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SHORT COMMUNICATION

A novel technique for relocating concealed insects

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Introduction

An understanding of the population dynamics of individuals requires knowledge of spatial behaviour (Turchin, 1991). Gathering information on the spatial behaviour of many smaller organisms such as insects is problematic because observing them in the field without disturbing their natural behaviour and habitat is sometimes very difficult. This problem is compounded by the small size, crypsis, and rarity of many insect species.

Various methods have been used to detect and record micro-habitat preferences of ground-dwelling insects in the field. These include pitfall trapping (Rijnsdorp, 1980; Best *et al.*, 1981), radio-active marking (Southwood, 1978; Baars, 1979), harmonic radar (Mascanzoni & Wallin, 1986; Lovei *et al.*, 1997), and radiotelemetry (Riley, 1989; Pride & Swift, 1992). Of the above methods, harmonic radar has perhaps shown the most promise for the detection of tagged individuals (Mascanzoni & Wallin, 1986; Roland *et al.*, 1996). This method is particularly useful for organisms that are relatively vagile, however the resolving power of harmonic radar does not lend itself to the study of relatively sedentary ground-dwelling animals. The method is also extremely costly, and the size of the tags used in harmonic radar studies is often a problem. Although lightweight, the diode tags used must have a trailing aerial (Mascanzoni & Wallin, 1986), which is a particular disadvantage for studies of small litter-dwelling insects as it is likely to be a hindrance to natural movement.

The novel technique detailed here involves the use of metallic tags and commercial metal detection equipment. It does not have the detection range of harmonic radar but is a much cheaper system that uses a smaller, more discrete tagging technique. In field experiments, the technique has proved to be effective in relocating released beetle larvae living in concealed situations. It was used to determine the mobility, micro-habitat preferences, and overwintering survivorship of leaf beetle larvae of the chrysomelid *Cryptocephalus coryli* (Linnaeus). *Cryptocephalus* larvae inhabit cases made of their own faeces that they carry around in the leaf litter. The cryptic cases of the larvae, their small size, and the habitat in which they reside make it very difficult to locate larvae using conventional techniques.

Cryptocephalus coryli is known from only two U.K. sites and is listed as RDB1 (endangered) (Hyman & Parsons, 1994). In common with other members of the genus, eggs are covered in faeces by the female and dropped to the ground beneath the host plant. Once hatched, the larva adds to the egg case to eventually form the characteristic larval case. The larvae are the overwintering stage for the genus and feed on leaf litter. There is no clear-cut diapause. The biodiversity action plan for this species (U.K. Biodiversity Steering Group, 1999) has the target of re-introducing this species to three sites by 2005. For such introductions to be successful, a thorough understanding of mortality and micro-habitat preferences of the larvae is essential.

Materials and methods

A detection system was employed that was based on the principle of the pulse field induction loop (Pulse Technologies, Oxford, U.K.). The device was designed originally to locate small amounts (< 2 mm) of non-ferrous and ferrous metal in fabrics. The metal detector consists of one small, hand-held unit (720 g), which for field use is connected to a 12-V lead acid gel battery (Yuasa, Reading, Pennsylvania). The tag used to label the insects was a strip ($1 \pm 0.2 \times 3 \pm 0.2$ mm, 0.35 ± 0.07 mg) of stainless steel, which was taken from the security labels that are attached to goods to deter shoplifters. Different-sized tags were tested and it was found that the device had a maximum detection range of about 7 cm. One small strip of metal was attached to each of the cases using a small drop of epoxy resin adhesive (Bostik, Leicester, U.K.). Laboratory-reared *C. coryli* larvae were then marked individually using a disc of acetate sheet (0.9 mm diameter) on which a unique code was printed (font size 2) using a laser printer. The disc was bored from the sheet using a modified mechanical pencil,

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and attached to the case of the larva using epoxy resin adhesive as before. Once the insect was detected in the field, the code on the disc could be read using a portable microscope (Specwell, Tokyo, Japan) and the insect returned quickly to the place of capture. The use of the acetate labels allowed the behaviour and fate of individual insects to be monitored. The range of the equipment in this trial was never more than 3 cm. In initial tests, detection was found to be possible through any type of litter/soil, and moisture did not seem to impede the location of tagged individuals. Further tests of the relocating technique were applied to the third-instar (30 ± 3.8 mg including case, $n = 20$) and final (fifth)-instar (92 ± 3.3 mg including case, $n = 20$) larvae of *C. coryli*. The mass of the larvae was recorded before and after tagging and for third-instar larvae the tag represented $3.26 \pm 0.48\%$ of larval body weight while for final-instar larvae the tags represented $1.18 \pm 0.36\%$ of their body weight. To test the effect of the tags on the mobility of the larvae, a 1×1 m box was filled with leaf litter to a depth of 4 cm. Twenty tagged larvae and 20 untagged larvae were allowed to disperse for 24 h (20 °C, LD 12:12 h). The distances moved by the larvae away from the release point were recorded.

Field tests were carried out using 40 tagged larvae released in October 1999 at Kirkby Moor, U.K. (54°15′N, 3°8.6′W). Prior to release, the larvae were acclimatised by placing them in successively cooler incubators (20, 13, 8, and 5 °C). Ten larvae were placed beneath each of four *Betula pendula* trees, the main host plant used by this species at the site. Over the first 48 h, the larvae were relocated in the morning and the evening, and the distance moved from the release point was recorded. For the rest of the test period, the larvae were retraced once a month until March 2000. During each visit, the micro-habitat in which the larvae were found was recorded, together with evidence of predation. Larvae were recorded as still alive if the head capsule was visible at the aperture end of the case.

Results and discussion

During laboratory tests, it was found that tagged larvae moved an average of 7.1 (± 7.59) cm over a 24-h period. Untagged larvae moved 7.6 (± 7.9) cm in the same time. A *t*-test showed that there was no significant effect of tagging on movement through the leaf litter ($t = -0.12$, $P = \mathrm{NS}$). In the field, the first 48-h monitoring of the released larvae revealed a nocturnal pattern of activity corresponding with observations on captive larvae, in which the majority of feeding appears to take place overnight. Relocated larvae were either partly buried in soil, secreted within small grass tussocks, or in coppice stools. Eighty-two per cent of the observations of larvae being partly buried in bare ground were of mature final-instar larvae, pointing to a change in micro-habitat prior to pupation (Fig. 1) and possibly the need for a small-scale mosaic of ground characters for successful development in this species. Individual marking enabled the micro-habitat selection and mortality of specific larvae to be studied (Fig. 1). During the study, many empty, damaged larval cases were found. These cases had been chewed or gnawed open and the larvae had been removed cleanly from inside, probably by a small mammal. At the end of the experiment, 87.5% of the relocated larvae had been eaten.

The technique enabled 90% of the released larvae to be relocated after a period of 5 months. During this time, the metal tags showed only minor corrosion. Only one of the tags had come free from the larval cases; this may have been a result of the action of the predator as it removed the larva from its case.

The field tests showed that metal detection is an efficient method for relocating relatively immobile larvae in semi-concealed locations. The technique enabled both behavioural and ecological aspects of *C. coryli* larvae to be understood in their natural environment.

The range of the detection system is small but is ample for the relocation of insect larvae that do not disperse very far. The technique enables tagged individuals to be found quickly with minimal habitat disturbance. With more sensitive equipment, the detection range could be increased and the tag size reduced. The system is many times cheaper than an alternative detector based on the harmonic radar principle [harmonic radar hardware (Recco, Lidingo, Sweden) costs ≈ £15 000, whereas a hand-held metal detector (Pulse Technologies) costs ≈ £400]. Furthermore, harmonic radar can have difficulty pinpointing separate signals from a group of released animals if they remain in close proximity to the release point, and water and humidity have also been shown to attenuate harmonic radar signals (Lovei *et al.*, 1997). Some organisms can also generate false signals that confuse the operator of the harmonic radar equipment (Lovei *et al.*, 1997). No such problems were encountered using the metal detector.

The use of a metal detector and metal tags would be useful for describing the habitat use and ecology of other small terrestrial invertebrates that are relatively sedentary and cryptic. For example, this method could be used to determine pupation sites of many leaf beetles whose larvae move away from the host plant and construct a pupal chamber in the top layer of the soil. The tag would be shed with the ecdysed cuticle of the final-instar larvae and deposited within the pupal chamber. The distance moved

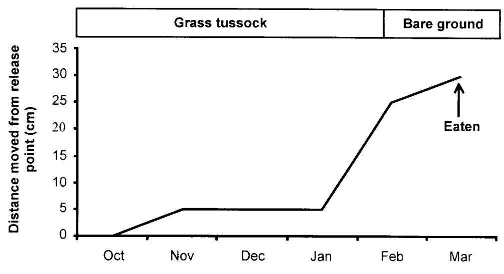

Fig. 1. Micro-habitat selection of a single *Cryptocephalus coryli* larva over a 5-month period.

away from the host plant could be described along with the micro-habitats chosen by the larvae. At present, recapturing released larvae of a cryptic nature usually entails destructive sampling of the habitat (Nicholls & Pullin, 2000). The over-wintering strategies of adult insects on or in the ground beneath their host plants could also be studied, and adult insects could be relocated in cryptic situations such as the fissures in tree bark. Finally, in principle this technique could be applied to any situation where individuals are otherwise difficult to locate but are present relatively close to the surface.

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