Report for Buglife: Violet Click beetle 2021

The violet click beetle (*Limoniscus violaceus*) is a saproxylic species of conservation status and interest across Europe. An extremely rare species, in the UK it is believed to be restricted to 3 areas: Windsor Great Park in Berkshire, (SU 93458 73992), Bredon Hill in Worcestershire (SO 95746 40310), and Dixton Wood in Gloucestershire (SO 97969 31357), with the larval habitat described as undisturbed wood mould in central cavities of veteran trees.

To date, therefore, determining presence of the species has relied on searching wood mould in tree cavities felt to have the appropriate characteristics to find larval specimens, or pre-emergent adults, which inevitably leads to disruption and potential habitat destruction. Furthermore, the larvae have the characteristic morphology of click beetles: orangey-brown exoskeleton and small, visible, mandibles, meaning expert identification skills are required to tease out the crucial differences that distinguish it from more common click beetle species. The adults, at approx. 12mm long and largely black with a subtle hint of violet, are very rarely seen in flight. When the above is combined with a habitat that is largely out of public view, namely ancient beech (*Fagus sp*.) such as found in Windsor Great Park, and ash (*Fraxinus sp.*) in Dixton Wood and Bredon Hill, the true distribution and population size of the species has been impossible to establish in a meaningful way.

Over the last decade, the use of pheromones (biologically active, species specific, volatile chemicals) isolated from insects of conservation status, has been used in the development of lures to monitor the adults of such species via capture mark release (Harvey *et al.,* 2018; Harvey *et al.* 2017; *Svensson et al*. 2004 ). The chemicals can be determined by dissection of the adult beetle's gland and identification of the extracted contents. This method has the disadvantage that it necessitates the sacrifice of specimens from already challenged species. The method used here, and by the papers cited above by Harvey *et al*, collects volatiles released by live specimens, utilising the 'head-space', or air above live insects, placed in a glass vial. It is a non-invasive technique which doesn't harm the species in question- making it particularly suitable for species which are in very low numbers, without further damaging populations. However, since insects produce these volatiles in very low quantities (parts per million) this technique relies on accessing enough individuals initially to be able to reliably collect and identify the correct chemicals (Harvey *et al*., 2011). The insects are placed in a small glass vial with a Solid Phase Micro-Extraction (SPME) fibre placed in the air above the specimens. Volatiles that are given off adsorb onto the fibre which can then be examined via Gas Chromatography – Mass Spectrometry, and a tentative identification made, using the NIST library (a scientifically recognised collection of spectra of known compounds to which isolated chemicals can be compared). Then, where possible, a standard solution is purchased, or synthesised where it is not commercially available, and the spectra of the standard and that produced by the species compared to confirm identification.

The SPME- GC-MS technique can also be utilised to collect volatiles from larvae. Once a definitive identification has been made, as outlined above, passive diffuser devices can be used in-situ to determine larval presence without recourse to habitat searching and potential destruction. The novel use of passive diffusers for larval identification was

pioneered by Dr Deborah Harvey and Dr Paul Finch (Harvey *et al*., 2011), at Royal Holloway University of London. The devices, which were developed to measure air pollution, utilise a carbon mesh, which adsorbs volatiles present in the area. The carbon mesh is enclosed in a cover, which has been selected for chemical nature and size of molecules given off. Once collected onto the mesh, the volatiles can be eluted using an organic solvent and the resultant liquid dried under nitrogen, re-suspended and identified using GC-MS.

To date, the above methods had not seemed viable for *Limoniscus violaceus,* owing to the extremely low numbers of specimens found from which to isolate the initial compounds. However, the sourcing of 7 adults and 10 larvae from France (via Dr Nicolas Gioux) has now allowed us to identify the volatiles released by adults and larvae. These will now be used for a potential monitoring scheme for both life stages.

The compound for the adult monitoring scheme requires synthesis and would normally been compared to a standard (as described above), and then used in the laboratory in bioassays to establish dosage for lures. However, considering the species rarity, and the chemical similarity of the compound isolated from other closely related species, we will pilot the lures next spring/summer across the UK.

For the adults, lures will be placed in aerial traps in the flight season of the adult, believed to be from early May, dependent upon average temperatures. Advice will be sought from Dr Gioux, to determine minimum temperatures required for flight. Traps will be checked daily, and specimens removed and released. To reduce chances of mortality, damp paper will be placed in the holding chamber of the trap. Specimens caught will be sexed, measured, and released. They will also be marked by a small dot made on elytra of the beetle using a permanent Sharpie pen, since these have been used by Harvey on other species of rare saproxylic beetles, are easy to apply, do not cause damage and are non-toxic to beetles, and do not make them more susceptible to predation.

We will also use Radiello passive diffusive devices, placed in tree cavities identified as suitable from habitat characteristics detailed from where previous specimens have been found. These will be placed within the cavity, but are small (6cm long and 1cm diameter) and can be placed, attached to a wire for ease of removal, by pushing through wood mould, minimising disturbance of surrounding areas. They are left in place for 2-3 weeks.

Together, it is hoped that these two systems will provide, for the first time for this species, a non-invasive, accurate, and chemically sound technique for monitoring the species, that can be employed by volunteers after minimum training. It will therefore be possible to carry out wide-ranging surveys, in order to determine distribution and population estimations of this elusive beetle both here and across Europe.

References

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