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Development of non-invasive monitoring methods for larvae and adults of the stag beetle, *Lucanus cervus*

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Abstract. 1. The stag beetle, *Lucanus cervus* is Nationally Scarce in the UK, yet no methods exist for monitoring the abundance of adults or presence of the subterranean larvae.

2. Here, we describe the design of an aerial flight interception trap that can be used to catch adults. Various lures were tested and ginger root was found to attract both sexes in equal numbers.

3. Road transect surveys of adults killed by vehicles were found to produce reliable estimates of the total abundance of both sexes in areas up to about 12 km from the survey.

4. A novel use of radial diffusive samplers is described to infer the presence of larvae. Both larvae and adult females produce longifolene, which is highly attractive to males.

5. Larvae produce a characteristic stridulation pattern, which can be recorded and distinguished from sounds produced by other saproxylic beetles that may co-occur with *L. cervus*.

6. We conclude that aerial traps baited with ginger, combined with road transect surveys can be used to monitor population abundance of adults, while detection of longifolene and the characteristic stridulation pattern can be used to reveal larval presence, without destroying their fragile habitat.

Key words. Acoustics, insect traps, longifolene, sound production, transects, volatiles.

Introduction

The stag beetle, *Lucanus cervus* L, is Britain's largest and most striking terrestrial beetle. Males have prominent enlarged mandibles, which seem to be used in courtship battles and mate guarding for the smaller females (Lagarde *et al*., 2005) as well as restraining the female during mating (Harvey & Gange, 2006). The adult flight period is of 4–8 weeks duration, beginning in mid-May, and eggs are laid in the soil close to decaying wood. Larvae feed underground, often up to 1 m deep, on decaying wood of stumps and roots from a wide range of broad-leaved trees and shrubs (Percy *et al*., 2000). The larval stage lasts between 3 and 7 years, with pupation occurring in autumn. The pupal stage lasts a maximum of 6 weeks (Harvey, 2007). Adults eclose, but remain underground until emergence in late spring of the following year. Although anecdotal evidence exists of adults visiting sap runs on trees, it appears that no feeding takes place in the adult stage (Mamonov, 1991; Harvey, 2007).

In the UK, the status of *L. cervus* is classified as 'Nationally Scarce, Category B', as it occurs in < 100 of the 10 km national grid squares (Percy *et al*., 2000). Successive national surveys have indicated that its range in Britain has declined, although some authors have reported increases in local distributions (Bowdrey, 1997; Pratt, 2001). However, no data exist on whether there have been changes in its abundance. It occurs throughout Europe; but evidence from several countries suggests that its range is decreasing and it has become extinct in Denmark (Tochtermann, 1987; van Helsdingen *et al*., 1995). From the conservation point of view, more certain knowledge of the status of this insect is of critical importance, for without such data, it is impossible to adequately service the Biodiversity Action Plan that has been written for the species in the UK or

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conservation plans in other European countries, such as the Netherlands (J. T. Smit & R. F. M. Krekels, unpubl. data).

The uncertainty surrounding its abundance undoubtedly stems from the fact that there is currently no rigorous recording method for monitoring either adult abundance or larval occurrence. Surveys of adult distribution have relied on records from casual observers, but these may always be subject to recorder bias. The lack of survey methods is a reflection of the life history characteristics of the insect; adults are not captured by traditional methods of trapping crepuscular insects, such as light or malaise traps, while the subterranean larvae cannot be found without destruction of the habitat (Harvey, 2007). Furthermore, the fact that adults do not feed means that traps baited with food are also unlikely to be successful. However, following a comprehensive investigation of the biology of the insect, (Harvey, 2007), several novel trapping and recording techniques have been developed and the aim of this paper is to present tests of these methods.

Although the adults do not feed, tests have shown that they do respond positively to the volatiles given off by decaying wood and various fruits (Harvey, 2007). Thus, it is possible that a lure can be developed which attracts both sexes in equal numbers. These need to be placed within an appropriate trapping device, which negates the requirement for constant monitor observation. Such traps are used widely for recording insects, since the only requirement is placement and periodic inspection (Wileyto *et al*., 1994). Here, we describe a flight interception trap which was used in tandem with standard pitfall traps, to accommodate the preferred methods of locomotion of the adult: flight in the male and seeking behaviour on the ground for the female (Harvey, 2007). Pitfall traps are often criticised because they are unquantifiable, as the area from which the insects have been captured is unknown (Woodcock, 2005). Nevertheless, they are widely used and especially so for insects such as carabid beetles (Spence & Niemelä, 1994), whose ground dwelling behaviour resembles that of the female *L. cervus*.

On occasions when females do take flight, this is always at a height of about 1–2 m above ground. This behaviour, coupled with their normal ground dwelling habit renders them particularly susceptible to encounters with road traffic and it has been noted that many beetles are found dead on roads each year (Hawes, 1998; Percy *et al*., 2000). As roads present a form of linear transect, we investigated whether these could be used to monitor beetle numbers, in a manner similar to mammals (Taylor & Goldingay, 2004) or the successful 'butterfly walk' method (New, 1998).

It is critical to develop *in situ* detection methods for larvae, without destroying their habitats. Many of the current methods for detecting saproxylic insects rely on hand searching the habitat (e.g. Ranius & Jansson, 2002; Meggs *et al*., 2003), while Bußler and Müller (2009), suggested using a vacuum cleaner to sample cavities in hollow trees. However, these methods are all liable to disrupt the fragile larval habitat and in the case of the stag beetle are impractical, since the larvae are subterranean. It is known that species of saproxylic insects produce pheromones, to which their predators respond (Svensson *et al*., 2004) and so we hypothesised that if similar volatiles are produced by *L. cervus* larvae, detection of these would indicate larval presence. Here, we describe a novel use of radial diffusive samplers (Radiellos). These consist of a cartridge of absorbent material housed within a porous cylinder which can be placed in a test environment, left for a period of volatile collection, then removed and examined by gas chromatography after thermal desorption or solvent extraction. They are used extensively to monitor environmental pollution (e.g. Bruno *et al*., 2007, 2008), but have, to our knowledge never been used for insect detection.

Many animals, including insects, produce sounds either to communicate with their conspecifics (non-incidental sounds) or as a result of eating, moving or flying (incidental sounds) (Chesmore & Ohya, 2004). The former have been used both to monitor and identify species, but the process is time and labour intensive. Advances in computer technology have allowed systems to be developed that can be used to measure the incidental sounds made by animals. The advantage of such technology is that it may be left unattended (Chesmore & Ohya, 2004). Although acoustic techniques have been widely used for recording birds (Anderson *et al*., 1996) and frogs (Taylor *et al*., 1996), acoustic recording of insects is far less common (Chesmore, 2001; Farr & Chesmore, 2007). Sprecher-Uebersax and Durrer (1998) and Harvey (2007) reported stridulation in stag beetle larvae, the purpose of which is unknown. However, if this sound could be detected *in situ*, we hypothesised that it might also provide an excellent non-destructive detection method for larvae.

Methods

Testing of adult lures

All behavioural experiments were conducted using choice chambers, comprising open plastic containers of 50 cm × 40 cm × 10 cm. In each trial, one adult beetle was placed in the middle of the container, equidistant from the substance under test and an equally sized, inert clay item. A sign of attraction was judged as the insect moving towards the substance and palpating it with the mouth parts. If an individual moved towards a lure, but remained near it without any positive interaction, this was discounted as a positive result. A beetle showing a positive response to a lure was replaced in the centre of the container, the position of the chamber and lure altered, and only if the insect repeated the attraction again, was this recorded as a positive result. After each trial, the containers were washed with distilled water. The experiments were carried out in the evening, at ambient temperature, between 20:00 and 22:00 h, using no artificial light, to encompass the activity period of the adults (Harvey, 2007). All experiments were conducted during the flight season, i.e. between late May and mid July. Since the beetle is rare and only a few live adults are found each year, it was necessary to use each beetle for more than one experiment, but this had the advantage of being able to test each individual on a variety of lures. To reduce errors, beetles were 'rested' on alternate nights, and marked on the elytra to ensure that the tested individuals were not specimens that were either 'reactors' (responding to all stimuli) or 'non-reactors'. Throughout the course of this study, experiments were carried out on approximately 25 males and 25 females each year.

Alpha copaene has been shown to be an attractant to other saproxylic insects (e.g. Vrkocova *et al.*, 2000) and to test this substance, 100 mg was placed on cotton wool in glass vials. In addition, certain fruits and roots are also known to emit this chemical, notably mango and avocado fruits (Siniyinda & Gramshaw, 1998; Shelly *et al.*, 2008) and ginger roots (Shelly, 2001), so these were also used. Other vegetable materials which may emit this chemical and so were tested included banana, strawberry, tomato, cherry and peach fruits and potato and carrot (tubers and roots),as well as red and white wine and beer. As adults may visit sap runs on trees, we also tested maple syrup. Finally, we tested 'stag wood' i.e. decaying wood, in which larvae had been feeding, larvae, larval frass and a fungus, *Trametes versicolor*, which is frequently found growing on stumps inhabited by larvae. For all solid substances, a 56 g sample was used, while for maple syrup, this was 20 ml.

Aerial trap design

For the aerial trap construction, materials had to be a compromise between weight and durability. Furthermore, the materials had to be inexpensive, as our aim was to design a trap that can be constructed easily by amateur recorders, who have little or no access to research funds. The final design was constructed from heavy duty plastic sheeting, which is light-weight and strong and was based on a conventional cross-vane flight interception trap (Young, 2005).

The final design comprised two parts, an upper part which contained the lure, and a lower funnel to direct trapped beetles into a holding container (Fig. 1). The trap consists of an upper set of rectangular blades set as to form an X, supporting a funnel at the bottom of which is attached the holding container. Each blade consists of a rectangular piece of plastic sheet, 500 mm high by 380 mm wide. A hem of 25 mm was created at both top and bottom using staples to provide a sleeve to accommodate 3 mm bamboo split canes. The blades were stapled together along the mid line bottom, such that each cane passed through one whole arm of the X, and held in place by two canes, fitted through a hem made at top and consisting of half of each sheet. Canes of length 480 mm were used at the top and 600 mm at the bottom of the X.

Approximately 80 mm from the top and central to the X, an aperture was cut to accommodate the lure holder. A clear plastic tub with removable lid was used for this purpose. This was held snugly in place by the plastic of the trap, requiring no other restraint, and could therefore be easily removed for placing the lures. Holes were made in a ring around the top and in the bottom of the tub, to allow dispersion of volatiles and to prevent the collection of rain water.

The bottom half of the trap consisted of four triangular sections of polythene sheet, with a basal dimension of 350 mm, stapled together to form an open-topped pyramid. An inverted plastic tub, with removable lid and capacity 800 ml, with a large hole cut into the bottom was then secured with split pins on to the four wings of the pyramid, such that the tub was flush with the wings, and excess polythene trimmed away. Holes were also made into the lid of the tub such that rain water could drain out. The size of the holding chamber was such that it could easily accommodate more than one beetle. For extra security in windy locations a piece of string was also fastened through the lid and anchored to the ground. The open end of the pyramid had a small hem (25 mm) stapled into it, such that four split canes (length 480 mm) could be placed through the trap, holding the aperture in the form of a square. The top of the trap was attached to the lower half by sliding the longer green canes from bottom of the veined section diagonally through the upper corners of the pyramid. Twine was used to suspend the upper green canes of the trap from a suitable height.

Fig. 1. Aerial trap in place, demonstrating placement of lure in upper chamber.

Traps were placed so that the veins of the upper segment were at least 2 m from the ground. Following the results of the attractant experiments, five different lures were tested in each of 10 replicate traps, over the period 2005–2008. In each case an identical control trap (but without a lure) was placed 2 m away from the test trap. In each locality, trap positions were exchanged after 3 weeks, with whole traps being moved rather than just the lure holders. Every beetle captured was sexed, marked with quick drying paint on the elytra and released at least 50 m from the trap.

Pitfall traps

Pitfall traps were constructed using flowerpots, of 180 mm diameter and 170 mm depth. The pots were buried such that the top of the pot was at a level with soil height. A plastic 'roof' was secured at a height of 30 mm above the trap rim, to prevent predator attack (Harvey *et al.*, 2011). Traps were baited with the same five lures used in the hanging design. Lures were fitted on the under surface of the trap roof. Dependent on the nature, lures were either secured in glass vials, 50 mm long × 25 mm diameter, or attached directly to the lid. Perishable lures were replenished at 2 day intervals. In each location, a blank control was placed, 2 m away from a baited trap and after 3 weeks placement of the two traps was exchanged. The entire baited trap was replaced, in case volatiles had adsorbed on to the trap surface. Every trap was checked twice a day for a period of 6 weeks and every insect captured, sexed, marked and released as above. A few other species (mainly Carabidae) were found in these traps, but they were of such limited occurrence that the data are not presented here.

Trapping was carried out across the distribution of the stag beetle in the United Kingdom. Traps were largely sited in urban gardens since this is the predominant habitat of the insect in this country. The placing of traps was determined by monitors from the People's Trust for Endangered Species national survey in 1998 (Percy *et al.*, 2000), to ensure that the species was present in the area where traps were set. This also meant that monitors were competent in identifying both sexes of the beetle and those species with which it might be confused (J. T. Smit & R. F. M. Krekels, unpubl. data), such as *Dorcus parallelopipedus*. Hanging traps and pitfall traps were not placed in the same locations, to maximise area sampled.

Road transect surveys

Road belt transect surveys were carried out along a 600 m stretch of Church Road, Bentley, Suffolk, UK, (grid reference TM 1130 3670), a C-category highway, which is approximately 4.5 m wide throughout. The road does not have street lighting. Surveys were undertaken daily, by walking along both sides of the road, at the same time (09.30–10.00), from mid-May to mid-August over 5 years, from 2004 to 2008. All beetles, whether dead or alive were counted and sexed. Dead specimens were removed and live specimens marked with a spot of quick drying paint and released nearby. In each year, the total number of beetles recorded by observers in an area centred on the transect was recorded and every record pinpointed by its post code. This was done because it was impractical to equip all recorders with a GPS system, thus records are accurate to the nearest 100 m. The total area sampled was a circle of radius 22 km.

Larval detection using volatiles

Radiello cartridges (Type 130, Supelco Analytical, Gillingham, UK) comprising a stainless steel net cylinder, with a 100 mesh grid opening and 5.8 mm diameter, packed with 530 ± 30 mg of activated charcoal (particle size 35–50 mesh) were placed inside white polyethylene diffusive bodies. The latter act to restrict contaminating particles above 25 μm diameter being absorbed from soil. In July 2008, one cartridge was placed in each of three tanks (depth 40 cm), each containing 10 stag beetle larvae, such that it was completely submerged in the soil, but not in contact with the larvae. Control tanks, containing decaying wood (taken from the same source as that given to larvae) and soil only were also set up and one cartridge placed in each.

The cartridges were left in place for 30 days. The carbon filters were washed through with dichloromethane, using three times the volume of the cylinder (3×0.5 cm^3), and the elutant was concentrated to 20 μl, using nitrogen gas. The solution was analysed by GC-MS, using a HP5890 Series II GC, coupled to an HP5970 MSD, operated in the electron ionisation mode at 70 eV with ion source and quadrupole temperatures of 180 and 250 °C respectively. Chromatography was carried out on a SGE HT5 25 m × 0.22 mm fused silica column with 0.1 μm film after splitless-split injection (delay 1 min) with helium carrier gas at a pressure of 10 psi. The GC column oven was programmed as follows: initial temperature 50 °C for 2 min, increase at 8 °C min^{-1} up to 350 °C, then held for 30 s. Compound identification was carried out by comparison with the literature data, injection of authentic compounds and by matching experimental spectra with the Nist 05.l library (http://nvl.nist.gov/).

Cartridges were also placed in known stag beetle breeding sites in Leatherhead, Surrey (TQ 150565) and Copdock, Suffolk (TM 104412), UK. In Leatherhead, seven diffusers were sited just above ground level (30 mm) in a hedge, within a stump and within a log pile where larvae were known to occur. In Copdock, six were placed just above ground level (30 mm) between cut logs stacked in a tree surgeon's wood yard, which has many years of accumulated decaying woodchip and sawdust lying on the ground beneath the logs. At each site, a control cartridge was placed within 10 m of each of the 'test' unit, in similar habitat, but where no stag beetles had ever been observed.

Larval detection using acoustics

A wooden frame, of dimensions 420 mm × 335 mm × 50 mm was constructed, Perspex™ was attached and the frame filled with soil and small pieces of 'stag wood' (see above). Two final instar larvae were introduced into the cavity and allowed to burrow. Two 10 mm diameter piezoelectric transducers (RS Components Ltd, Corby, UK) were placed on to the Perspex, and small microphones in rubber finger cots were placed inside the terrarium. Leads from the microphones and piezoelectric discs were connected to an amplifying unit, which was in turn plugged into a PC and viewed using the Goldwave audio editing programme (http://www.goldwave.com). Recordings were made over a period of 3 h. Following the measurements recorded in the terrarium, a stump (approx. 450 mm diameter × 600 mm depth), known to contain larvae, was buried in a 450 mm diameter deep plastic container to ensure recordings were from within the stump. The stump was monitored using small microphones attached to an amplifying unit which was then plugged into a minidisc recorder (Sony Recording Minidisc Walkman MZR700). Microphones were sealed into rubber finger cots to stop water damage, placed into the soil next to the stump at a depth of approximately 50 mm, and left *in situ* for 1 h. The recording was then copied onto a PC, studied, and edited with the Goldwave audio editing programme. As it is critical to determine if any sound produced by *L. cervus* larvae is unique, recordings from the terrarium were repeated, using larvae of the lesser stag beetle, *Dorcus parallelipipedus* and the rose chafer, *Cetonia aurata*. Larvae of these two species are frequently found in the same stump as *L. cervus* and being morphologically similar, are often mistaken for them.

Statistical analysis

The chi-squared test was used to determine whether male and female beetles displayed a positive attraction to any of the substances tested. This test was also used to compare responses of male and female beetles to each substance. For the road transect data, the total number of beetles found in each year was summed. For each year, the total number of all beetles seen (not including the transect numbers) was calculated by summing all observation records for a series of radiating circles 1–22 km away from the survey site. The Pearson correlation coefficient was used to examine the relation between beetle numbers on the transect and those found in the circle, using years as replicates.

Table 1. Summary of adult responses and significance to various potential attractants.

Substance tested	Male response (%)	Female response (%)	Males vs. females
Larvae	100.0***	0.0	**
Ginger	92.6***	81.5**	NS
Alpha copaene	91.9***	92.3**	NS
Avocado	90.0**	81.8**	NS
Mango	85.9***	87.9***	NS
Heptanol	75.0**	0.0	**
Larval frass	66.7*	87.5**	*
'Stag wood'	62.2**	63.6*	NS
Maple syrup	50.0	33.3	*
Fungus	50.0	50.0	NS
Nonanol	50.0	0	**
Caryophyllene	50.0	78.6*	NS
Peach	40.0	66.7	NS
Carrot	25.0	0	NS
Strawberry	20.0	6.3	NS
Cherry	20.0	0	NS
Cucumber	16.7	0	NS
Tomato	12.5	0	NS
Banana	0	0	–
Beetroot	0	0	–
Potato	0	0	–
Red wine	0	0	–
White wine	0	0	–
Beer	0	0	–

Data are the percentage of all male or female beetles responding positively to a particular substance. The males vs. females column indicates any difference between the sexes, with all $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; NS, not significant.

Results

Testing of adult lures

Adult beetles responded positively to many of the substances tested (Table 1). No substance elicited a 100% response in both sexes, but every male tested was attracted to larvae. Interestingly, larvae held no attraction for females, and a similar (though less pronounced) response was found with heptanol and nonanol, both of which attracted males but not females.

Of the various plant parts tested, ginger root and the fruit of mango and avocado were particularly attractive and in all cases, there was no difference between the responses of males and females (Table 1). In each case, the proportion of beetles responding to these substances was not different to that for alpha copaene. Meanwhile, both sexes responded to the frass produced by larvae and the wood that larvae had been feeding in. In these cases, there was no difference between the responses to these substances and that of the fungus, *T. versicolor*.

In the search for an inexpensive and attractive lure to test in the field, ginger, mango, larval frass, 'stag wood', and *T. versicolor*, were selected for use in the aerial and pitfall traps.

Aerial and pitfall traps

Both trap types caught adults of both sexes, and the total number of beetles caught was 30 males and 25 females, over the 4 year period. The proportion of males caught in each trap type, baited with each substance, is shown in Fig. 2a. This shows that ginger was the only lure that performed equally well in both trap types ($\chi^2 = 0.6$, d.f. = 1, $P > 0.05$). While mango attracted significant numbers to pitfall traps, no beetle was caught in a hanging trap with this lure. Both larval frass and the fungus, *T. versicolor*, failed to attract beetles in any numbers.

Figure 2b shows the response of female beetles. Ginger again attracted beetles to both trap types and there was no difference between the traps ($\chi^2 = 0.5$, d.f. = 1, $P > 0.05$). However, female beetles appeared to be much more responsive to larval frass than males ($\chi^2 = 6.8$, d.f. = 1, $P < 0.01$), mirroring the response seen in the laboratory trial (Table 1). Females did not respond to mango to the same extent that they did in the laboratory, or to the same extent as males, which was different to that observed in the laboratory (Table 1). Unbaited traps captured two beetles over the entire period and area.

Road transect surveys

A total of 153 beetles were found on the road transects over the 5 year period, with an average of 30.6 ± 4.6 individuals per year. Over the five survey years, the average ratio of females to males found was 3.5:1, a significant departure from equality

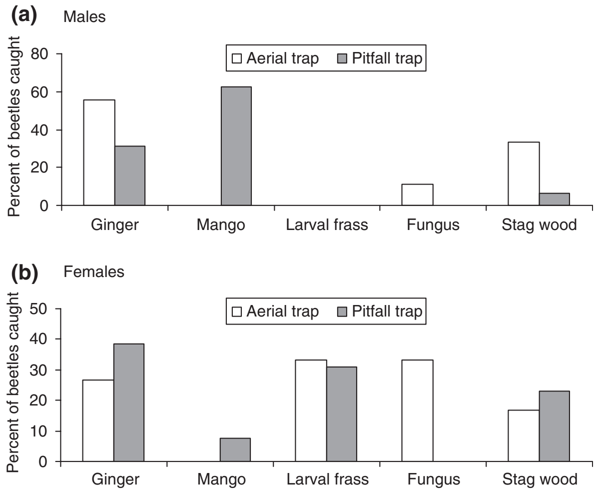

Fig. 2. Percent of all beetles attracted to different lures in aerial and pitfall traps over a 4 year period (2005–2008).

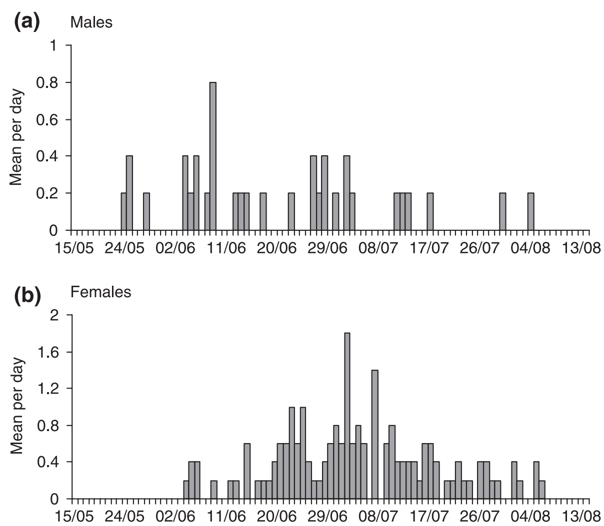

Fig. 3. Mean number of adult beetles observed each day on the road transect, averaged over a 5 year period (2004–2008).

($\chi^2 = 76.5$, d.f. $= 1$, $P < 0.001$). Very few live specimens were found; 87% of females and 76% of male beetles were dead.

The average number of male beetles found per day over the 5 year study period is shown in Fig. 3a. Males were found over a 2 month period, from 24 May to 5 August, with a peak on 9 June. Greater numbers of female beetles were found (Fig. 3b), and were recorded over a similar period to males, from 4 June until 7 August. Peak numbers occurred on 3 July.

The correlation between total beetle transect numbers and the total found in the local area varied with distance from the transect (Fig. 4). In females, 90% of the correlations were significant at $P = 0.05$ and there was a clear pattern in that the relations with totals were weak in the immediate area (up to 5 km from the transect), but thereafter were highly significant, and remained so with increasing distance. However, in the males, only 36% of relations were significant. Weak correlations again existed in the local area, with significant effects only being detected in the 5–12 km band. As distance increased from 12 km away from the survey site, correlations became progressively weaker.

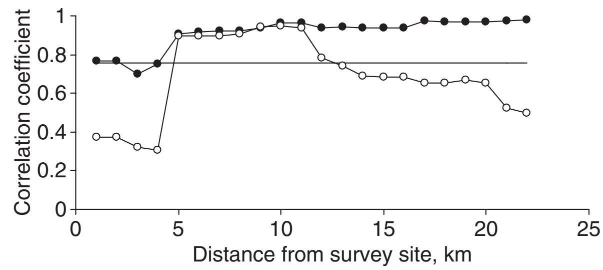

Fig. 4. Pearson correlation coefficient between road transect numbers and total numbers in circles of increasing radius, plotted against distance from the survey site. Filled circles represent female and open circles represent male beetles. Horizontal line represents value required for the correlation to be significant at $P = 0.05$.

Larval detection using volatiles

The three most abundant chemicals detected in the laboratory containers were alpha pinene (a monoterpene, molecular formula $C_{10}H_{16}$), caryophyllene and longifolene (both sesquiterpenes, molecular formula $C_{15}H_{24}$). However, while the former two chemicals were detected in tanks with and without larvae, longifolene was only detected when larvae were present. In the two field situations, longifolene was again detected by all cartridges when larvae were present, but was not found in any site when larvae were absent. Thus, it appears highly likely that this chemical is emitted by the larvae and that its presence can be used to infer their presence.

Larval detection using acoustics

Sounds produced by the larvae of *L. cervus* could be clearly detected in both the terrarium and the stump using custom-made low cost piezoelectric sensors. Larvae of *L. cervus* and *D. parallelipipedus* could be detected biting the wood, and each produced a different and characteristic audio pattern (Fig. 5). No clear recording of *C. aurata* biting could be obtained. Perhaps of greater interest was the discovery that larvae of all three species of beetle stridulate and that the pattern is characteristic of each species (Fig. 6). Each is represented by short bursts of sound, but that of *D. parallelipipedus* is of much longer duration. The time and frequency characteristics of the three species are as follows: *L. cervus* has a duration of approximately 1 s with a dominant frequency of 700 Hz and frequencies between 600 Hz and 1.8 kHz; *D. parallelipipedus* has a duration of more than 3 s with a dominant frequency of 400 Hz (range 400 Hz to 3 kHz)

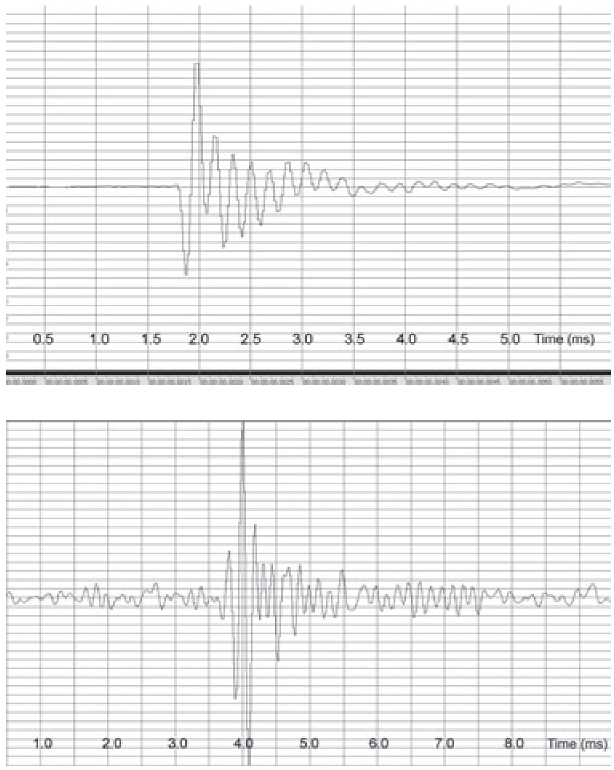

Fig. 5. Acoustic patterns produced by biting of *Lucanus cervus* (upper graph) and the lesser stag beetle, (*Dorcus parallelipipedus*) larvae.

and *C. aurata* duration is 1.5 s with a frequency span of 400–600 kHz. As the biting and stridulation patterns were detected from within the stump, acoustics can be used to detect larvae of *L. cervus in situ*, without destruction of the habitat.

Discussion

Perhaps one of the most interesting observations in the search for attractants was the fact that adult male beetles were highly responsive to larvae. In other behavioural experiments, adult males were found to approach larvae and attempt to mate with them (Harvey, 2007). This behaviour is not unique and has occasionally been reported in Scarabaeidae (Haynes *et al*., 1992; Haynes & Potter, 1995). It has been proposed that this may explain the evolutionary development of sex pheromones being derived from non-communicative chemicals, which are lost in adult males but retained in females (Haynes *et al*., 1992). In *L. cervus*, the chemical responsible for male attractant behaviour appears to be longifolene. In controlled studies, air entrainment from both larvae and adult females produced this compound (Harvey, 2007). Longifolene would only be of use as a lure for trapping male beetles, but we did demonstrate that it can be used to detect larval presence in buried wood.

To date, we are unaware of any entomologists using thermal desorbable radial diffusive samplers (Radiellos) for the detection of insect-produced volatiles. Our results show that these have great potential in both controlled and field conditions, confirming the efficacy of these samplers in environmental monitoring (Swaans *et al*., 2007). Longifolene was detected in every situation in which larvae were present and we conclude that the use of Radiellos represents a novel, effective and non-destructive method of larval detection in the field.

Longifolene is a volatile sesquiterpene, present in a number of plants but not widely found in trees or insects. Interestingly, it has been reported as being emitted from mango (*Mangifera indica*) fruits (Hernandez-Sanchez *et al*., 2001). This may explain the attractiveness of mango to adult males, but is less likely to be an explanation for the positive response of females to this fruit. It has been reported to occur in the frass produced by larvae of *Monochamus alternatus* (Coleoptera, Cerambycidae) (Li & Zhang, 2006), and may be an oviposition deterrent in this species. Meanwhile, it is also implicated as an oviposition deterrent in a mosquito, *Anopheles albimanus* (Torres-Estrada *et al*., 2005) and as a wound-induced attractant to insect parasitoids (Kopke *et al*., 2008). Given the complete lack of attraction to female beetles, longifolene may also act as an oviposition deterrent to *L. cervus*.

We decided that if any substance was to be successful as a lure then it needed to fulfil the following criteria: (1) attracted both sexes with a frequency of over 80%; (2) attracted both sexes in equal numbers; (3) was equally successful in both trap types and (4) was inexpensive to use in large quantities. Once these criteria were applied, the only material that satisfied all four was ginger root. Although alpha copaene was successful in the laboratory, it was deemed too expensive to use on a large scale and so was omitted from the trapping programme. This chemical is likely to be the reason why ginger was successful, as the roots are known to emit it (Shelly, 2001). Alpha copaene occurs in a wide range of plants and is a known attractant for many insects, including saproxylic species (Vrkocova *et al*., 2000; Crook *et al*., 2008). We conclude that both types of trap can be used to monitor adult numbers and if baited with ginger are likely to be a successful method in future population abundance studies of this insect. Future studies using these traps could be integrated with spatial modelling (Thomaes *et al*., 2008), to provide a much-needed accurate picture of the population dynamics of this insect. We do not believe that such a trapping method would be detrimental to the abundance of an already rare insect. This is because mating occurs in a frenzy of activity at the end of the flight season (Harvey, 2007) and any trapping before this would be highly unlikely to reduce reproductive potential.

Both sexes responded to the presence of larval frass, decaying wood and the fungus *T. versicolor*. In our experiments, we did not measure whether beetles were actively repelled by the substrates, but Harvey (2007) discovered that female beetles were deterred from oviposition by the presence of large quantities of wood that had been chewed by the beetle. This is a likely mechanism to avoid competition in the larvae. The stumps in which larvae are found represent a patchy and limited resource and a reasonable strategy would be for females to lay in stumps in a suitable state of decay (hence their attraction to fungi) but not where larvae are already present (hence their avoidance of larvae and eaten wood).

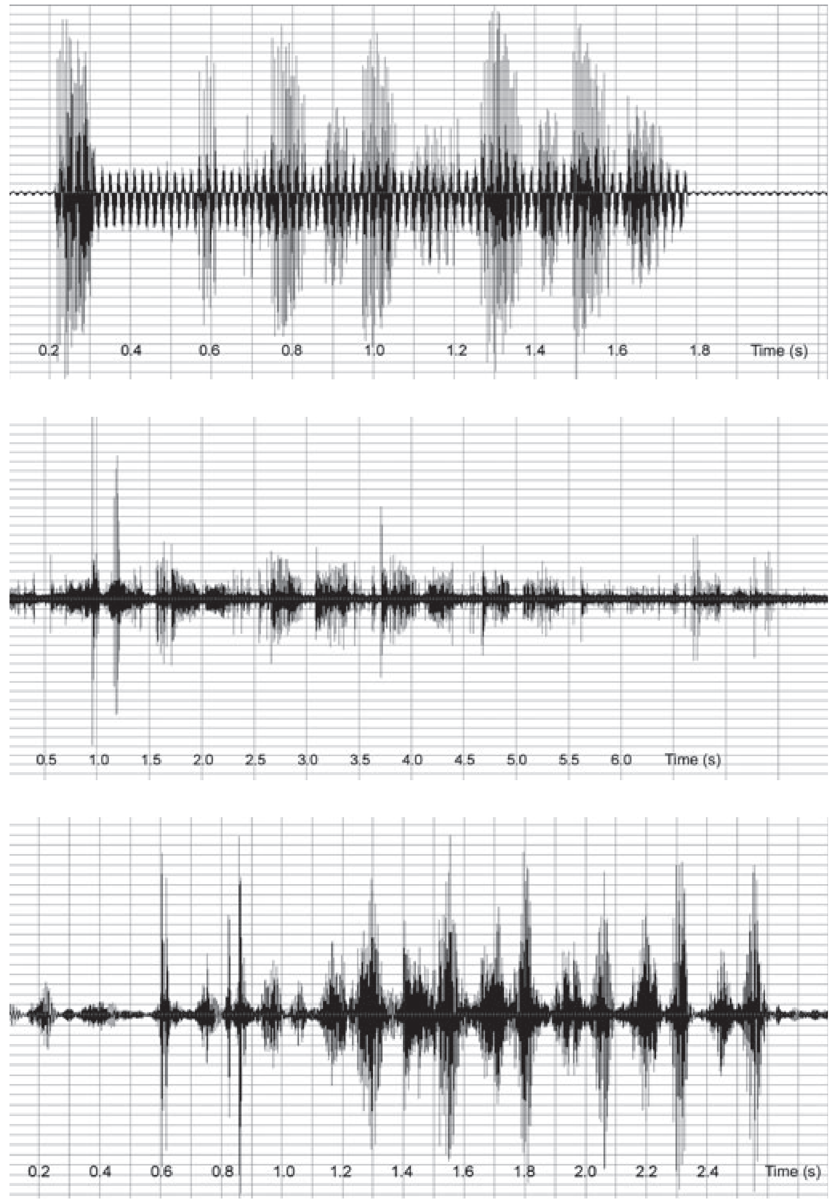

Fig. 6. Acoustic patterns produced by stridulation of larvae of *Lucanus cervus* (upper graph), *Dorcus parallelipipedus* (middle graph) and rose chafer, *Cetonia aurata* (lower graph).

Perhaps unusually, we chose to examine methods that would allow monitoring of both sexes of the beetle. This might seem excessive, but our aim was to adopt a protocol that encompassed the known differences in dispersal behaviour of the sexes: females predominantly pedestrian and males by flight. Furthermore, by recording numbers of males and females, we were able to accurately determine the sex ratio of the beetle and to determine whether both sexes exhibited the same degree of size variation (Harvey & Gange, 2006).

The road transect surveys also proved to be a useful method by which beetle presence and abundance could be monitored. If such a technique is to be used, it is essential that it is not biased in any way and provides a fair reflection of total numbers in the wider area. Road transects are certainly biased towards females, as shown by the sex ratio (3.5:1) of specimens found. In much wider surveys, this ratio is closer to unity (Hawes, 2005; Harvey, 2007). This bias is certainly due to the flight behaviour of the adults. Males have a greater propensity to be on the wing than females and usually fly at a height of 3 m or above (Mamonov, 1991; Hawes, 2005). Females spend most of their active time on the ground searching for suitable habitat in which to oviposit, and if they take flight, this is at 1–2 m above ground (Hawes, 2008). One encouraging feature of the road transect data is that the seasonal pattern of occurrence of both sexes mirrors closely that seen for all records in the UK (Percy *et al*., 2000). A second encouraging aspect was the close correlation between the numbers found and those in the wider area. For both sexes, there was little relation between transect numbers and total numbers in the immediate local area (up to 5 km distant). This lack of agreement is difficult to explain, for with limited dispersal distances (Rink & Sinsch, 2006), one might expect a good correlation in the local area. The most likely reason is that it is an artefact of there being fewer recorders and hence records in this zone. Of greater interest are the strong correlations between transect numbers and total counts for both sexes in the area > 5 km from the study site. This is good evidence that road transect numbers are an unbiased reflection of the total population in any 1 year. The fact that after a distance of about 12 km, the correlations become less significant in males probably represents differences in the dispersal ability of the adults. Males move over considerably longer distances than do females, with the longest single flight for a male recorded at 1.7 km (Rink & Sinsch, 2006). It is likely that at the boundary of the study zone, total numbers were confounded by beetles flying into and out of the area, whereas a distance of 12 km is likely to exceed the total dispersal distance of males. Females do not show such variation, because their dispersal distances are much smaller, even as low as a few metres (Rink & Sinsch, 2006).

Sampling of subterranean insects without destroying the larval habitat is notoriously difficult (Gange, 2005). We have shown that the detection of longifolene can be used to infer larval presence, but the fact that this can be produced by plants that grow in close proximity to stag beetle larval habitat (e.g. Kite, 1995) does not make it an infallible method. However, if longifolene detection was coupled with acoustic recording, then errors in detection are most unlikely. Acoustic detection of insects as a sampling method is very underused (Chesmore, 2001), but we believe it could have great potential in detecting larvae in the field. Stridulation patterns of *L. cervus* were clearly different from those of the species likely to co-occur as larvae (*D. parallelipipedus* and *C. aurata*). Stridulation by larvae has been reported before (Sprecher-Uebersax & Durrer, 1998; Harvey, 2007) and is most likely a form of communication between larvae. It was noted that stridulation increases if larvae are handled or placed in solitary confinement (Harvey, 2007), but also occurs in groups of larvae. Whatever the reason for its occurrence, it is frequent and has great potential as a non-destructive larval monitoring method.

In conclusion, we have developed various methods which offer genuine promise for population monitoring of an elusive and rare insect, one that is thought to be in decline across much of its European range (Harvey *et al*., 2011). Hanging and pitfall traps baited with ginger can provide reliable monitoring tools for adults and we recommend that both are used in any location, so that both sexes are recorded. Additionally, they provide a cheap, (under €5), weather proof method of monitoring a large area and should enable accurate population estimates to be obtained, thereby avoiding the problem of recorder distribution, associated with amateur surveys (Percy *et al*., 2000; J. T. Smit & R. F. M. Krekels, unpubl. data). Road transect surveys provide a measure of beetle presence, and total numbers can be an accurate reflection of those in the wider area, but they are more labour intensive. We recommend that road transects are used to determine if the insect is present in an area, prior to a detailed trapping programme. The road transects confirmed that the adult flight season is relatively short, thus traps offer a permanent sampling technique during this time; an improvement over the current situation of chance observation by surveyors.

We also recommend that once adults are detected, suitable habitats may be examined for larvae, using Radiello cartridges, coupled with acoustic recording. Critically, these do not involve disturbance of the habitat and can be used at any time of the year. These methods may have great potential for use in habitat surveys aimed at providing information on rare species for planning enquiries. Radiellos can be used *in situ*, to detect longifolene, are weather proof and the diffusive body ensures that the carbon mesh is unaffected by soil particles or insect interference. Once a potential habitat has been identified, the device can be placed and left unattended. These units cost approximately €26 each, but may be washed and reused. A more rapid and cheaper detection of larvae can be performed with acoustic recording of larval stridulation, but we recommend that both methods are used to accurately determine larval presence.

We began this work mindful that monitoring in insect conservation is often reliant on the good will of amateur entomologists and is not well funded. We believe that the inexpensive and simple methods reported here offer real promise for the engagement of amateurs in the conservation of this insect. However, most importantly they take into consideration the behavioural features of both adults and larvae, and provide a way of monitoring the insect without killing adults or destroying the larval habitat.

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