Applied Soil Ecology 53 (2012) 39–48

Contents lists available at SciVerse ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Understanding the relationship between adult and larval *Agriotes* distributions: The effect of sampling method, species identification and abiotic variables

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ARTICLE INFO

Article history:
Received 1 August 2011
Received in revised form 7 November 2011
Accepted 9 November 2011

Keywords:
Click beetles
Wireworms
T-RFLP
Redundancy Analysis
Pest management
Risk assessment

ABSTRACT

For successful management of invertebrate pest species it is important to understand the relationship between the distribution of above-belowground life stages, and the factors influencing them. Here we test the hypotheses that observed aboveground adult male click beetle (Coleoptera: Elateridae) and belowground larval (wireworm) distributions are related, species-specific, and influenced by sampling method and abiotic variables. Adult male and wireworm *Agriotes* species were sampled from grass/cropped fields in the South Hams, Devon, UK, using sex pheromone traps, soil cores and bait traps. A range of abiotic data was also collected. Redundancy Analysis (RDA) with forward selection was used to assess the influence of abiotic variables and the effect of sampling method on observed species distributions. Analyses were implemented with wireworm species grouped as a pest complex, and with wireworms identified genetically to species to determine the effect of grouping on the analysis outcome. There were no straightforward relationships between aboveground adult and belowground wireworm *Agriotes* species distributions, especially for *A. lineatus*, and these differed depending on the wireworm sampling method. Interspecific differences were observed between *Agriotes* and 'non-*Agriotes*' wireworm distribution, otherwise masked when wireworms were grouped together. Significant associations were found between adult and wireworm species' distributions and specific abiotic variables. These results suggest that risk assessment of wireworm damage should be based on the collection of wireworm rather than adult samples, though there are still inconsistencies between wireworm sampling methods and knowledge gaps which need to be addressed to increase the reliability of these techniques.

1. Introduction

Until recently the aboveground and belowground components of the terrestrial ecosystem have generally been considered separately, but there is growing evidence that interactions between the biodiversity in these two spheres play an important role in community and ecosystem processes (Schroter et al., 2004). Numerous studies have investigated interactions between above- and belowground invertebrates in terms of their influence on plant dynamics, e.g. succession and species diversity (Binns et al., 2000; De Deyn et al., 2003; van Ruijven et al., 2005), induced plant defence strategies (Wackers and Bezemer, 2003; Erb et al., 2008), and their indirect influence on each other in terms of growth, survival, oviposition and host plant selection due to their effect on the quantity and quality of resources that plants produce (Wardle et al., 2004; Soler et al., 2009). However, few studies have considered how the spatial distributions of species with life stages in both spheres are linked despite many species of ecological and economic importance having both above- and belowground life cycle stages. Examples include click beetles (e.g. *Agriotes* spp.), crane flies (*Tipula paludosa* Meigan and *T. oleracea* Linnaeus), some Carabidae (e.g. *Pterostichus melanarius* Illiger), and clover root weevil (*Sitona lepidus* Gyllenhal). The significant economic implications associated with the activity of root herbivore pest species dictate that it is important to understand the distributions, influencing factors and relationships between all life stages in order to assess risk of future damage accurately, and to target control measures at the correct species, thus reducing pesticide application and increasing sustainability. Indeed, improving the sustainability of agricultural crop and grassland systems has been identified as a key area for future work (van der Putten et al., 2009).

This study focuses on *Agriotes* species of click beetles (Coleoptera: Elateridae), which are significant crop pests in Europe and North America, as an example of a pest for which relatively little is known of above-belowground species distributions, yet

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doi:10.1016/j.apsoil.2011.11.004

knowledge of which is highly important for successful management. Although damaging to many crop species, they are particularly known for their effects on potatoes, where even low population densities can cause economic damage (Parker and Howard, 2001). In the UK a complex of three *Agriotes* species (*Agriotes obscurus* Linneaus, *A. sputator* Linneaus and *A. lineatus* Linneaus) is thought to cause the majority of damage to crops (Parker and Howard, 2001); adults oviposit in the soil of agricultural fields where the resulting larvae, known as wireworms, feed on crop roots until they pupate and emerge 3–7 years later, depending on species, region and conditions.

Assessing the risk of infestation, and potential crop damage, is an important part of effectively managing below-ground wireworms, and there are currently three methods used to estimate their presence and/or abundance within fields; soil core sampling, bait traps and adult sex pheromone traps. Soil core sampling is widely used but is labour intensive (in both the field and processing in the laboratory), and can underestimate abundance where populations are low (Yates and Finney, 1942). Bait traps can be processed relatively quickly in the field, commonly using vegetable and cereal baits, and have been found to be as effective, if not better, than soil cores at detecting the presence of wireworms (Parker, 1996). However, it can be difficult to separate wireworms from germinating bait, and the trap catches cannot be used for predicting subsequent crop damage since the effective range of the trap can be affected by a number of site-specific variables (Parker, 1996). Female sex-pheromones have been identified for a number of economically important *Agriotes* species in Europe and Canada (Toth et al., 2003) and traps developed to capture adult males (Furlan et al., 2001; Vernon, 2004). Adult trap catches have been used as an indicator of wireworm presence/absence as an alternative to direct wireworm sampling, but there are limitations in relating adult trap catches to wireworm (as a complex) distribution assessed using soil core or bait trap sampling (Furlan et al., 2001; Blackshaw and Vernon, 2008). For sex pheromone traps, as for bait traps, the range of attraction in the field has not been confirmed and adults are therefore accumulated from an unknown area, whilst soil cores directly sample the damage causing wireworm phase. Only with the recent development of genetic methods to identify *Agriotes* wireworms (Ellis et al., 2009; Staudacher et al., 2011) has it been practical to study wireworm species distribution; there are problems with the morphological identification of very small larvae and larger larvae for which the mandibular structures have worn down, and a large amount of time and expertise is needed, particularly for large sample sizes. As such no published studies to date have been able to relate the distributions of adult *A. obscurus*, *A. lineatus* and *A. sputator* to wireworms of these species.

Previous studies have considered the influence of abiotic variables on wireworm distribution, and factors such as soil moisture (Campbell, 1937; Evans, 1944), humidity (Lees, 1943) and temperature (Campbell, 1937; Evans, 1944; Falconer, 1945) have been shown to affect wireworm behaviour, but there may also be differences in species distributions related to different abiotic variables which are not apparent when wireworms are grouped as a pest complex.

Here, three aspects of above-belowground distribution of click beetle life stages were investigated:

1. The suitability of soil core, bait trap and sex pheromone sampling methods for assessing the relationship between *A. obscurus*, *A. sputator* and *A. lineatus* adult and wireworm distributions.
2. The influence of a range of environmental, chemical, physical and cultural abiotic variables on adult and wireworm distributions.
3. Whether or not it is appropriate to group wireworms as a species complex for risk assessment and management purposes, as has been the case historically.

2. Materials and methods

2.1. Wireworm and click beetle sampling

Wireworms were collected from 99 organic fields on six farms in the South Hams, Devon, UK, between February and April 2004 using both soil cores (10 cm deep, 10 cm wide) and bait traps (1:1 wheat–barley seed mixture inside a 300 ml plastic pot, predrilled with 25 mm × 2 mm holes) (Parker and Howard, 2001). All six farms grew vegetables in a livestock-based grass rotation involving beef, dairy, and/or sheep production, and the fields sampled represented the variety and proportions of crops and pasture found in these rotations. Twenty soil cores were sampled from each field in a 'W' formation (Parker and Howard, 2001), and 10 bait traps were then placed inside every other hole left by the soil cores, backfilled with loose soil from another part of the field, and collected 1 week later (Parker, 1996; Seal et al., 1992). However, warm weather conditions meant that the seed bait germinated and grew rapidly making the traps difficult to extract and sort. Therefore only bait traps in fields sampled early in the season (the first 41) were processed. The bait itself and the surrounding loose soil were hand sorted for specimens, though the majority of wireworms were found in the surrounding soil. The contents of the soil cores were processed using wet sieving within 48 h of sampling. From 7th May to 12th August 2004 adult males were trapped using sex pheromones for each species in Yatlor traps (Furlan et al., 2001; Toth et al., 2003) from the centre of 92 of the 99 fields (seven fields were excluded for practical reasons). Traps were emptied weekly and replaced in the same position, and pheromones were replaced after 6 weeks. Click beetles and wireworms were stored at −20 °C within 12 h of sampling.

2.2. Abiotic data

Soil chemical properties were determined from soil cores taken randomly in each field (3 cm diameter, 25 cm deep, 20 per field). Samples were air dried, bulked and analysed using standard methods (Anon, 1986). Cultural attributes were obtained from interviews with the farmers, and environmental properties were taken from Ordnance Survey maps (Appendix A).

2.3. DNA extraction and species identification

DNA from wireworms and adult males was extracted using a standard salt/chloroform method (Rico et al., 1992). Wireworms of the species *A. obscurus*, *A. sputator* and *A. lineatus* were identified using terminal restriction fragment length polymorphism (T-RFLP) which targets the mitochondrial 16S rRNA gene, using adult males (identified as *A. obscurus*, *A. sputator* or *A. lineatus* using morphological characteristics) as positive controls, following the protocol of Ellis et al. (2009). In cases where no restriction fragment was produced (12 samples), samples were sequenced directly at the16S rRNA region of mitochondrial DNA (mtDNA), following the protocol of Ellis et al. (2009).

2.4. Sequence data

Sequences (254–312 bp fragments of mitochondrial 16S rRNA) from wireworms unidentified from the T-RFLP method were edited and aligned using BioEdit v.7.0.9.0 (Thompson et al., 1994; Hall, 1999), and compared to published sequences from this region (Ellis et al., 2009) for *A. obscurus* (three haplotypes, GenBank accession