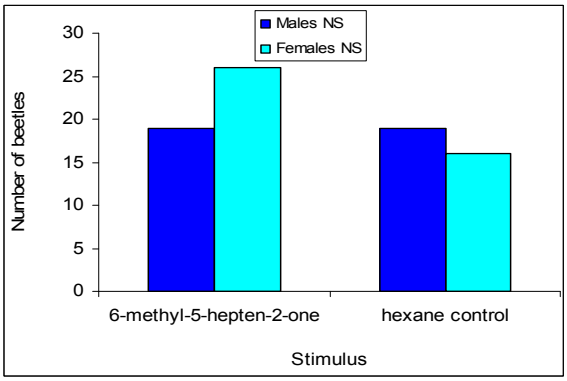
Females NS (Not significant χ^2 = 0.00; p = 1.00); Males NS (χ^2 = 3.09, p = 0.08).



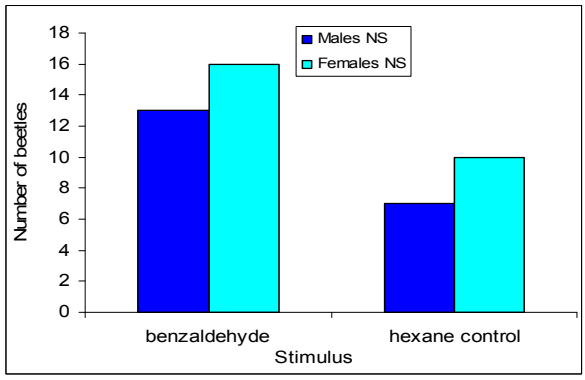
Females NS (χ^2 = 1.34; p = 0.25); Males NS (χ^2 = = 0.00, p = 1.00).



Females NS (χ^2 = 0.03; p = 0.87); Males NS (χ^2 = 0.42, p = 0.52).



Females NS (χ^2 = 1.89; p = 0.17); Males NS (χ^2 = 0.05, p = 0.83).



Females NS[1] (p = 0.17); Males NS[1] (p = 0.13).

Figure 5.11. Movement of diapaused adult *L. lilii* into the odour streams of 10 µl of 1 mg/ml laboratory standard chemicals and 10 µl hexane in a linear-track olfactometer. NS = Not significant. ** significant at p<0.05. [1]Replication too low for analysis by χ^2 and Fishers exact test carried out.

5.3.3. Analysis of vacuum distillate from adult beetles

Initial analysis of the vacuum distillate of beetle extract showed the presence of a large peak in both male and female extract, but it was thought this may be due to contamination. GC-MS of vacuum distillation products confirmed the presence of a contaminant which was provisionally identified as 1,2-Benzenedicarboxylic acid. This compound may have masked the presence of any differences in the volatile profile between male and female beetle extract.

5.4. DISCUSSION

5.4.1. Identification of potentially biologically-active compounds from headspace volatiles collected from an infested *Lilium regale* plant

Differences in volatile profile between intact and infested plants: Analysis of headspace volatiles did not show clear differences between intact *L. regale* and plants damaged by *L. lilii*. However, further investigation is warranted. It has been widely reported that herbivore damage induces changes in the volatile profile of plants compared to intact or mechanically damaged plants (see Bolter et al. 1997 and references therein). The changes in odour profile can be specific to the herbivore, and may consist of an increase in the emission of a blend of compounds qualitatively similar to that produced by intact plants, or of volatiles that are released only after herbivore damage (including those which are present at trace quantities before damage). These induced changes in odour profile often affect the odour-induced behaviour of the herbivore. For example, the Colorado potato beetle, *Leptinotarsa decemlineata,* shows a preference for odour-streams from conspecific-damaged potato plants over undamaged or mechanically damaged hosts (Bolter et al. 1997). The odour-induced movement of female *L. lilii* to conspecific damaged plants over that of intact plants in olfactometer studies has been demonstrated (Chapter 4). Therefore it is likely that there are differences in the headspace volatile composition emitted by intact and *L. lilii* damaged *L. regale*. Possible explanations for the lack of detection of clear differences in volatile composition in these experiments include the properties of the absorptive polymer (Porapak Q); in GC analysis the solvent peak may have masked some of the volatiles and the low sensitivity, as only a fraction of the volatiles are absorbed and analysed (see section 5.1). It was necessary to gain a liquid sample from the entrainments in order to carry out GC-EAG and co-injections (see Agelopoulos and Pickett 1998), and this negated the use of some other absorptive materials such as Tenax TA. The lack of sensitivity of the absorbent may be overcome

by increasing the time period or the quantity of plant material that is entrained, thus collecting a greater quantity of headspace volatiles. Alternatively the entrainment samples could be concentrated in a flow of nitrogen, as this would result in larger peaks in GC and therefore any differences between headspace volatiles of infested and intact plants may be more apparent, although this would decrease the volume of sample available for identification experiments. What is clear is that more repetition is required in the collection of headspace volatiles, from both infested and intact *L. regale*. Additional samples would enable a statistical comparison of the ratio of volatiles produced by intact and beetle damaged plants.

Identification of volatile chemicals eliciting responses from L. lilii *antennae:* Twelve peaks were identified as active by GC-EAG and the probable identity of five of these peaks has been established. For one of the peaks, two possible compounds were identified (nonanal and linalool), both having activity in EAG experiments (Table 5.3). The GC and GC-EAG traces indicate that the headspace volatiles emitted from infested *L. regale* plants are a complex mixture, with those compounds identified as biologically-active present at low concentrations accompanied by relatively large amounts of inactive compounds. This is consistent with current knowledge (see Bruce et al. 2005).

It is clear that more identification work is required, by GC co-injection and EAG. Compounds corresponding to peaks 5 and 6 were identified, but found to be inactive in EAG experiments (Figure 5.6, Table 5.3). Compounds which may correspond to two other peaks (Indole, peak 9, and (*E,E*)-α-farnesene, peak 10) were not tested in EAG experiments. Some identification of compounds relating to peaks 1 and 12 has been carried out (GC-MS) but identities remain unconfirmed by GC co-injection and no compounds relating to peak 11 have been identified. Additionally, there is some evidence that further GC-EAG experiments will identify more compounds in headspace volatiles of *L. regale* that elicit a response in *L. lilii* antennae, as a significant response in EAG tests was observed with *cis*-jasmone. This compound (RI 1373 on HP-1) did not correspond to any of the peaks identified in GC-EAG experiments

For the seven compounds that gave a significant or close to significant response in EAG experiments it can be concluded that these chemicals are detected by the antennae of *L. lilii* at the dose tested ($1g \times 10^{-6}$). The dose of a compound is known to affect the intensity (mV) of response in EAG recording; for example EAG studies have demonstrated dose response relationships for a number of compounds with the chrysomelids *L. decemlineata* (Visser 1979), and *Diabrotica virgifera virgifera* (Cossé and Baker 1999). The above studies indicate that if a response is observed to

a compound the intensity increases with the amount (concentration) used as a stimulus, and in both the above studies responses to compounds were observed at lower doses than those used with *L. lilii* and did not decline with increasing concentration. Therefore it is likely that if *L. lilii* antennae do respond to the chemicals tested it would have been apparent at the concentration used.

In the EAG experiments the sex of the beetle from which the antennae originated was not determined and it is theoretically possible that the response of male and female antennae differ. However, in other chrysomelids, such as *L. decemlineata* marked differences between sexes have not been detected in the responses of the antennae (Visser 1979).

5.4.2. Assessment of behavioural activity (behavioural bioassay)

Five of the chemicals identified as EAG-active were tested in the linear-track olfactometer at a concentration of 10 µm of 1 mg/ ml in redistilled hexane (i.e. 1g x 10^{-6}) using 80 replicates. One EAG-active compound was tested at 48 replications (benzaldehyde) and one possible EAG-active compound was not tested (β-pinene), the availability of beetles limiting the number of experiments that could be carried out. Thus the number of replicates with Benzaldehyde needs to be increased and a bioassay with β-pinene should be carried out before conclusions can be made on these compounds.

One of the tested compounds elicited a behavioural response in *L. lilii*; females moving into the odour stream from clean air in preference to an odour stream containing *cis*-jasmone. However, it would be premature to assume that these chemicals elicit these responses in the field. Unlike EAG responses where a high concentration of a compound is likely to result in a response if the antennae detect it (see 5.4.1), there may be a lack of behavioural response or an atypical response with unnaturally high or low concentrations of a compound. For example subtle alterations in the concentration of green leaf volatiles can switch off attraction of *L. decemlineata* (Visser and Avé 1978). Thus it cannot be concluded that the substances used will have the observed response if tested at different concentrations. It is also possible that compounds which gave no significant response in these tests may elicit a response at other concentrations. The beetles physiological state may also affect the response, and differences in the odour-mediated behaviour of different physiological states of *L. lilii* have been demonstrated (Chapter 4). In addition, the use of bioassays with single compounds may provide a misleading picture of the actual situation, and single compounds may not result in a behavioural response whereas a mixture might (Ma and Visser 1972); it is known that the odour-mediated behaviour of many insect herbivores is in response to a specific mixture of volatiles (see section 5.4.4).

5.4.3. Analysis of vacuum distillate from adult beetles

Behavioural bioassays with *L. lilii* have indicated that a pheromone is produced by one or both sexes (Chapter 4) and the male-produced pheromone of closely-related beetles has been identified (*Oulema melanopus* see Chapter 4). The vacuum distillate in this case proved too contaminated for meaningful analysis. It was not possible to isolate the source of the contamination; 1,2-Benzenedicarboxylic acid is a commonly occurring compound and frequent contaminant (A. Mohib pers. com, 2007). Therefore further exploration for a pheromone of *L. lilii* is worthwhile, be that by vacuum distillation or volatile entrainment. Some initial volatile entrainment of single beetles was attempted early in this work, however initial GC analysis of elutions gave no peaks of interest. It is more likely that volatile entrainment of a number of beetles of the same sex would provide adequate quantities of volatiles, as was used in the identification of the pheromone of *Oulema melanopus* (Cossé et al. 2002). However, before this method is attempted a method of determining the sex of adult *L. lilii* without dissection needs to be developed.

5.4.4. Conclusions

As the entrainment samples analysed were collected from *L. regale* infested with *L. lilii* it is not possible to be certain of the source of the identified compounds. They may be produced by *L. regale*, whether infested or intact, from infested plants only or the beetles themselves. However, the volatiles identified have been found to be produced by a variety of plants and often are emitted in greater quantities following herbivore damage. These compounds have been implicated in the odour-mediated behaviour of a variety of insects. It is generally thought that odour-mediated host location can be due to compounds that are plant-specific but more often specificity is achieved by a particular ratio of volatiles, the constituent compounds of which may be produced by many plant species (the green leaf volatiles (GLV), Visser 1986). In this respect the results of these experiments correspond with the knowledge of the effects of plant-produced volatile compounds identified as having behavioural effects within the Chrysomelidae. For example linalool, nonanal, methyl salicylate, 6-methyl-5-hepten-2-one and indole are produced in greater quantities by potato plants when fed upon by adults or larvae of *L. decemlineata* compared to intact plants (Visser et al. 1979, Dickens 1999). In bioassays, mixtures of these chemicals resulted in behavioural responses, whereas the individual chemicals did not: *L. decemlineata* preferentially moves into airstreams containing various combinations of (*E*)-2-hexen-1-ol, *cis*-3-hexen-1-ol, linalool, nonanal, methyl salicylate and indole over that of clean air (Dickens 1999). Benzaldehyde along with the GLVs *cis*-3-hexenyl acetate and *cis*-3-

hexenol is emitted in increased amounts by willows (*Salix* spp.) when fed upon by *Phratora vulgatissima* (Peacock et al. 2001a). Benzaldehyde, nonanal and indole have also been isolated from extract of buffalo gourd (*Curcurbita foetidissima*) and were GC-EAG-active along with several other compounds with the rootworms *Diabrotica undecimpunctata howardi* and *D. barberi* (Cossé and Baker 1999). No reference to chrysomelids responding to β-pinene could be found, however β-pinene is produced by a wide variety of plants and has behavioural effects on some beetles, for example many Cerambycidae and Scolytidae associated with coniferous trees (Chenier and Philogene 1989). *cis*-Jasmone is considered a herbivore-induced plant volatile that repels insect attack and has an indirect defensive effect by attracting predatory and parasitic insects that prey on the herbivores (Bruce et al. 2003). Thus it is possible that movement away from an odour stream containing this chemical is beneficial to *L. lilii* and this may be the first record of a behavioural response to this compound in the Chrysomelidae.

Therefore the evidence gathered in these experiments suggests that the compounds identified as eliciting a response from *L. lilii* antennae are likely to have been produced by the plant and may at least in part be produced following beetle damage. As with other chrysomelids it is likely that it is a ratio of compounds that leads to specificity in host location, as is the case with *L. decemlineata* (Visser and Avé 1978, Visser 1979, Visser et al. 1979). However, additional identification work is required (GC, GC-EAG, GC-MS, GC co-injection), followed by bioassays and field testing using both single components and different blends of identified EAG-active compounds before the behavioural significance of the compounds can be fully elucidated. It is also possible that if a pheromone is produced by *L. lilii* this will affect behaviour, as with *L. decemlineata* where a male-produced aggregation pheromone is being used in the development of a field lure (Dickens 2006). Further work is required to isolate any sex-specific volatile compounds. The results of these experiments indicate that that it should be possible to develop a synthetic blend of compounds to which *L. lilii* respond and this may lead to improved methods of control using semiochemical-based methodologies.

CHAPTER 6. GENERAL DISCUSSION

6.1. INTRODUCTION

The lily beetle (*Lilioceris lilii*), is a pest of lilies (*Lilium* spp) in the UK and parts of North America (Salisbury 2003b, Casagrande and Kenis 2004). A multi-disciplined approach has been employed to study the primary objectives and aim of the project (section 1.13). The results of the studies are discussed with reference to these objectives and aim and potential avenues for further research which may lead to improved control measures for the beetle highlighted.

6.2. OBJECTIVE 1: TO INVESTIGATE THE BIOLOGY AND ECOLOGY OF *L. LILII*

Investigations into *L. lilii* were initiated by a review of the literature (Chapter 1), this highlighted inconsistencies and deficiencies in knowledge of the beetle's biology and ecology. Some information on the life cycle and host range of *L. lilii* in the literature is contradictory; early publications refer to two or more generations of the beetle a year (e.g. Lataste 1931), and indicate that this species is polyphagous (e.g. Fox Wilson 1942). However, recent studies (e.g. Haye and Kenis 2004) demonstrate that the beetle is univoltine, with a long oviposition period (April to August) so that overlapping life stages often occur together and that the beetle has a host range limited to *Lilium*, *Fritillaria* and *Cardiocrinum*. Some of the early misconceptions are still widely reported in pest control literature (e.g. Alford 1995).

Laboratory culture (Chapter 4) and phenology data collected from a field trial and an established population of the beetle at Wisley Garden, Surrey (Chapter 3), corroborates recent studies. Additional observations were made, including that reproductive *L. lilii* can colonise new areas. Phenology observations were compiled over three years and the mean numbers and patterns of occurrence of the different life stages between the field trial and the established population showed some differences, including that mean numbers of adults per plant were higher at Wisley Garden than at the field trial site; this may have been due to the field trial being a newly colonised site where populations of the beetle are still building up. Statistical analysis of field trial data also illustrated that between-year differences in beetle occurrence were significant. Therefore inferences drawn from the data should be considered with care. Nevertheless, a valuable a baseline of data, to which future observations can be compared was obtained.

Evidence from the field trial, which involved six different *Lilium* (Chapter 3), in combination with the literature suggests that all *Lilium* can act as hosts for the beetle, although there are differences in susceptibility, as has been found by other authors

(Livingston 1996, Conjin pers. com, 2000, Casagrande and Tewksbury 2007a). The susceptibility of different *Lilium* does not appear to relate to host taxonomic group or hybrid division, however further trials are required to investigate groups and divisions of *Lilium* not yet tested. In addition, the susceptibility of the other plant genera that can be hosts for *L. lilii* (*Cardiocrinum* and *Fritillaria*) should be similarly assessed. It was apparent when making comparisons with the results of tests conducted by other authors that a standard host is needed against which others can be compared; *L. regale* is a suitable candidate as it provided consistent results in the field trial and is a plant on which *L. lilii* can be reared without difficulty in the laboratory.

The field trial ran for three years, however it is possible that the lily beetle population and the patterns of damage will continue to change with time and so the trial should be retained for further monitoring. This may also enable some observations on parasitoid occurrence. Two species of larval parasitoid occur at Wisley Garden (Salisbury 2003a), but neither species was observed in the field trial and it is possible that the parasitoids have yet to locate the trial plots.

6.3. OBJECTIVE 2: TO INVESTIGATE THE CHEMICAL ECOLOGY OF THE BEETLE

This project investigated the odour-mediated behaviour of *L. lilii* by the use of olfactometer bioassays (Linear track olfactometer (LTO), Chapter 4). Some identification of the behaviourally-active volatiles emanating from beetle-infested host plants was achieved, using methods of volatile entrainment, electrophysiology and initial tests with behavioural bioassays (Chapter 5).

The LTO experiments indicated that host odour is, at least in part, utilised by *L. lilii* to locate host plants and conspecifics, supporting Southgate's (1959) statement that "it is obvious that smell plays a large part in the location of these insects with their food-plant". In tests with diapaused (reproductive) beetles, females were more responsive than males and the evidence suggests that the odour of conspecifics that are already damaging hosts has a synergistic attractive effect. The behavioural responses were consistent with those of chrysomelids that produce aggregation pheromones, and odour may be at least part of the mechanism of host and mate location following winter diapause in *L. lilii*. The aggregation pheromones identified in the Chrysomelidae are all male-produced and several have a synergistic effect when combined with beetle-induced plant-damage volatiles, e.g. in the cases of *Acalymma vittatum* (Smyth and Hoffmann 2003), *Phyllotreta cruciferae* (Soroka et al. 2005), and *Diorhabda elongata* (Cossé et al. 2006). However, the observed behaviours are also consistent with the behaviour of *Phratora vulgatissima* (Chrysomelidae) for which an aggregation

pheromone probably exists but the sex producing it is unknown (Peacock et al. 2001a). It is also possible that male *L. lilii* produce a sex pheromone, as when reproductive individuals were investigated only females showed a significant response in tests with plant material and conspecifics. However, in the Chrysomelidae sex pheromones have only been identified from female *Diabrotica* spp. (Table 4.2). The movement of diapaused females into the odour stream of an intact host in preference to that of a host with *L. lilii* larvae provides evidence of odour-mediated competition avoidance, a response likely to induce dispersal of reproductive females to plants with a low level of conspecific larval infestation. Within the Chrysomelidae similar behaviour has been observed with *Plagiodera versicolora,* whose larvae repel conspecific females with volatile compounds, which also repel competitors (Raupp et al. 1986).

Tests with pre-diapause (non-reproductive) individuals demonstrated that the odour-mediated behaviour of *L. lilii* differs with physiological state. Significantly more pre-diapause males moved into an odour stream containing intact host odour in preference to the odour of a host plus five conspecific adult beetles. If this behaviour is analogous with that in the field it is likely to result in dispersal of pre-diapause males to hosts that have low levels of infestation. It is possible that once diapaused (perhaps close to the plants fed upon in the previous season), these males colonise plants with the subsequent release of volatile chemicals to which other reproductive adults respond. It is thought that initial host location by chrysomelids producing aggregation pheromones is carried out by 'pioneer' individuals, and once a suitable host is found and feeding initiated an aggregation pheromone is produced to which individuals of both sexes respond (Landolt 1997).

Some care needs be taken before these conclusions can be accepted. *Lilioceris lilii* are polygamous (Nolte 1939) and it is possible that the behavioural effect of odour on individuals changes after contact with the opposite sex, as is the case with *Leptinotarsa decemlineata* (Dickens 2007). These tests also highlight the challenge of working with a beetle that cannot practically be sexed without dissection, sexing the beetles would enable experimental designs that would have facilitated clearer conclusions on the behaviours and responses of the sexes, particularly concerning evidence for the presence of a pheromone.

Identification of some of the volatile chemicals, which may at least in part elicit the behavioural responses observed in LTO bioassays, was achieved, using volatile collection (volatile entrainment) from *L. regale* plants infested with *L. lilii* and identification by electrophysiological techniques (Electroantennography, EAG), Gas Chromatography and Mass spectrometry (Chapter 5). The identity of six compounds (benzaldehyde, 6-methyl-5-hepten-2-one, β-pinene, nonanal, linalool and methyl

salicylate) present in entrainment samples and one further plant volatile (*cis*-jasmone) were found to elicit antennal responses in *L. lilii*. *cis*-Jasmone was not identified as present in the headspace volatiles of *L. regale*, highlighting that there may be additional compounds that elicit responses from *L. lilii* antennae which were not identified as present in entrainment samples and further volatile collection and identification is required.

Six of the seven EAG-active compounds were tested in LTO bioassays with diapaused beetles. One compound elicited a behavioural response at the concentration tested; females moving into a clean air odour stream in preference to one containing *cis*-jasmone. It would be premature to conclude that this chemical elicits this response in the field, or relate to the odour-mediated behaviours observed in olfactometer tests that used plant material and beetles as a stimulus. There may be a lack of response or an atypical behavioural response with unnaturally high or low concentrations of a compound. For example, subtle alterations in the concentration of some green leaf volatiles (GLV) can switch off the behavioural responses of *L. decemlineata* (Visser and Avé 1978). Therefore it is possible that compounds which gave no significant response in the bioassays will elicit a response if tested at other concentrations. In addition the use of bioassays with single compounds may provide a misleading picture of the actual situation, and single compounds may not result in a behavioural response whereas a mixture might (Ma and Visser 1972). Additional volatile entrainments are required so that an estimate of the ratios of the electrophysiologically-active volatile components in the headspace of *L. regale* can be achieved. This would enable the testing of artificial lily odour in olfactometer experiments. It is also feasible that testing the same volatiles with beetles at different physiological states would provide different responses; LTO experiments showed differences in the odour-mediated behaviour of reproductive and non-reproductive *L. lilii*.

The entrainment samples were collected from *L. regale* infested with *L. lilii* and it was not possible to be certain of the source of the identified compounds (plant or insect). However, the identified compounds are known plant-produced volatile chemicals, and some are produced in greater quantities following herbivore damage (see Dickens 1999, Peacock et al. 2001b). It is generally thought that odour-mediated host location can be due to compounds that are plant-specific but more often specificity is achieved by a particular ratio of volatiles, the constituent compounds of which may be produced by many plant species (Visser 1986, Bruce et al. 2005). The behavioural response of *L. lilii* to *cis*-jasmone is of particular interest. This compound is a herbivore-induced plant volatile that can repel insect attack and act as an indirect defensive mechanism by attracting predatory and parasitic insects that prey on herbivores (Bruce

et al. 2003), this may be the first record of a chrysomelid having a behavioural response to this compound.

One species of host plant was used in these investigations (*L. regale*), however although the literature and a field trial indicate that all *Lilium* are potential hosts of *L. lilii* and the beetle is also able to develop on *Fritillaria* and *Cardiocrinum.* However, *Lilium* vary in susceptibility to the beetle (section 6.2), and it is feasible that this variation is odour-mediated, but behavioural bioassays and electrophysiological investigations are necessary to determine if this is the case. Other factors that can affect host choice include nutritional components, the presence of secondary structures on leaves, and morphological characteristics such as leaf colour and structure of the plant (see Fernandez and Hilker 2007), so these should also be investigated as potential causes of variation in host susceptibility.

An unsuccessful attempt was made to extract a sex-specific volatile compound (potential pheromone), which the results of behavioural bioassays had indicated as present, but further work will be required to isolate and identify such a compound.

6.4. OBJECTIVE 3: IN CONJUNCTION WITH SURVEY-BASED DATA, TO ASSESS THE RISK TO THE LILY INDUSTRY RELATING TO POT PLANTS, CUT FLOWERS AND OUTDOOR BULB PRODUCTION

The assessment of the risk that lily beetle poses to the UK lily industry was primarily made by circulating a postal survey to lily growers (Chapter 2) with additional information gained from RHS members and the literature (Chapter 1) as well as from other aspects of the project. Confined to south east England between 1939 and 1989 *L. lilii* it is now found throughout England and Wales and has become established in Scotland and Northern Ireland. The beetle is continuing to spread and may in future occur everywhere its host plants are grown in the UK (and possibly the rest of the world). Results from the survey and the field trial (Chapter 3) indicate that all *Lilium* are at risk from *L. lilii*, and that it can infest plants under any growing regime. In commercial production *L. lilii* infestations are likely to increase insecticide use and production costs. However, the surveys indicated an increase in sales of lilies despite many *Lilium* users stating that they would stop growing the plants and so the current and likely future economic impact of the beetle remains unclear. There is also a theoretical risk to the native *Lilium* of North America and the rare native *Fritillaria meleagris* of England (Sutton 2004, Ernst et al. 2007), which requires further investigation.

6.5. AIM: BASED ON OUR UNDERSTANDING OF THE BIOLOGY, ECOLOGY AND CHEMICAL ECOLOGY DEVELOP INTEGRATED PEST MANAGEMENT STRATEGIES FOR CONTROL OF *L. LILII*.

A review of the literature and the results of the postal survey (Chapters 1 and 2) indicate that current management options (manual removal or use of broad spectrum insecticides) for *L. lilii* are unsatisfactory. Biological control with parasitoids alone is unlikely to be achievable in the UK; despite two parasitoids being present, *L. lilii* continues to be a problem and the deliberate introduction of further natural enemies to the UK is unlikely to be practical due to the requirement for extensive quarantine testing to satisfy a plethora of government advisory bodies (see HMSO, 1981 and section 1.11).

An alternative pest management approach would be to manipulate the behaviour of *L. lilii* by using semiochemicals for example by placing semiochemical-baited traps at glasshouse entry points to monitor or prevent beetle invasion of commercial production systems. Other management strategies involving semiochemicals include mass removal trapping, attract-annihilate, behavioural disruption, the use of antifeedants and the attraction of natural enemies (see Foster and Harris 1997, section 4.1.3). Within the Chrysomelidae some success has been achieved with *D. u. howardi* using bait containing insecticide, a cucurbitacin feeding stimulant and several floral volatiles in fields of melon, which gave comparable yield increases to pesticide use alone (Brust and Foster 1995) and the use of an attract-annihilate strategy, based on host kairomones and a pyrethroid insecticide has shown the potential to reduce the commercial application rate of pesticide against *L. decemlineata* larvae (Martel et al. 2007).

Studies with *Diabrotica* spp. highlight the need for detailed knowledge of the behaviour and ecology of a pest and its hosts if semiochemical-based management strategies are to be successful. Temporal and spatial placement of baits and traps is critical and dependent on the species concerned, as is colour of the lures: for example adult *Diabrotica* spp. are more attracted to yellow traps than to other colours (Hoffmann et al. 1996, Metcalf et al. 1998, Jackson et al. 2005). In this respect progress has been made in the development of semiochemical-based strategies against *L. lilii*. Laboratory rearing and phenology observations have added further to our knowledge of the beetle's univoltine life cycle and host preferences (section 6.2). Behavioural bioassays have provided insights into the odour-mediated behaviour of *L. lilii* and the process of identifying key volatile chemicals that may elicit the observed behaviours has been initiated (Chapters 4 and 5). Variations in the susceptibility of *Lilium* have been demonstrated and this may be utilised in management of *L. lilii*; for example using more

susceptible varieties as trap crops to which control measures can be targeted. The effectiveness of a trap crop may be enhanced by the addition of a semiochemical, for example the use of the male-produced aggregation pheromone of *L. decemlineata* to draw beetles to a point source (e.g. trap crop) has been demonstrated (Kuhar et al. 2006); it is likely that a pheromone is produced by one or both sexes of *L. lilii* (see section 6.3).

Much additional work is required before a semiochemical-based control programme against *L. lilii* can be instigated. However, the results of these studies indicate that it should be possible to develop a synthetic blend of compounds to which *L. lilii* respond. Further investigation is more likely to be successful if combined with research into the chemical ecology of the beetle's parasitoids (see Chapter 1), which may lead to methods of control utilising semiochemical-based methodologies and reduced reliance on synthetic pesticides.

6.6. CONCLUSION

This project has made significant progress in confirming the univoltine life cycle and limited host range of *L. lilii*; reports of multivoltine life cycle and wide host range should be discounted. Progress has been made in identifying the behaviours of the beetle that may be mediated by host and conspecific odour. Some identification of the volatile chemicals that may mediate these behaviours has been achieved. These studies indicate that the odour-mediated responses of *L. lilii* are consistent with other chrysomelids that produce a male aggregation pheromone to which reproductive individuals of both sexes respond, although sex-specific volatiles have not yet been isolated from lily beetles. Whilst it is clear that further research is required into the chemical ecology of *L. lilii* and its natural enemies it has been demonstrated that it should be possible to develop a semiochemical-based control system for this beetle as an alternative to broad spectrum insecticides.

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A.1. Lily beetle fact sheet, sent with surveys

Lily beetle survey 2006 - Fact sheet

The lily beetle (*Lilioceris lilii*) can be a serious pest of lilies (*Lilium* and *Cardiocrinum*) and fritillaries (*Fritillaria*). Both adults and larvae cause damage, primarily by defoliation, but heavy infestations can damage flowers, seed capsules and stems. The beetle became established in England during the 1940s and until the early 1990s was largely confined to Surrey. However, over the past 15 years the beetle has spread rapidly and is now found in every English county from Yorkshire southwards, has become widespread in Wales and is established in Glasgow and Belfast. Despite its increasing occurrence very little scientific work has been carried out on the beetle, and the current and future impact on the *Lilium* industry in the UK has not been assessed.

The adult beetle (Fig. 1) is 8 mm long, bright red with a black head and legs. The fully grown larvae (Fig. 2) are 8-10 mm long, dirty orange-red with a black head, but they are normally covered by their own slimy black excrement and could be mistaken for birds' droppings. Adult beetles are active from late March through to October, larvae are found between May and September.

At present management of this pest relies on chemicals or hand picking, however the long period over which the beetle is active can make this difficult. Adequate control may only be gained if measures are repeated regularly in areas where the pest is abundant.

Research joint funded by the HDC and RHS. A three year Ph.D. research project is being undertaken which could pave the way for improved management of the lily beetle. Part of the project is investigating the chemical ecology of the beetle to get an understanding of, among other things, how it is able to locate lilies when they are planted together with a range of other plants in the garden. A field trial is also underway, nearly 1000 lily bulbs representing six lily groups have been planted to investigate whether any display resistance to the beetle. The work is being done in collaboration with Rothamsted Research in Hertfordshire and Imperial College London.

How you can help. One of the primary aims of the project is to ascertain the risk that the lily beetle poses to the lily and fritillary industry in the UK. A large part of this assessment will be made using results from the attached survey, therefore the more growers that fill in the survey, the more accurate and useful this assessment will be.

Fig 1. Adult lily beetle

Fig 2. Lily beetle grubs on lily

A.2. Survey form sent to lily providers

Lily beetle risk assessment Survey 2006

Section A

1 Contact details

Your name/ nursery	
Address	
Post code	
Telephone	
E-mail	

Section B – The lily beetle

2a Have you ever had a problem with lily beetle? If so, please state what was done.

……...

……..

2b Have you ever had enquiries or complaints from customers about the lily beetle? If possible please give details.

……..

2c To your knowledge, is lily beetle present in gardens locally (within 5 miles)? ……...

Yes	**No**

2d A number of those who contacted the RHS for advice on lily beetle in 2005 will not purchase any new lilies or fritillaries for their garden due to the beetle problem. In light of this information and the other information provided with this survey, what impact do you think the beetle may have on the lily or fritillary part of your business?

Factor	**Please tick one**	**Additional notes**
Increase sales		
No effect		
Small decrease in sales		
Significant decrease in sales		
Make lily /fritillary growing unviable		

Lily beetle risk assessment survey 2006

3 Please list the *Lilium/ Fritillaria/ Cardiocrinum* that you supply

……..

……..

Section C – Plant production methods

4a Are you a producer, retailer or wholesaler of *Lilium*/ *Fritillaria*/ *Cardiocrinum*? Please tick all appropriate

Producer (including growing on)	**Retailer**	**Wholesaler**

If retail or wholesale only please go to question 5.

4b Where do you source new stock? Please tick all appropriate

Propagation	**Purchased externally**

If only purchase externally please go to question 4e.

4c How do you propagate? Please tick all appropriate

Seed	**Bulb division**	**Bulb scales**	**Stem bulbils**	**Tissue culture**	**Other** (please specify)

4d Are you breeding new varieties/ cultivars?

Yes	**No**

……

4e What is your current growing regime? Please tick all appropriate

Under glass (no artificial heat/ light)	**Under glass** (with heat/ light)	**Outdoors**	**In pots**	**In open ground**	**Other** (please specify)

Section D – Source of stock

5a Where do you source new stock? Please tick all appropriate

UK	**Overseas** (please list country(ies) of origin)

5b What stage of plants do you source? Please tick all appropriate

Seed	**Bulbs**	**Plants in pots**	**Other** (please specify)

Section E – Plant sales

6a At what stage do you sell plants? Please tick all appropriate

Seed	**Bulbs**	**Plants in pots**	**Cut flowers**	**Other** (please specify)

6b What is your approximate turnover of plants (number of bulbs, seeds or stems) each year?

……..

6c Has this volume been increasing or decreasing over the past five years?

……..

6d Who do you sell plants to? Please tick all appropriate

Large retailers (eg B&Q)	**Small retailers** (independent plant centres)	**On site** (open to the public)	**Mail order / internet**	**Other** (please specify)

Lily beetle risk assessment survey 2006

Section F – Pest and disease control

7a Have you had any pest or disease problems? Please tick all appropriate

Pests:

Aphids	**Thrips**	**Vine weevil**	**Other** (please specify)

Diseases:

Bulb rots	**Virus**	**Other** (please specify)

7b What chemicals are used in production / storage? Please list

Chemical	**Product name**
Pesticides	
Fungicides	
Fertilisers	
Other	

7c What other pest / disease control strategies are used? Please list

……..

……..

Completion and return of this form

Please add any other comments or information which you consider important

.....

.....

Please check that you've completed all sections and return in the pre-paid envelope provided to the RHS Wisley by Friday 10 March 2006.

A.3. Survey form sent to gardens open to the public

Lily beetle risk assessment survey 2006

Once completed please return to Andrew Salisbury, Entomology Laboratory, **Freepost**, RHS Garden Wisley, Woking, Surrey, GU23 6BR in the envelope provided.

Contact name:
Address:
Post code:
Tel:
E-mail:
Date:

1a.	**Are *Lilium/ Fritillaria/ Cardiocrinum* grown in the garden?** Yes No If no please go to question 5
1b.	Approximately how many varieties and bulbs of lilies/ fritillaries are grown. **<10** **10-50** **50-100** **100+**

2.	**The lily beetle.**
2a.	**Have you ever had a problem with lily beetle?** **Yes** **No** (Go to question 2c.)
2b.	**What control measures were taken against lily beetle.** (Please circle all appropriate). **None** **Manual removal** **Pesticide** (Please specify product used) Other **(Please specify)**
2c.	**To your knowledge, is lily beetle present in other gardens locally (within 5 miles)?**
2d.	If lily beetle becomes a problem (or is already a problem) what effect will this have on lily/ fritillary use in the garden. **Please circle.** No change **(Plants will be replaced as necessary or as new planting demands)** ***More lilies/ fritillaries will be planted***

Fewer lilies/ fritillaries will be planted

Lilies/ fritillaries will no longer be planted in the garden

Other (Please specify)

3	Lily growing
3a.	**Where do you source new stocks of lilies/ fritillaries for the garden?** Please circle all appropriate. Own propagation Purchased wholesale/ direct from grower (UK) ***Imported from outside UK*** ***Purchased from retail outlets*** ***Other*** *(Please specify)*
3b.	**What stage of plants do you source?** Please circle all appropriate. **Bulbs** **Plants in pots** **Seed** **Other** (Please specify)
3c.	**How do you grow lilies/ fritillaries?** Please circle all appropriate. **Under glass** (no artificial heat/ light) **Under glass** (with heat/ light) ***Outdoors*** ***In pots*** ***In open ground*** **Other** (Please specify)

4.	**Pest and disease control**
4a.	**Have you had any pest or disease problems other than lily beetle on lilies/ fritillaries?** Please circle all appropriate. ***Pests:*** ***Aphids*** ***Thrips*** ***Vine weevil*** ***Slugs*** **Other** (Please specify) **Diseases:** **Bulb rots** **Virus** **Lily disease** **Other** (Please specify)
4b.	**Are there any chemical inputs into lily/ fritillary growing?** Please list. **None** **Pesticides:** **Fungicides:** **Fertilizers:** **Other:**

5.	**Plant sales**
5a.	**Do you have a plant centre on site?** (If no go to question 6)
5b.	**Does the plant centre sell lilies/ fritillaries?** If so please circle all appropriate stages sold. No Bulbs Plants in pots Seed Cut flowers **Other** (please specify)
5c.	**Has the volume of sales of lilies/ fritillaries been increasing or decreasing over the past five years?**
5d.	Have plant sales staff received queries about the lily beetle from customers?

6.	**Please add any other comments or information which you consider important**

Please check that you've completed all sections and return in the pre-paid envelope provided to the RHS Wisley by Friday 10 March 2006.

Thank you for your co-operation.

Andrew Salisbury
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