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Dispersal of soil-dwelling clover root weevil (*Sitona lepidus* Gyllenhal, Coleoptera: Curculionidae) larvae in mixed plant communities

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ABSTRACT

Insect pests that have a root-feeding larval stage often cause the most sustained damage to plants because their attrition remains largely unseen, preventing early diagnosis and treatment. Characterising movement and dispersal patterns of subterranean insects is inherently difficult due to the difficulty in observing their behaviour. Our understanding of dispersal and movement patterns of soil-dwelling insects is therefore limited compared to above ground insect pests and tends to focus on vertical movements within the soil profile or assessments of coarse movement patterns taken from soil core measurements in the field. The objective of this study was to assess how the dispersal behaviour of the clover root weevil (CRW), *Sitona lepidus* larvae was affected by differing proportions of host (clover) and non-host (grass) plants under different soil water contents (SWC). This was undertaken in experimental mini-swards that allowed us to control plant community structure and soil water content. CRW larval survival was not affected either by white clover content or planting pattern or SWC in either experiment; however, lower clover composition in the sward resulted in CRW larvae dispersing further from where they hatched. Because survival was the same regardless of clover density, the proportion of infested plants was highest in sward boxes with the fewest clover plants (i.e. the low host plant density). Thus, there is potential for clover plants over a larger area to be colonised when the clover content of the sward is low.

1. Introduction

Insect pests that have a root-feeding larval stage often cause the most sustained damage to plants because their attrition remains largely unseen, preventing early diagnosis and treatment (Brown and Gange, 1990; Hunter, 2001; Blackshaw and Kerry, 2008). The spatial distribution of soil dwelling insect larvae is determined by a number of factors. These include the host specificity of the above-ground adult stage; the oviposition site of the parent, the host specificity of the below-ground larval stage, its ability to move through the soil matrix, and soil physical conditions (Villani and Wright, 1990). These factors typically contribute to the highly aggregated populations of insects found below-ground (Brown and Gange, 1990).

Characterising movement and dispersal patterns of subterranean insects is inherently difficult due to the problems in observing their behaviour. Laboratory approaches to study small scale movements have been reviewed by Dawson and Byers (2008) and include Petri-dish 'arenas' (Johnson et al., 2005), nutrient slant-boards (Kendall and Leath, 1974; Murray and Clements, 1992a; Dawson et al., 2004) and 'soil olfactometers' (Johnson et al., 2004a). More recent innovations have also included X-ray tomography (Johnson et al., 2004b) where it is possible to visualise larval movement in specially packed soils in small soil columns, and acoustic techniques (Mankin and Fisher, 2002) which have been used for detecting the presence of assemblages of large insect larvae in natural soil systems. Whilst both of these approaches are useful, the former can only be used on a small scale (<50 mm) with extremely simple root systems and the latter is only sensitive to coarse movements of large invertebrates (Mankin et al., 2008).

Our understanding of dispersal and movement patterns of soil-dwelling insects is therefore limited compared to above-ground insect pests (Stinner et al., 1983), and tends to focus on vertical movements within the soil profile (e.g. Dowdy, 1944; Pacchioli and Hower, 2004) or assessments of coarse movement patterns taken from soil core measurements in the field (e.g. Gange et al., 1991; Blackshaw and Vernon, 2006). In particular, little is known about how soil-dwelling insects disperse in terms of plant community composition and soil conditions. The objective of this study was to assess how the dispersal of the clover root weevil (CRW), *Sitona lepidus*, Gyllenhal (Coleoptera: Curculionidae) larvae was affected by differing proportions of host (clover) and non-host (grass) plants under different soil water contents (SWC). This was undertaken in

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experimental mini-swards that allowed us to control plant community structure and soil water content.

The CRW is common in temperate grasslands in Northern latitudes (Bright, 1994; Murray and Clements, 1995) and has recently arrived in New Zealand (Barratt et al., 1996). This species is injurious to both white clover (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.) and is one of the main reasons why these species often fail to thrive in grassland systems. Both the adult and the soil dwelling larval stages of the insect attack the host plant. The adults have an above-ground habit and feed on the foliage of the plants where they oviposit. The eggs are transferred to the soil by the action of rainfall and/or wind. The eggs hatch in the soil and the 1 mm long larvae tend to feed on the nitrogen-fixing nodules on the roots (Murray and Clements, 1992b; Hackell and Gerard, 2004). Johnson et al. (2004b) determined that the larvae actively seek out nodules, responding to cues from the target host plant, and that they could discriminate between host plant and non-host plant roots from up to 60 mm away (Johnson et al., 2004a). The adult weevil disperses either by flying or crawling, although the extent to which the larvae disperse below-ground is not known. In this study we hypothesised that *S. lepidus* larvae will disperse further at lower host plant densities.

2. Materials and methods

Adults of the CRW were collected from a field site at North Wyke Research in the South West of England (Lat. 50° 46′ Long. 3° 54′). The weevils were held in oviposition cages in the laboratory and supplied with fresh clover leaves. Eggs were collected daily and stored in Petri-dishes on moist filter paper in a refrigerator at 2 °C until required (Murray et al., 2007). In this study, we used clonal plants of *T. repens* (white clover cv Gwenda), which were established from stolon cuttings and perennial ryegrass plants (*Lolium perenne* L.) which were established from tillers. All plants were grown and maintained in segmented seed trays under ambient conditions in the glasshouse. When required, the plants were transferred into 400 mm × 600 mm sward boxes packed to a depth of 65 mm at a standard bulk density of approximately 1 g cm^{-3} with a loam-based compost containing 7 parts loam: 3 parts peat: 2 parts sand (John Innes No. 3) which had been air dried and sieved to <2 mm. A mini-sward was created in each box by planting with a total of 24 plants in a 4 × 6 matrix. Two experiments were carried out in this study.

2.1. Experiment 1

Three planting patterns of grass and clover plants were established to give clover/grass proportions of 75/25, 50/50 and 25/75% (high, medium and low densities, respectively, Fig. 1). The soil in each box received 2 L of water on each of two consecutive days prior to planting with the grass and clover plants. Two levels of soil water content (SWC), ca. 40 (±5) and 20 (±5)% by volume, were maintained by monitoring with Theta probes (Delta-T devices Ltd., Cambridge, UK) embedded in the soil, and watering as necessary over the experimental period. In total, 18 boxes (6 of each planting pattern) were established in the glasshouse. After an 18 d establishment phase the boxes were randomly assigned to treatments with three replicate boxes of each planting pattern and SWC. Ten viable eggs of *S. lepidus* were added to clover plants at positions denoted by the black cells shown in Fig. 1. These positions were chosen so that the resultant larvae only had the options of lateral or forward movement. Eggs were removed from the Petri-dish and placed in 5 ml of water which was then added around the base of the clover plant using a pipette. This plant was designated the 'start' plant.

After 98 d, the boxes were destructively sampled by individually removing the entire (soil and plant) contents of each

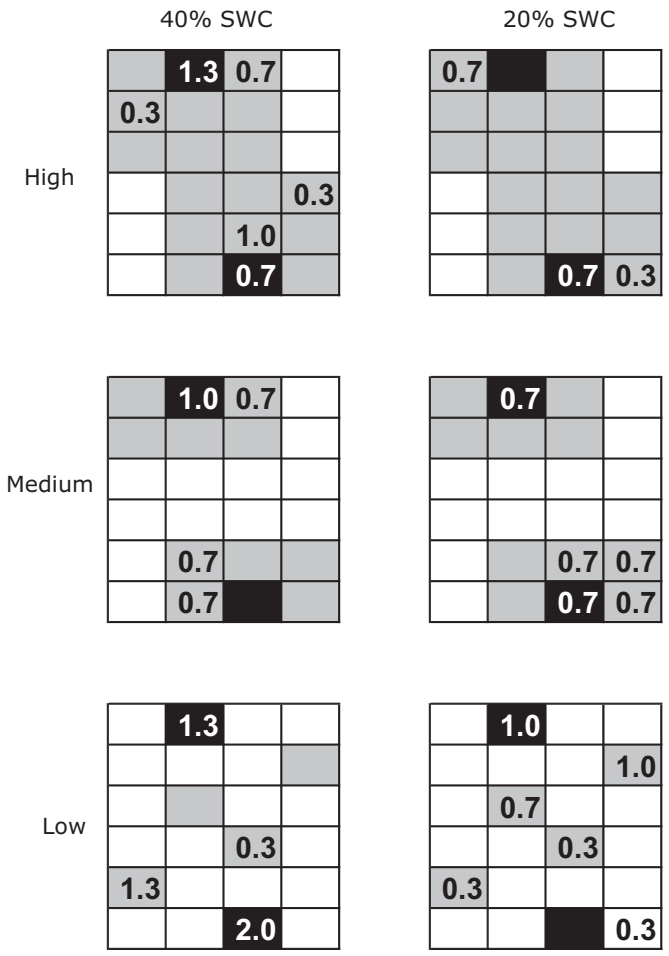

Fig. 1. Planting patterns of the mini-swards in Experiment 1. The diagrams each represent a sward box 60 cm × 40 cm divided into 24 cells each containing a single plant. Shaded cells contained a white clover plant, unshaded cells a perennial ryegrass plant, black cells contained a clover plant infested with 10 *Sitona lepidus* eggs. Mean numbers of larvae found on each plant are shown within that cell ($n = 3$).

100 mm × 100 mm cell. To extract the larvae the plant and soil from each cell was immersed in a saturated solution of $MgSO_4$ and agitated gently by hand; this caused the mineral and organic fractions to separate and the larvae float to the surface where, being white, they are easily spotted and collected. All larvae which were collected were stored in 70% ethanol solution.

2.2. Experiment 2

This experiment was conducted over the same duration as the previous study, to test whether systems with smaller colonies of plants, spatially separated, are more likely to aid dispersion of insects than systems with larger colonies of plants. Sward boxes were established using the same methods as above but using only one level of SWC (30%). The grass and clover plants were again planted in three (high, medium and low combinations): 71/29, 41/59, 21/79%, clover/grass, respectively with 5 replicates of each combination and 30 viable eggs were added to a single plant in each box (Fig. 2).

2.3. Statistical analysis

The effects, larval dispersal (defined as number of cells moved), soil water content, clover density, and their interactions were analysed in ANOVA using GENSTAT (VSN ltd., Hemel Hemp-