Overwintering sites of ladybirds, shieldbugs and allied species in Hertfordshire woodland: how important is aspect?

Joe Gray and John R. Healey

About this article
The fieldwork on which this article is based was conducted by Joe Gray for the dissertation component of his part-time MSc in Forestry with Bangor University. The dissertation was supervised by John Healey, Professor of Forest Sciences, Bangor University.

Introduction

For insects that live in temperate environments, hibernal conditions can limit continuous reproduction and normal metabolic functions (Leather *et al.*, 1993). Many species thus possess a suite of mechanisms unique to the winter period. These not only reduce the risk of cold injury but also obviate the danger of starvation (a significant cause of mortality in winter-active insects). Furthermore, overwintering strategies that involve moving to a less open microhabitat and entering a state of dormancy may provide the further benefit of a reduced risk of detection by winter-active predators. This must be balanced, however, against the increased vulnerability, if found.

Overwintering adaptations in insects

Broadly speaking, the mechanisms involved in insect overwintering can be grouped into those that are behavioural and those that are physiological, although many insects will employ the two in tandem. A key behavioural mechanism exhibited by many overwintering insects is the selection of a microhabitat in which the full effect of the adverse conditions will not be experienced. Soil, litter, and plant tissue all act as insulators, and insects may thus burrow or oviposit in these materials (Gillott, 2005). Other insects may crawl under stones or move into dense grass tussocks, hedgerows, or small hollows (Leather *et al.*, 1993). These behaviours are thought to be controlled by negative phototaxis (movement away from light), positive thigmotaxis (movement in response to a touch stimulus), or a combination of the two (Leather *et al.*, 1993).

Turning to physiology, during the winter period many insects enter diapause, a phenomenon in which physiological systems become largely inactive (Gillott, 2005). Diapause is more elaborate than other forms of insect hibernation (namely, quiescence, which is a response to a sudden deviation in conditions, and oligopause, which is a fixed period of dormancy in response to a cyclic climatic change). Not only does diapause involve a preparatory phase triggered by a temperature-independent factor (*e.g.* decreasing day-length), in which the insect undergoes metabolic changes and ceases feeding, but, additionally, the state of dormancy does not instantly terminate when more favourable conditions return (Leather *et al.*, 1993).

In evolutionary terms, one explanation for diapause is that 'natural selection has favoured the development of a safety margin against prematurely unseasonal conditions' (Gillott, 2005, p.668). A further fitness advantage provided by diapause is that the preparatory phase gives insects time to substantially reduce their vulnerability to cold damage. When insects freeze, the physical disruption of cell contents, coupled with the disturbance of enzymatic activity through dehydration, can result in mortality. To avoid this, there are two main physiological defences used by insects in environments with sub-zero temperatures (Gillott, 2005):

- 'Freezing-intolerant' insects reduce the freezing point in their body fluid using antifreeze proteins, as well as low-molecular-weight polyhydroxyl substances such as glycerol. In one remarkable example, the overwintering larvae of *Bracon cephi*, a parasitic wasp, are able to maintain haemolymph (the insect equivalent of blood) in a liquid state down to -47°C.
- 'Freezing-tolerant' insects can experience freezing of extracellular body fluids without damage to their cellular content through the deployment of ice-nucleating proteins (the formation of ice in the extracellular fluid is accompanied by heat release and thus reduces the rate at which body tissue cools).

It is thought that freezing-intolerant insects are the predominant type in the UK's temperate climate (Leather *et al.*, 1993). While the individual species for which freeze-protection mechanisms have been documented form a very small minority of all insects, there is at least some published literature relevant to insects that overwinter as adults in the UK. Taking the shieldbugs and allies as an example, there are at least four species for which freeze-protection mechanisms have been partially described. Bashan and Cakmak (2005) found that the Hairy Shieldbug *Dolycoris baccarum* and the Gorse Shieldbug *Piezodorus lituratus* underwent changes in fatty acid composition during the winter, an observation also made by Hodkova *et al.* (1999) for the Fire Bug *Pyrrhocoris apterus*. The Birch Shieldbug *Elasmostethus interstinctus*, on the other hand, was shown by Duman *et al.* (2004) to be able to synthesise antifreeze proteins.

Another physiological mechanism employed by overwintering insects is the development of a darker body colour, which has at least three potential advantages (Leather *et al.*, 1993): it allows more radiation to be absorbed during the winter; it enables a more rapid warming, and thus a quicker return to an active state, in spring; and it reduces detectability by predators that hunt by sight, such as birds.

Overwintering in woodland habitat

For those insects that use behavioural mechanisms, it is well established that woodlands offer sheltered overwintering microhabitats with protection from wind and predation, and that these can be important factors in survival (Leather *et al.*, 1993). For instance, decayed stumps (Baust, 1976), as well as logs and leaf litter (Holmquist, 1931), have been shown to offer insulation that can dampen the oscillations in ambient temperature.

Another cause of variation in conditions between different woodland microhabitats is the full or partial shade cast by habitat elements such as tree trunks and evergreen foliage. According to Holmquist (1931, p. 398), 'differences as great as 25°C may exist between temperatures of the north and south side of trees.' The question thus arises as to whether hibernating insects would have a preference for south-facing over north-facing hibernacula as a strategy to keep body temperature higher through the increased exposure to solar radiation and thus maximise winter survival. The reduced humidity of warmer microsites might also be associated with a lower burden of fungal infections and a lesser likelihood of a wet body surface, which would lead to internal freezing at a higher temperature (Raak-van den Berg *et al.*, 2012). A potential preference for the south-facing side of trees for hibernacula is consistent with empirical evidence from non-woodland settings for ladybirds (Takahashi, 1993; Raak-van den Berg *et al.*, 2012).

The present study explores the question of orientational preference in a woodland setting. The research focuses on two groups of insects for which all, or at least the majority of, species overwinter as adults and for which identification to species level in the field is mostly straightforward: ladybirds (beetle species in the family Coccinellidae); and shieldbugs and allies (species in the superfamilies Coreoidea, Rhopalidea, and Pentatomoidea). The UK Ladybird Survey has compiled a list of overwintering sites (hibernacula) for many of the more conspicuous species of Coccinellid (www.ladybird-survey.org/habitat.aspx; a listing adapted from Majerus and Kearns [1989]). However, no equivalent list exists for shieldbugs and allies.

Among the major biological texts that have been published on the UK's shieldbugs and allies, only Southwood and Leston (1959) and, to a lesser extent, Butler (1923) provided detailed coverage of overwintering. In contrast, Douglas and Scott (1865) and Saunders (1892) gave no information on the topic, Dolling (1991) covered it only broadly and briefly, and Kirby (1992), while offering some useful general information, provided species-level detail for just the small number of scarce and threatened insects that formed the main subject of his report. Nevertheless, it is possible from the existing literature to build up a partial picture of how the shieldbugs and allied insects use woodland for overwintering. The Parent Bug *Elasmucha grisea* has been described as using hibernacula within Birch woodland, the Pied Shieldbug *Tritomegas bicolor* as favouring sites near woodland, the Bishop's Mitre Shieldbug *Aelia acuminata* as preferring sites sheltered by woodland, and the Bronze Shieldbug *Troilus luridus* as always overwintering within or near woodland (Southwood and Leston, 1959). Of particular interest in that list is *A. acuminata*, which spends at least the summer part of its life-cycle in grassland away from trees. Another grassland species that has been described as moving into woodland to overwinter is the Scarce Tortoise Shieldbug *Eurygaster maura* (Kirby, 1992). In terms of the microhabitats used by shieldbugs and allies, a general observation made by Butler (1923) was the importance of the refuge provided by evergreen conifers during the winter, while Kirby (1992) observed that leaf litter and bark may be useful hibernacula. A more detailed, but non-exhaustive, list of probable hibernacula related to woody plants for different shieldbug and allied species is presented in Table 1. The species in Table 1 are restricted to those present in Hertfordshire (Ryan, 2014; Gray, 2015; Gray, 2016) that are known to overwinter as adults or nymphs.

Aim

The overarching objective of this study was to improve ecological understanding of the microhabitats used by insects that overwinter as adults in semi-natural woodland, with a focus on ladybirds and shieldbugs and allies. A specific aim within this was to investigate if the focal species exhibited a preference for hibernacula that face south with respect to a shade-casting habitat element, as compared with those having a north-facing aspect.

Methods

Study sites

Three woodland sites were selected, based on the following criteria, as assessed from Ordnance Survey maps in the GIS programme ArcMap™ 10.1 (Esri®; Redlands, CA, USA):

- A species mix of deciduous and evergreen trees.
- Sufficient space to run 150-metre transects in north-west, north-east, south-east, and south-west directions from a central point within the woodland interior.

Table 1. *Hibernacula of shieldbugs and allied species present in Hertfordshire*, as identified in the literature search.*

Species	At the base of trees	In bark crevices	In buildings	In conifer foliage	In empty seed-cases or pods	In grass tussocks or among roots	In holly foliage	In ivy	In leaf litter or detritus	In or under moss	In stumps or rotting wood	In the ground	On forbs	Under bark	Under logs and stones
ACANTHOSOMATIDAE															
Acanthosoma haemorrhoidale		X[1]			X[2]	X[1]	X[2]			X[3]	X[3]			X[1,4]	
Cyphostethus tristriatus				X[2]				X[2]							
Elasmostethus interstinctus				X[2,4]				X[1]		X[1]				X[1]	X[2]
Elasmucha grisea									X[1]						X[2]
COREIDAE															
Arenocoris fallenii						X[1]									
Coreus marginatus									X[5]				X[1]		
Coriomeris denticulatus										X[2]					
Gonocerus acuteangulatus				X[2]				X[2]							
Leptoglossus occidentalis			X[6]												
Syromastus rhombeus	X[1]					X[1]									
CYDNIDAE															
Legnotus limbosus												X[1]			
Tritomegas bicolor										X[1,7]		X[1]			X[4,7]
PENTATOMIDAE															
Aelia acuminata				X[4]		X[1,2]				X[2]					
Eysarcoris venustissimus									X[1]		X[2]				
Neottiglossa pusilla				X[4]											
Piezodorus lituratus	X[1]			X[4]	X[1]										
Podops inuncta						X[1,2]			X[2,4]	X[2,4]			X[2]		
Troilus luridus		X[1]		X[4]	X[2]		X[2]		X[4]	X[1,4]					
Zicrona caerulea	X[4]								X[4]	X[4]					X[4]
RHOPALIDAE															
Corizus hyoscyami				X[4]						X[4]					
Rhopalus subrufus									X[2]						
STENOCEPHALIDAE															
Dicranocephalus medius									X[8]					X[8]	

*No details were found in the literature for the following species. Cydnidae: *Sehirus luctuosus*. Pentatomidae: *Dolycoris baccarum, Eurydema oleracea, Nezara viridula, Palomena prasina*. Rhopalidae: *Liorhyssus hyalinus, Rhopalus parumpunctatus, Stictopleurus abutilon, Stictopleurus punctatonervosus*. Scutelleridae: *Eurygaster testudinaria*. Thyreocoridae: *Thyreocoris scarabaeoides*. *Picromerus bidens* (Pentatomidae) is not listed as it typically overwinters as an egg. *Pentatoma rufipes* is described by Southwood and Leston (1959) as overwintering 'on trees'.

[1] Southwood and Leston (1959). [2] Hawkins (2003). [3] Dusoulier and Mouquet (2007). [4] Butler (1923). [5] Hrušková *et al.* (2005). [6] Malumphy *et al.* (2008). [7] Halászfy (1953). [8] Kirby (1992).

- Low slope angle (assessed by measuring contour spacing), in order to keep the comparisons between north- and south-facing aspects as fair as possible.

The three selected woodlands were Balls Wood (which was also used as the site for a pilot survey), Bricket Wood Common, and Mardley Heath Local Nature Reserve. The centre point from which the four 150-metre transects ran was selected with the aims of minimising slope angles and ensuring that none of the transects reached the edge of the woodland (Figure 1), although canopy openings were permitted. In the case of Balls Wood, the transects were also located to avoid trees already sampled during the pilot survey.

Sampling was conducted between 8 January and 5 February 2016. The sequence in which the transects were sampled was randomly ordered for each site.

Sample unit selection

On each field day, a single 150-metre transect was surveyed from the centre-point outwards. A GPS unit was used to find the start and a compass was then used as a directional guide. Any tree or shrub on the transect meeting the criteria, as specified below, was sampled up to the point when the sampling target was reached. The target for each transect was 15 deciduous trees for leaf litter sampling and 15 trees or woody shrubs for evergreen foliage sampling (including evergreen trees and deciduous trees covered in Ivy *Hedera helix*). This gave a maximum of 180 trees for leaf litter sampling and 180 trees or shrubs for foliage sampling.

Trees were selected for leaf litter sampling if they met the following criteria:

- A trunk circumference at 1.3 metres above the ground of at least 0.75 metres (measured with a tape) – equivalent to 0.24 metres diameter at breast height.
- No obvious slope to the ground on which they stood.
- Accessibility of both sides of the tree.
- No marked imbalance in the volume of leaf litter on the north and south sides of the base.
- A distance of at least 5 metres from any previously sampled tree (measured with paces).
- A distance of no more than 15 metres away from the transect line (along a perpendicular and measured with paces).

Trees and shrubs were selected for evergreen foliage sampling if they met the following criteria:

- A height of at least 4 metres for a tree (estimated using a 2-metre measure placed upright at the base of the trunk) or a crown width of at least 2 metres for a shrub.
- Accessibility of both sides of the tree or shrub.
- No marked imbalance in the volume or density of foliage within reach on the north and south sides of the tree or shrub.
- A distance of at least 5 metres from any previously sampled tree or shrub (measured with paces).
- A distance of no more than 15 metres away from the transect line (along a perpendicular and measured with paces).

Trees and shrubs were excluded from the sample if their north and south sides were differentially shaded owing to the location of neighbouring vegetation. If necessary for achieving the sampling goals, and where the size of the woodland permitted this, transects were extended beyond 150 metres.

Sampling of the units

Leaf litter was sampled in one arc of approximately 120° on the northern side and one on the southern side of each tree, with sampling taking place within 0.5 metres of the trunk (Figure 2). Each side was sampled for 4 minutes (measured by a timer), with the order determined by coin flip. Leaves were manually searched at a steady pace, clump by clump working from the middle of the sampling zone towards the bottom.

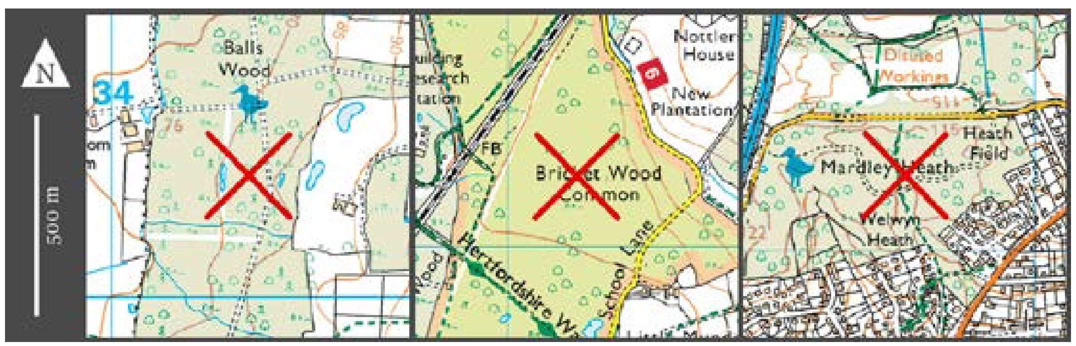

Figure 1. *Transect locations for the three study sites (Balls Wood, left; Bricket Wood Common, middle; Mardley Heath, right). The grid references (British National Grid) of the central point of the transects for each woodland were TL3435010310, TL1318001180, and TL2466018195, respectively. Maps: © Ordnance Survey (GB) and licensed through the EDINA Digimap Service for educational purposes.*

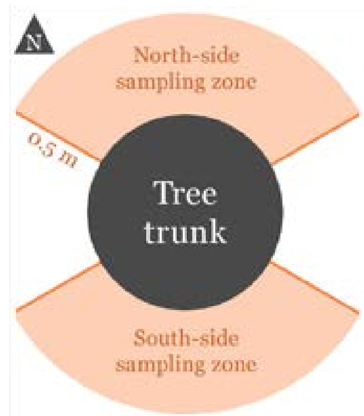

Figure 2. *Diagram indicating the zones for leaf litter sampling.*

edges, with a pale cloth sheet used to aid detection by increasing the contrast with the background (Ausden and Drake, 2006). For each sampling unit, the tree species was recorded along with any insects of interest found.

For foliage sampling, a large cloth sheet (approximately 2 metres by 1.5 metres) was laid out under the foliage being sampled and ten firm blows were administered to the foliage with a 2-metre metal pole, working from the highest reachable point down to the bottom of the foliage (Speight, 2005). The foliage on the northern and southern sides of each tree or shrub was sampled separately, with the order determined by coin flip. For each sampling unit, the tree or shrub species was recorded along with any insects in the target taxa landing on the sheet. After sampling, replacement of leaf litter was carried out as far as was practical in order to minimise disturbance to the habitat.

Data analysis

Several hypotheses were tested, and so in order to reduce the risk of 'type I' error (a false positive) associated with examining multiple questions within the same study, a hierarchy for testing was pre-specified. In this approach, which is becoming increasingly popular in medical research, the order of testing for the hypotheses is pre-stated, and as soon as one fails to reach significance, the testing stops and all hypotheses below it in the hierarchy are automatically rendered non-significant.

The testing hierarchy for this study was designed on ecological grounds. Firstly, since tree trunks provide absolute shade while evergreen foliage only provides partial shade (and deciduous crowns very little in the winter), the leaf litter hypotheses were placed at the top of the hierarchy and the foliage hypotheses at the bottom. A further division of hypotheses was based on the expected number of non-zero samples, using data from the pilot phase. Since the Orange Ladybird *Halyza 16-guttata* was the dominant species in the pilot, especially from the foliage sampling, it was included in a species-level hypothesis as well as within the two hypotheses evaluating all ladybirds. The final hierarchy of hypotheses (expressed in the null form) was as follows, with this order reflected in the results section:

- Ladybirds overwintering in leaf litter at the base of deciduous trees do not exhibit a preference for a south-facing aspect over a north-facing one.
- Shieldbugs and allies overwintering in leaf litter at the base of deciduous trees do not exhibit a preference for a south-facing aspect over a north-facing one.
- Ladybirds overwintering in evergreen foliage do not exhibit a preference for a south-facing aspect over a north-facing one.
- Orange Ladybirds overwintering in evergreen foliage do not exhibit a preference for a south-facing aspect over a north-facing one.
- Shieldbugs and allies overwintering in evergreen foliage do not exhibit a preference for a south-facing aspect over a north-facing one.

For each of these hypotheses, one-tailed Wilcoxon matched-pairs testing was performed. A result was considered statistically significant if its *P*-value, and all others above it in the hierarchy, were less than 0.05. For a *post hoc* test of whether the Birch Shieldbug showed a preference for leaf litter of its foodplants, Chi-squared testing was conducted. All statistical analysis was carried out using R 2.6.2 (R Foundation for Statistical Computing).

Results

In total, leaf litter was sampled for 146 trees and evergreen foliage for 112 trees and shrubs. High winds curtailed the sampling (the final transects at Balls Wood and Bricket Wood Common could not be carried out, while the final transect at Mardley Heath was cut short). Furthermore, the limited nature of suitable sampling units for evergreen foliage, especially at Bricket Wood Common, hindered attainment of the target number of samples. Overall, 278 insects were found during the main study period. Few of these showed instant activity but most showed signs of life within a minute.

Among the species of tree for which at least 10 trees were sampled, Beech *Fagus sylvatica* (n=15) supported the greatest number of overwintering insects, with a mean of 0.87 insects per tree found in the leaf litter sampled. Hornbeam *Carpinus betulus* (0.82, n=34) and Birch *Betula* sp. (0.81, n=31) had similar levels, while Oak *Quercus* sp. had the lowest (0.61, *n*=56). No statistical testing for difference between tree species was conducted as environmental covariates (*e.g.* litter moisture) were not captured, and so the possibility of strong confounding was high. The equivalent values are not presented for evergreen foliage as high variability in the volume and thickness of foliage meant that sampling effort differed substantially between species of tree and shrub.

Leaf litter insects

The leaf litter sampling yielded 27 ladybirds from northern aspects and 46 from southern aspects (70% more on the southern side). The comparison of paired values revealed that the difference was statistically significant (*P*=0.026). The maximum number of ladybirds found within the sampled leaf litter for any tree was three (two triplets were found under Oak and one under Sweet Chestnut *Castanea sativa*). No one ladybird species was dominant (Figure 3). Table 2 provides the range of hibernacula in which each ladybird species was found.

For shieldbugs, the sampling yielded 10 individuals from northern aspects and 25 from southern aspects (150% more on the southern side, *P*=0.007). The maximum number of shieldbugs found for any tree was three (a triplet under Oak and a triplet under Birch). Two species were dominant (Figure 3): the Birch Shieldbug (51%) and the Hawthorn Shieldbug *Acanthosoma haemorrhoidale* (31%). Table 3 shows the range of hibernacula in which each shieldbug species was found.

Evergreen foliage insects

The evergreen foliage sampling yielded 60 ladybirds from northern aspects and 87 from southern aspects (45% greater), a difference that was statistically significant when the paired data were analysed (*P*=0.024). The large majority (88%) of these were Orange Ladybirds. For this species, there were 52 and 77 from northern and southern aspects, respectively (*P*=0.021). Two trees among the 112 sampled had a particularly high abundance of overwintering ladybirds: a Douglas Fir *Pseudotsuga menziesii*, with 22 insects, and a Holly *Ilex aquifolium* tree with 19 (in both cases all were Orange Ladybirds).

Only 23 shieldbugs were found during the foliage sampling, including 10 Hawthorn Shieldbugs (43% of the total) and six Bronze Shieldbugs (26%). Only one tree yielded more than a solitary bug (a Holly with two). The difference between aspects was small (10 from northern and 13 from southern aspects) and non-significant (*P*=0.263). Thus the fifth and final null hypothesis in the hierarchy could not be rejected, although it should be noted that the test was underpowered statistically.

Post hoc analysis

A *post hoc* analysis was conducted to explore if the Birch Shieldbug showed a preference for the leaf litter of its foodplants. Birch was the only one of its foodplants (as listed on http://britishbugs.org.uk/) that was represented within the sampling. Such a relationship might just be a result of proximity, if the insect entered the leaf litter from a foodplant, but it could also be

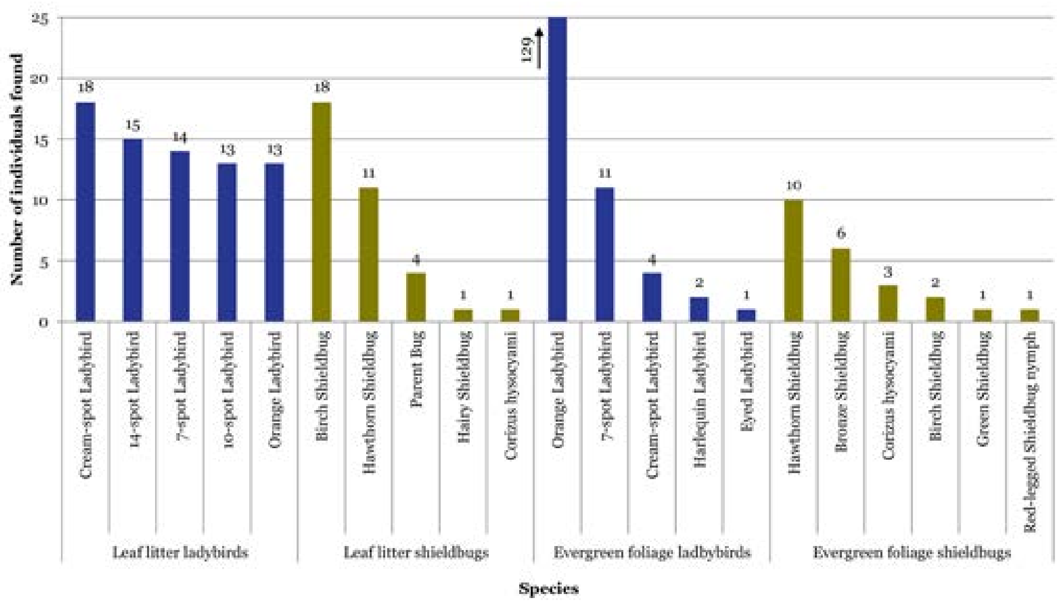

Figure 3. *Counts of individuals found per species, by microhabitat, during the main phase of study. Ladybirds are shown as blue bars and shieldbugs as green bars.*

Table 2. *Species of ladybird found in the study's pilot phase (Balls Wood only) or main phase (three sites) in Hertfordshire, along with their associated hibernacula.*

Species	Hibernacula*	Sites
7-spot Ladybird *Coccinella 7-punctata*	In base of grass clump In conifer foliage (Norway Spruce, Yew) **In leaf litter** On Gorse On Holly On sedge	BW, BWC, MH
10-spot Ladybird *Adalia 10-punctata*	**In conifer foliage** (Norway Spruce) In leaf litter	BW, BWC, MH
14-spot Ladybird *Propylea 14-punctata*	In leaf litter	BW, BWC, MH
Cream-spot Ladybird *Calvia 14-guttata*	**In conifer foliage** (Douglas Fir, Yew) In leaf litter **Inside standing dead wood** **On Holly** **On Ivy**	BW, BWC, MH
Eyed Ladybird *Anatis ocellata*	**In conifer foliage** (Norway Spruce) **On Holly**	BW, MH
Harlequin Ladybird *Harmonia axyridis*	**In conifer foliage** (Douglas Fir, Norway Spruce)	BW
Orange Ladybird *Halyza 16-guttata*	**In base of grass clump** **In conifer foliage** (Douglas Fir, Norway Spruce, Yew) In leaf litter **Inside fallen dead wood** On Cherry Laurel **On Ivy** **On Gorse** On Holly **On sedge**	BWC, BW, MH
Pine Ladybird *Exochomus 4-pustulatus*	In conifer foliage (Douglas Fir)	BW

*Emboldened hibernacula are those not listed on the website of the UK Ladybird Survey (www.ladybird-survey.org/habitat.aspx; a listing adapted from Majerus and Kearns [1989]).

BW=Balls Wood. BWC=Bricket Wood Common. MH=Mardley Heath.

advantageous in providing closer access to a suitable food source when emerging from the starved state of overwintering, which might influence shieldbug behaviour to positively select this litter type. While the results revealed a trend towards a foodplant preference for the Birch Shieldbug, this did not reach statistical significance (*P*=0.11). The test had limited statistical power, and so it is not possible to draw a firm conclusion from this. No other insects with strong foodplant preferences occurred in sufficient numbers to test.

Other findings

An interesting ecological question that can be asked about overwintering adult insects concerns the level of predation. While it is not possible to estimate predation rates from the sampling method that was applied here (any insects consumed would, of course, have been missed), it is interesting to note that the overall level of predation damage observed was very low. Of the 281 insects for which at least a complete abdominal section was found, 278 appeared to be fully intact and were counted in this study. The exceptions were all shieldbugs in leaf litter: a Parent Bug and a Hawthorn Shieldbug with their heads removed; and a Green Shieldbug missing its legs, antennae and the tip of its abdomen. Several ladybird forewings were found in the leaf litter but no effort was made to identify them to species level.

While the study focused on ladybirds, shieldbugs, and allies, some other records were made during the course of the fieldwork, especially during the pilot survey. Other true bugs that were found are listed in

Table 3. *Shieldbugs and allied species found in the study's pilot phase (Balls Wood only) or main phase (three sites) in Hertfordshire, along with the associated hibernacula.*

Species	Hibernacula*	Sites
Birch Shieldbug *Elasmostethus interstinctus*	In conifer foliage (Douglas Fir) **In leaf litter** On Holly On Ivy	BW, BWC, MH
Blue Shieldbug *Zicrona caerulea*	In leaf litter	BW
Bronze Shieldbug *Troilus luridus*	In conifer foliage (Norway Spruce) **In senescent Oak foliage** On Holly **On Ivy**	BW, BWC, MH
Common Green Shieldbug *Palomena prasina*	**On Holly**	MH
Corizus hyoscyami	In conifer foliage (Douglas Fir) **On Gorse** **In leaf litter**	BW
Hairy Shieldbug *Dolycoris baccarum*	**In leaf litter**	MH
Hawthorn Shieldbug *Acanthosoma haemorrhoidale*	**In conifer foliage** (Douglas Fir, Norway Spruce, Yew) **In leaf litter** **On Cherry Laurel** **On Gorse** On Holly **On Ivy**	BW, BWC, MH
Parent Bug *Elasmucha grisea*	In leaf litter	BW, MH
Red-legged Shieldbug *Pentatoma rufipes*	In conifer foliage (Yew)†	BW

*Emboldened hibernacula are those not encountered in the literature search (Table 1) for the species in question.
†Early instar.

BW=Balls Wood. BWC=Bricket Wood Common. MH=Mardley Heath.

Table 4. Of these, the most abundant was the Birch Catkin Bug *Kleidocerys resedae*, with approximately 80 individuals found on a single young, understory Douglas Fir, near a mature Birch tree during the pilot. However, its distribution was highly patchy, with only five individuals found during the main study (all in leaf litter with a southern aspect).

It was possible to submit some non-Coccinellid beetle records to the county recorder. A particularly interesting find was a ground beetle *Carabus nemoralis*, which is a species that has become rare in the county. The beetle found appeared to be a gravid female. Finally, a number of records were collected for the 2015–19 Hertfordshire Mammal, Amphibian and Reptile Atlas. These included a Smooth Newt *Triturus vulgaris* overwintering under a log, a Common Frog *Rana temporaria* overwintering in leaf litter, and several Fallow Deer *Dama dama*.

Table 4. *Other true bugs found during the pilot or main phase of the study.*

Species	Microhabitat
Anthocoris nemorum	In leaf litter and among Ash keys
Drymus brunneus	In leaf litter
Drymus sylvaticus	In leaf litter
Kleidocerys resedae	In leaf litter and evergreen foliage
Lygus pratensis	In evergreen foliage
Lygus rugulipennis	In leaf litter, at base of grass clump, and among Ash keys
Nabis ferus	At base of grass clump
Stenodema laevigata	At base of grass clump

Discussion

The study revealed statistically significant preferences of ladybirds and shieldbugs for leaf litter on the south side of trees over that on the north side. It also demonstrated statistically significant preferences of ladybirds, including the Orange Ladybird when considered on its own, for evergreen foliage with a southern aspect over that with a northern aspect. The equivalent analysis of shieldbugs in evergreen foliage was underpowered statistically.

The most plausible explanation for the leaf litter results is that there was a sufficient direct shading effect of trunks within 0.5 metres on their northern side (Figure 2) that the leaf litter on the southern side of trees had higher temperatures due to greater insolation. Similarly for the evergreen foliage, the most plausible explanation is that there was sufficient physical separation between the crowns of the target trees or shrubs and major sources of shade (*e.g.* the crowns of the nearest neighbouring evergreen trees or shrubs) to cause a similar effect of higher temperature (and resulting lower moisture) of the foliage on the southern than the northern side of the sampled crowns. Even in winter the crowns of deciduous, as well as evergreen, trees and shrubs have some shading and sheltering effect, and so the proximity between the sampled trees and the crowns of neighbouring trees in the three studied woodlands (a product of the tree density) is likely to have had a moderating effect on both mechanisms.

The sampling methods employed were minimally destructive, non-expensive, and efficient. An indication of the overall sampling scheme's effectiveness can be gained by comparing the species of Acanthosomatid and Pentatomid bugs (families with the most conspicuous members) that were found over the course of the study (including the pilot) against the list of known species for the 1-km grid squares covered (taken from http://ecoforestry.uk/map-sp.php). Six of seven Pentatomids on the combined species list for the grid squares were found. The exception was the Gorse Shieldbug. Similarly, three of the four Acanthosomatid species were found. The exception here was the Juniper Shieldbug *Cyphostethus tristriatus*. Interestingly, a Juniper Shieldbug was found at Balls Wood by JG during early April 2015, in a Douglas Fir, and it might have been an overwintering insect.

The study yielded a data-set on hibernacula used by insects that overwinter as adults in woodland in Hertfordshire. These findings add to the limited existing literature in this subject area, as reviewed earlier, and will hopefully inspire other naturalists to publish further additions.

Limitations

A southern aspect has the potential to benefit insects only in as much as it offers a favourable microclimate. Therefore, it would be more ecologically informative to examine how hibernacula preference varied with specific microclimate variables (*e.g.* daytime temperature, night-time minimum temperature, and moisture level), rather than using aspect as a surrogate. To do so would require substantially more resources for environmental monitoring, and on a larger sample of trees, than were available for this study. Another improvement would be to link hibernacula conditions to the probability of survival and emergence in the spring. One study that has made inferences about overwintering survival is that of Raak-van den Berg *et al.* (2012) conducted in the Netherlands. They found relatively high winter survival of the Harlequin Ladybird *Harmonia axyridis* for hibernacula with a south-western aspect and also for sheltered sites compared with exposed sites.

Another limitation of the present study was the imperfect standardisation of evergreen sampling effort if the two sides of a tree or shrub had differing foliage densities – a fixed beating effort applied to both sides would have sampled a greater amount of foliage on the denser side. While trees and shrubs with marked imbalances were excluded, no plant is perfectly symmetrical. The leaf litter sampling offered a fairer comparison within each sampling pair (and across pairs), as the amount of litter sampled was standardised by time, but this was still potentially subject to unconscious bias in effort. A superior method would be for one researcher to bag up litter samples and label them with a code and another researcher to examine the contents of each without knowledge of the codes. This method would be more time-consuming and more destructive to the habitat, but if the litter was also being examined for environmental variables such as moisture content, the effort would be justified.

A third consideration relates to the effect of aspect on litter and foliage being potentially stronger where the density of trees and shrubs was lower and where the crowns of adjacent trees had space between them (allowing penetration of skylight and wind). An improvement on the study methods would be to quantify the tree density and crown spacing around each sampling unit for inclusion in a statistical model. Again, though, it would be necessary to assemble a larger data-set for such an analysis.

Finally, it is important to note that some insects are more conspicuous in leaf litter than others. While this does not undermine the validity of the comparison of aspect, caution is needed in interpreting the relative numbers of species found from the leaf litter sampling.

Conclusion

Findings from the study support the hypothesis that insects overwintering as adults in woodland favour hibernacula (both on live evergreen foliage and in leaf litter on the ground) with a beneficial microclimate, as reflected by preference for southern aspects over northern aspects. Future work is recommended to explore the link between hibernaculum preference and the temperature and moisture of the microhabitat, as well as its impact on survival rate.

Full data set

The full data set is available on request by emailing joe@ecoforestry.uk.

Acknowledgements

Thanks are due to Trevor James for confirmation of several ladybird identifications during the pilot phase, as well as for his careful review of this manuscript. Thanks should also go to Peter Kirby for encouraging comments while the study was being conceived.

References

Ausden, M. and Drake, M. (2006). 'Invertebrates'. In: Sutherland, W.J. (ed.) *Ecological Census Techniques: a Handbook*. Cambridge: Cambridge University Press, pp. 214-249.

Bashan, M. and Cakmak, O. (2005). 'Changes in composition of phospholipid and triacylglycerol fatty acids prepared from prediapausing and diapausing individuals of *Dolycoris baccarum* and *Piezodorus lituratus* (Heteroptera: Pentatomidae)'. *Annals of the Entomological Society of America*, 98: 575-579.

Baust, J.G. (1976). 'Temperature buffering in an Arctic microhabitat'. *Annals of the Entomological Society of America*, 69: 117-119.

Butler, E. (1923). *A Biology of the British Hemiptera-Heteroptera*. London: H. F. & G. Witherby.

Dolling, W.R. (1991). *The Hemiptera*. Oxford: Oxford University Press.

Douglas, J.W. and Scott, J. (1865). *The British Hemiptera. Vol. I. Hemiptera-Heteroptera*. London: R. Hardwicke.

Duman, J.G., Bennett, V., Sformo, T., Hochstrasser, R. and Barnes, B.M. (2004). 'Antifreeze proteins in Alaskan insects and spiders'. *Journal of Insect Physiology*, 50: 259-266.

Dusoulier, F. and Mouquet, C. (2007). 'Clé de détermination des Acanthosomatidae Signoret, 1864 du Massif armoricain (Hemiptera, Heteroptera)'. *Invertébrés Armoricains*, 1: 7-13.

Gillott, C. (2005). *Entomology* (3rd edition). Dordrecht, The Netherlands: Springer.

Gray, J. (2015). 'A new species of Lygaeidae for Herts and other updates to the Atlas of the Hemiptera-Heteroptera of the British Isles'. *The Hemipterist*, 2: 46-47.

Gray, J. (2016). 'Some records of Hemiptera-Heteroptera from Hertfordshire (VC20)'. *The Hemipterist*, 3: 56.

Halászfy, E. (1953). 'A synopsis of the Heteroptera of Hungary and the neighbouring areas. I. 1. Brachyplatidae; 2. Cydnidae'. *Annales Historico-Naturales Musei Nationalis Hungarici*, 45: 187-195.

Hawkins, R.D. (2003). *Shieldbugs of Surrey*. Woking: Surrey Wildlife Trust.

Hodkova, M., Simek, P., Ckova, H.Z. and Nováková, O. (1999). 'Seasonal changes in the phospholipids composition in thoracic muscles of a heteropteran, *Pyrrhocoris apterus*'. *Insect Biochemistry and Molecular Biology*, 4: 367-376.

Holmquist, A. M. (1931). 'Studies in arthropod hibernation. III. Temperatures in forest hibernacula. *Ecology*, 12: 387-400.

Hrušková, M., Honěk, A. and Pekár, S. (2005). '*Coreus marginatus* (Heteroptera: Coreidae) as a natural enemy of *Rumex obtusifolius* (Polygonaceae)'. *Acta Oecologica*, 28: 281-287.

Kirby, P. (1992). *A review of the scarce and threatened Hemiptera of Great Britain*. Peterborough: Joint Nature Conservation Committee.

Leather, S.R., Walters, K.F.A. and Bale, J.S. (1993). *The Ecology of Insect Overwintering*. Cambridge: Cambridge University Press.

Majerus, M. and Kearns, P. (1989). *Ladybirds*. Oxford: Richmond Publishing.

Malumphy, C., Botting, J., Bantock, T. and Reid, S. (2008). 'Influx of *Leptoglossus occidentalis* Heidemann (Coreidae) in England'. *Het News*, 12: 7-9.

Raak-van den Berg, C.L., Stam, J.M., de Jong, P.W., Hemerik, L. and van Lenteren, J.C. (2012). 'Winter survival of *Harmonia axyridis* in The Netherlands'. *Biological Control*, 60: 68-76.

Ryan, R.P. (2014). 'The county distribution of the Hemiptera-Heteroptera of the British Isles'. *The Hemipterist*, 1: 38-103.

Saunders, E. (1892). *The Hemiptera Heteroptera of the British Islands*. London: L. Reeve & Co.

Southwood, T.R.E. and Leston, D. (1959). *Land and Water Bugs of the British Isles*. London: Frederick Warne & Co..

Speight, M. (2005). 'Sampling insects from trees: shoots, stems, and trunks'. In: Leather, S.R. (ed) *Insect Sampling in Forest Ecosystems*. Oxford: Blackwell, pp. 77-115.

Takahashi, K. (1993). 'Overwintering and aestivation sites of *Coccinella septempunctata brucki* Mulsant (Coleoptera: Coccinellidae) and its migration to alfalfa fields'. *Applied Entomology and Zoology*, 28: 473-478.