



Identification and distribution of leatherjackets (*Tipula* spp.) in the Republic of Ireland

A. Moffat^{1,2,3†}, M. T. Gaffney⁴, F. Brennan⁵, L. Cole², G. Jackson³, A. Konkolewska¹ and L. McNamara¹

¹Teagasc, Crop Research Centre, Oakpark, Carlow, Ireland

²Scottish Rural University College, Ayrshire, Scotland

³The School of GeoSciences, University of Edinburgh, Edinburgh, UK

⁴Horticulture Development Department, Teagasc, Ashtown, Dublin, Ireland

⁵Teagasc, Soils, Environment and Landuse Department, Johnstown Castle, Wexford, Ireland

Abstract

The soil-dwelling larval stage of crane flies, commonly known as leatherjackets, are classified as agricultural pests in Europe, and pests of turf in North America and Canada. They cause significant damage and yield loss in many cropping systems through their feeding on plant roots and stems at ground level. The effective chemical control for these pests, chlorpyrifos (available since 1965), was prohibited across Europe in 2019. This has left severely restricted control options for growers. Unlike Northern Ireland and Great Britain, no leatherjacket surveys or routine identifications have been conducted across Ireland. Therefore, the leatherjacket species of agronomic importance has not been confirmed. Since lifecycles, feeding behaviour and damage periods differ between species, identifying the most common species is a vital first step in any pest management strategy. Here we report key findings from a 2-yr structured survey of Irish crops, conducted in 2019 and 2021, where 135 sites were sampled. Both grassland and cereal crops were inspected. Soil cores and soil samples were collected and larval abundance determined. The European crane fly, *Tipula paludosa* Meigen, accounted for approximately 70% of larvae collected and identified ($n = 337$). In 2019, 40% of grasslands exceeded the threshold of 1 million larvae/ha, while only 3.3% of cereal fields were over the threshold of 600,000 larvae/ha. These results indicate that agricultural grasslands in Ireland have the potential to be significantly impacted by leatherjacket damage, although this may vary temporally and geographically across the island. Without effective control options, yield losses will be highly probable.

Keywords

Cereal • grass • leatherjacket • pest • survey • *Tipula*

Introduction

Leatherjackets are the larval stage of *Tipula* adults, also known as crane flies. They are apodous, soil-dwelling grubs that feed on the roots and shoots of a wide range of crops. Two species are known to be of agricultural importance: *Tipula paludosa* Meigen, known to affect mostly permanent pastures and spring cereals, and the marsh crane fly, *Tipula oleracea* Linnaeus, widely reported to affect winter cereals planted after oilseed rape (Benefer *et al.*, 2017). *Tipula paludosa* larvae are present from October until late July, and so have the ability to inflict serious feeding damage, especially in spring when they are most active. In contrast, *T. oleracea* are bivoltine, meaning they have two active larval stages per year, targeting crops from December to March and June to August.

Chlorpyrifos, a broad-spectrum organophosphate insecticide, was previously used for leatherjacket control through spray application and became the only chemical control option. This insecticide came under review within the European Commission in 2016 and has since been prohibited for use in European agriculture since 2019, due to its genotoxic potential. Similar restrictions are being applied elsewhere, with the Environmental Protection Agency (EPA) prohibiting use in some American states, such as California, Hawaii, New York, Maryland and Oregon. This severely restricted the options available to farmers for leatherjacket control. While it is known that ploughing can reduce populations by up to 50% (Evans, 2003), current European Union (EU) policies, such as the Farm to Fork Strategy and the Zero Pollution Action

†Corresponding author: A. Moffat
E-mail: aislingmoffat@gmail.com

Plan, urgently emphasise the need for the maintenance of existing soil carbon stocks, which are protected by minimum or no tillage agricultural practice. These practices also reduce the production of methane and greenhouse gases, and are therefore promoted (van der Putten *et al.*, 2018; Veerman *et al.*, 2020; Köninger *et al.*, 2022).

In grasslands, larvae can live undisturbed and build in population over time, increasing the risk of serious economic damage. Total sward destruction has been recorded at populations of 4.9 million/ha (Blackshaw & Coll, 1999). However, measurable yield loss has also been detected within populations ranging from 0.5 to 1.14 million/ha, as summarised in Table 1. The degree of damage inflicted is associated with the overall condition of the sward itself. Shallow rooting soils and heavy applications of slurry in the autumn were often linked to high levels of leatherjacket damage in Northern Ireland (Blackshaw & Coll, 1999). Both of these factors increase plant stress, subsequently suppressing the plant's defence system. They also observed the additional damage inflicted by predatory birds searching and feeding on the larvae. Leatherjackets thrive in maritime temperate climates, as temperatures do not drop sufficiently in winter to induce a non-feeding diapause. In Ireland, grass growth stops when temperatures drop below 5°C (Brereton, 1981). With leatherjackets continuing to feed in such temperatures, this can increase the potential for yield losses and damage to occur. Aside from cultivation, the other control option available for leatherjacket control is the use of commercially available nematodes, such as *Steinernema feltiae* and *Steinernema carpocapsae* (Oestergaard *et al.*, 2006). Apart from being costly, nematodes are also species specific and need to be applied at precise stages of each species' lifecycle. For example, *T. oleracea* have been deemed more susceptible to *S. feltiae* infection compared with *T. paludosa* (Peters & Ehlers,

1994). Therefore, if the larval species are not identified, these biological control options may prove ineffective and extremely costly for the farmer. As Ireland has ideal conditions for leatherjacket survival, and because there are limited control options available, there is a need for research into alternative and sustainable management options.

An economic threshold sets a population level where the damage expected (i.e. yield loss) is equal to the cost of the control measures. Over the years, different economic thresholds have been suggested for leatherjackets in both grassland and cereal crops. The thresholds given in Table 1 summarise those recorded previously (from 1953 to 1994) and take into account the cost of fertiliser and insecticide application. While costs associated with yield loss and control vary, threshold tables remain static and thus are always out of date. Blackshaw (1984) calculated the average grassland losses incurred in Northern Ireland to be greater than half a tonne of DM per hectare per year. These losses were even higher in north-east England (French, 1969) and south-west Scotland. French (1969) reported losses of 200 kg DM/ha at populations of 1 million larvae/ha, while Newbold (1981) states losses of 400 kg DM/ha for first cut silage with populations of 2.5 million/ha. Previously, annual surveys of leatherjacket occurrence in agricultural settings were conducted in northern England (1948–1963) (Cohen, 1953; White, 1963), south-west England (1963–1974) (Mayor & Davies, 1976), Northern Ireland (1970–1988) (Blackshaw, 1983; Blackshaw & Perry, 1994) and Scotland (1987–2018) (Ahmed, 1968; McCracken *et al.*, 1995; Blackshaw & Petrovskii, 2007). Data collected from these studies helped to establish the limiting factors in relation to larval survival. However, to determine any new and alternative integrated pest management (IPM) strategies, knowledge of the specific pest species, its distribution and ecology is paramount. In this study, we conduct the first

Table 1: Summary table of all previous records regarding leatherjacket populations where (i) yield loss is evident (damage) and (ii) population densities are said to have reached an economic threshold (economic), where cost of control measures is equal to the damage inflicted

| Threshold (per/ha) | Production type | Economic/damage | Reference |
|--------------------|-----------------|-----------------|---------------------------------|
| 1×10^6 | Grassland | Economic | Maercks (1953) |
| 1×10^6 | Grassland | Damage | Lange (1963) |
| 0.5×10^6 | Grassland | Damage | Strickland (1965) |
| 2.5×10^6 | Grassland | Economic | French (1969) |
| 0.95×10^6 | Grassland | Damage | French (1969) |
| 2×10^6 | Grassland | Economic | Newbold (1981) |
| 1.14×10^6 | Grassland | Damage | Newbold (1981) |
| 1×10^6 | Grassland | Economic | Blackshaw (1984) |
| 0.25×10^6 | Cereals | Economic | Golightly (1967); Rayner (1969) |
| 6×10^5 | Cereals | Economic | Anon (1994) |

nationwide survey of Ireland to determine (i) the *Tipula* species of agronomic importance and (ii) the population levels of leatherjackets in Irish agricultural grasslands and cereals.

Methods

Surveying took place across 45 farms (135 fields; 75 grassland and 60 cereal) in 2019 and 2021, from February to May in both years. Field areas ranged between 0.2 and 20.4 ha in grassland and 0.7 and 19.1 ha in cereal. A total surface area of 640 ha was sampled across Ireland. Fields previously affected by leatherjacket damage were prioritised, and two additional fields were sampled per farm to include a broad range of observations, including soil types and characteristics, vegetation types and geoclimatic gradients. The fields chosen at each farm were uniform in terms of production system, that is, they were either all grassland fields or all cereal fields. Details of farm management techniques as well as individual field histories were recorded from participants at the time of sampling.

Survey techniques

All fields were walked in a standardised “W” pattern. In the grassland fields, 25 soil cores were taken evenly along this “W” pattern using a cylindrical pipe and a stainless steel corer, adapted slightly from McCracken *et al.* (1995). The cores were 6.5 cm in diameter and 12 cm in depth (the total volume of soil sampled was 331.9 cm³). Using the metal corer, the piping was pushed into the soil to a depth of 10 cm, which captured the zone where leatherjackets actively feed on grass roots. Within the cereal fields, a row scratching technique was instead used, as soils were generally too loose to collect using the core method. Row scratching involved measuring a 30 × 13 cm² area of soil and digging to 10 cm depth (Blackshaw *et al.*, 1996), and this was repeated 20 times throughout the field. The soil and any crops present were dug up, collected and returned to the laboratory.

Extraction process

All grassland cores were heat extracted following the “Blasdale” funnel method (Blasdale, 1974). A temperature of at least 40°C was necessary. At this temperature, the invertebrates moved down the soil column to escape the heat, and were collected in flasks below. Soil and roots collected from cereal fields were manually sorted in the laboratory to extract the leatherjackets. Invertebrates collected from both methods were retained in vials of 70% ethanol and stored at –20°C.

Morphological identification of larvae

64.5% of the larval samples collected from the 2-yr survey ($n = 526$) were visually identified under a standard stereomicroscope using a taxonomic key (Smith, 1989), noting

the shape of the lateral and ventral anal papillae. Blackshaw & Coll (1999) previously stated that this visual identification method is not wholly accurate and can be quite subjective. Therefore, to determine the accuracy of morphological identification, a subsample of 64 larvae additionally underwent molecular identification.

DNA barcoding of larval samples

Following the steps detailed by Benefer *et al.* (2017), a DNeasy[®] Blood and Tissue Kit (Qiagen, Ireland) was used for all extractions. As larval specimens were stored in ethanol, they first underwent a cleaning step where they were steeped in water and dried using blotting paper. A sterilised scalpel blade was used to cut a small abdominal segment from each specimen. The abdominal segments were placed in 1.5 mL tubes and were freeze-dried for 24 h. Following this, they were ground for 10 min. The DNA was then extracted following the advised protocol recommended by the manufacturer.

Aliquots of 8 µL were taken from the extracted DNA for quality control analysis, while the rest of the DNA was stored at –80°C for further amplification and sequencing. Quantification of the DNA was conducted using the Qubit (dsDNA HS Assay[®] Kit, ThermoFisher, Ireland), while DNA quality was assessed by Nanodrop (ThermoFisher, Ireland) and gel electrophoresis. All DNA with yields above 20 ng/µL underwent polymerase chain reaction (PCR).

PCR and amplification

A Qiagen Taq PCR Core Kit (Qiagen, Ireland) was used for performing the PCR on a Peltier Thermal Cycler (200). The cytochrome c oxidase subunit I (COI) primers used were those adapted by Benefer *et al.* (2017), from Ramirez-Gonzalez *et al.* (2013) at 28.4 nmol concentration (COI forward primer “TTTCAACAAATCATAARGAYATYGG” and COI reverse primer “TAAACTTCNGGRTGNCCAAAAAATCA”). The primers were diluted to 10 µM and the magnesium chloride to 3 µM. For the volumes used, see Table 2. The PCR cycle used consisted of 3 min of initial denaturation (94°C), 1 min of denaturation (35 cycles at 94°C), 30 s of annealing (at 52°C) and a 1-min extension phase (72°C) followed by 10 min of a final extension phase (at 72°C). The gel electrophoresis showed two bands per sample (Figure 1). In order to send the purest DNA for sampling, all samples underwent a further cleaning step, using a MinElute[®] Gel Extraction Kit (Qiagen, Ireland), and extracting the specified base pair (bp) region, 590–662 bp (Benefer *et al.*, 2017).

Sequencing

Once the DNA was amplified using PCR, and cleaned using the MinElute, solutions of 14 µL product were mixed with 4 µL of the forward primer (10 µM) and sent to LGC Genomics (Germany) for sequencing in the forward direction only. These

Table 2: Master mix for the DNA barcoding PCR

| Product | Amount/sample (µL) |
|--|--------------------|
| PCR buffer | 2.5 |
| dNTP | 0.5 |
| Forward primer | 2.5 |
| Reverse primer | 2.5 |
| Taq | 0.125 |
| MgCl | 3 |
| BSA | 2 |
| Distilled and deionised H ₂ O | 10.9 |
| DNA | 1 |

BSA = bovine serum albumin; dNTP = deoxynucleotide triphosphate; PCR = polymerase chain reaction.

primers target specifically the COI mitochondrial protein coding gene. The returned sequences were cleaned using FinchTV (Chromatogram viewer software) and were subsequently entered into the GenBank database in BLASTn (Basic Local Search Tool) to compare nucleotide sequences received with

sequences in the database. Using the “Percent ID” and “Query Cover” results generated from BLAST, comparisons to the visual assessment results were made and accuracy was calculated.

Results

Field populations

The mean leatherjacket populations across the 2 yr was 467,658/ha in grassland and 47,497/ha in cereal. Populations ranged from 0 to 3,408,750/ha in grasslands and 0 to 807,660/ha in cereal. Population levels and their distributions across Ireland are visible in Figures 2 and 3. It is evident that populations in cereal fields were considerably lower. The highest population recorded from a cereal site was recorded in April 2019, where winter wheat had followed oilseed rape, a crop notorious for trapping crane fly adults in its canopy. In 2019, 3.3% of cereal fields were above the given economic threshold of 600,000 leatherjackets/ha, followed by zero fields in 2021 (Table 3). Approximately 90% of all cereal fields were under some method of cultivation. Whereas in 2019, 40% of grassland fields had populations above the 1 million larvae/ha economic threshold level (Table 3).

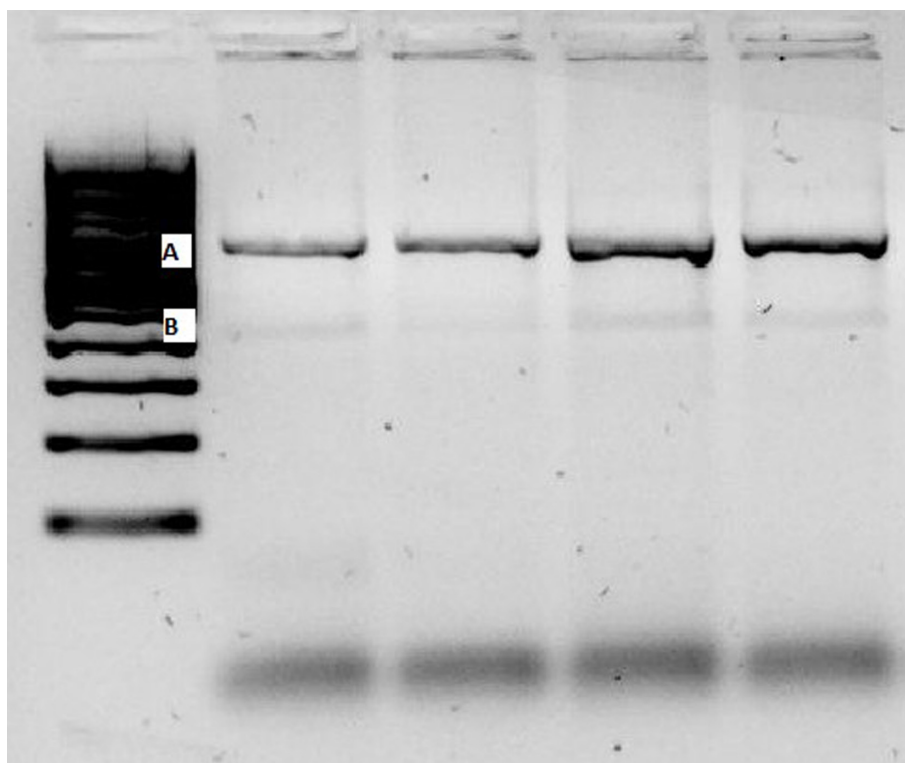


Figure 1. Image of gel electrophoresis showing two distinct bands (A and B), hence indicating the need for a further MinElute DNA clean-up step.

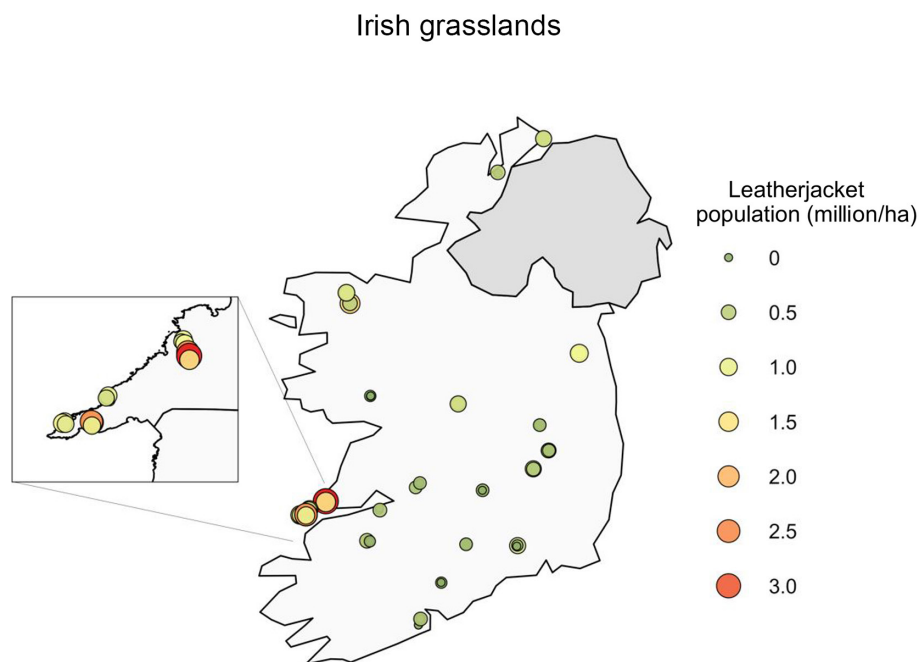


Figure 2. Map of Irish grassland sites sampled in 2019 and 2021, with a continuous coloured scale indicating leatherjacket population densities (million/ha). The threshold for grasslands is 1 million/ha. Sites on or above this are coloured yellow or red, respectively.

Species identification

Visual identification

Tipula paludosa represented 74.2% of the specimens visually identified, while *T. oleracea* accounted for 5.3%; 19.6% were classified as physically damaged, such that identification was not possible, while 0.9% were grouped as other possible *Tipula* species. These results are summarised in Table 4.

Molecular identification – DNA barcoding

Of the 64 samples sent for sequencing analysis, 76.6% were identified as *T. paludosa*, with an average query cover and percentage ID score of 95.9% and 98.3%, respectively; 10.9% were identified as *T. oleracea* with an average query cover and percentage ID score of 95.4% and 99.1%, respectively. Finally, 7.8% of samples were identified as neither *T. paludosa* nor *T. oleracea*, one of which was another crane fly species: *Nephrotoma flavescens* Linnaeus. A total of 4.7% ($n = 3$) of samples returned as unreadable, since no similarities of their sequences were found on the BLAST database. When these unreadable samples were excluded, the sequence lengths ranged from 160 to 790 bp, reflecting the differing quality of the sequence reads at either end.

In terms of visual accuracy for larval identification, of the 49 confirmed *T. paludosa* samples, 37 (75.5%) had identical

taxonomic and molecular identifications and four (8.2%) were wrongly identified as *T. oleracea* via visual assessment of morphological features. Of the seven confirmed *T. oleracea* samples, four (57.1%) matched with molecular assessment, and two (28.6%) did not. One *T. oleracea* identified sample was visually classified as damaged, accounting for the final sample (14.3%).

In all, 17.2% ($n = 11$) of the samples sent for sequencing had been visually classified as damaged. These consisted of samples in which the anal papillae were mutilated, making identification impossible. BLAST results for these samples are outlined in Table 5. Four samples were identified as *T. paludosa*, one as *T. oleracea* and two as *N. flavescens*. The BLAST database also detected one fungal species, *Saprolegnia megasperma* Coker, and one herb species, *Boechera divaricarpa* (A.Nelson) Á.Löve & D.Löve. Two samples were unreadable within the BLAST database.

A total of 9.4% of samples ($n = 6$) were classified as unknown, where visual identification was unattainable under the microscope. Four of these samples were then identified through BLAST to be *T. paludosa*. One sample was identified as a *Drosophila* chemical tag through the BLAST database system (synthetic construct clone UAS-7xHalo7::CAAX). The remaining sample was unreadable in the BLAST database. Unreadable samples represented 4.7% ($n = 3$) of samples sent for sequencing.

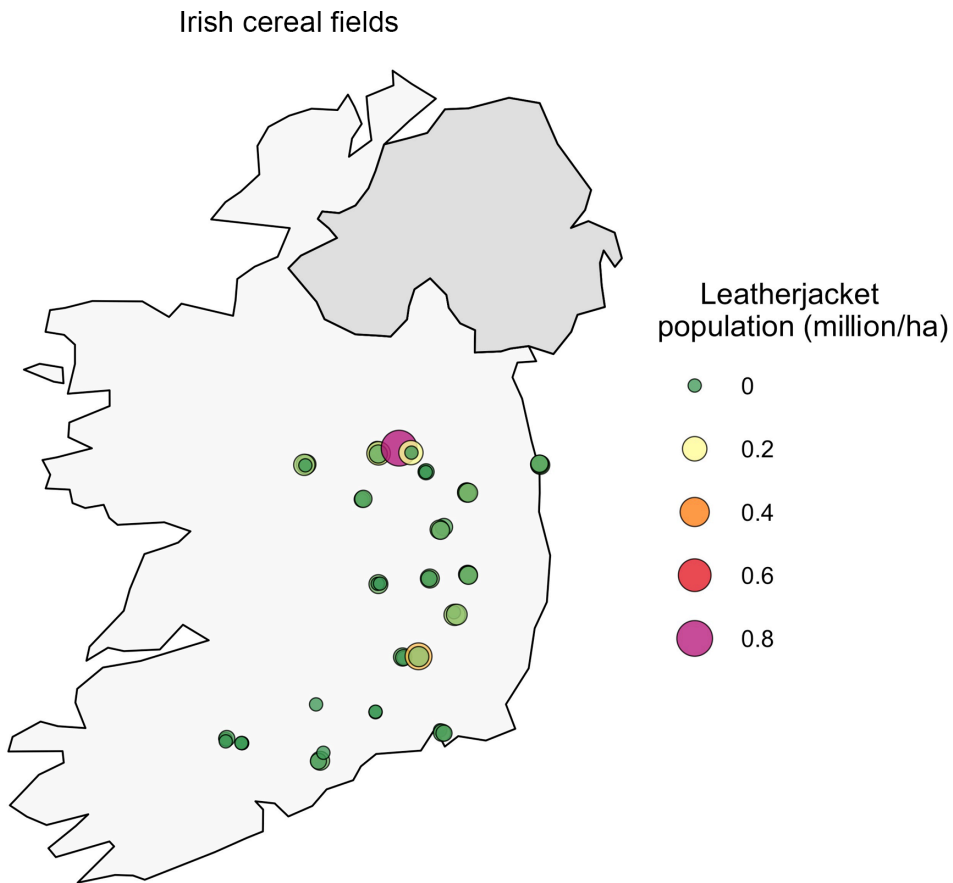


Figure 3. Map of Irish cereal/tillage sites sampled in 2019 and 2021, with a continuous coloured scale indicating leatherjacket population densities (million/ha). The threshold for cereal fields is 0.6 million/ha. Sites on or above this are coloured red or purple, respectively.

Table 3: Mean annual leatherjacket populations from the 2-yr Irish survey, allocated into population size categories and production system

| Production system | Year | Calculated mean population/ha | No. of fields sampled | % of fields in category (1×10^6 /ha [Blackshaw, 1983]) | | |
|-------------------|------|-------------------------------|-----------------------|--|------|------|
| | | | | <1 | 1–2 | >2 |
| Grassland | 2019 | 887,958 | 30 | 60.0 | 26.7 | 13.3 |
| | 2021 | 263,722 | 45 | 97.8 | 2.2 | 0 |
| | | | | % of fields in category (6×10^5 /ha [Anon, 1994]) | | |
| Tillage | 2019 | 656,500 | 30 | 96.7 | 3.3 | 0.0 |
| | 2021 | 298,792 | 30 | 100.0 | 0.0 | 0.0 |

Category 1 for grassland and tillage is any population under their respective thresholds (1×10^6 /ha and 6×10^5 /ha). Categories 2 and 3 are allocated as per Blackshaw (1983).

Table 4: *Tipula* species identification results from visual assessment of 337 larvae

| | Total (n) | <i>T. paludosa</i> | <i>T. oleracea</i> | Other species | Damaged samples |
|---------------|-----------|--------------------|--------------------|---------------|-----------------|
| Grassland (n) | 194 | 151 | 9 | 0 | 34 |
| Grassland (%) | | 77.8 | 4.6 | 0.0 | 17.5 |
| Tillage (n) | 143 | 99 | 9 | 3 | 32 |
| Tillage (%) | | 69.2 | 6.3 | 2.1 | 22.4 |
| Total (n) | 337 | 250 | 18 | 3 | 66 |
| Total (%) | | 74.2 | 5.3 | 0.9 | 19.6 |

Table 5: Results of the sequences entered into the BLAST database, including the average query cover (%) and average percentage ID (%)

| Species | Number of samples | Average query cover (%) | Average percentage ID (%) | Average base pair (bp) length |
|---|-------------------|-------------------------|---------------------------|-------------------------------|
| <i>Tipula paludosa</i> | 49 | 95.9 | 98.3 | 628.2 |
| <i>Tipula oleracea</i> | 7 | 95.4 | 99.1 | 665.1 |
| <i>Nephrotoma flavescens</i> | 2 | 95.0 | 99.45 | 660.0 |
| <i>Boechera divaricarpa</i> | 1 | 67.0 | 83.0 | 401.0 |
| <i>Saprolegnia megasperma</i> | 1 | 92.0 | 92.9 | 210.0 |
| Synthetic construct clone UAS-7xHalo7::CAAX | 1 | 98.0 | 99.5 | 790.0 |

Discussion

From the survey results, we can conclude that the main leatherjacket species of agronomic importance within Ireland is *T. paludosa*, accounting for approximately 70% of larvae collected. Compared with the most recent findings from south-west England, there is some variation. Of 142 larvae and 18 adults identified in a study by Benefer *et al.* (2017), 94% were *T. paludosa*. While the sample size for molecular identification was larger, sampling was focused only on grassland. The study described in this paper incorporates both grassland and cereal fields and of the 143 larvae visually identified from cereal fields, 69.2% were identified as *T. paludosa*, which from the literature was unexpected, as 42% ($n = 26$) of these fields were under winter sown crops, which are known to be targeted by *T. oleracea* (Blackshaw & Coll, 1999). Older studies from the UK and Northern Ireland report similar findings to Benefer, *et al.* (2017). Humphreys *et al.* (1993) used isoelectric focusing to determine leatherjacket species from grasslands across western Scotland and Northern Ireland in the 1990s. This is a method of separating larval proteins using silver staining. Once within the pH range of 5–6, two distinct bands could be seen for *T. oleracea* and only one for *T. paludosa*. From this technique it was determined that from a total of 411 larvae collected in western Scotland in 1990/1991, 93.3% were identified as *T. paludosa* and 4.4% as *T. oleracea*. In the same

year, 100% of larvae ($n = 57$) were identified as *T. paludosa* in north-eastern Scotland and 93.9% ($n = 723$) in Northern Ireland (Humphreys *et al.*, 1993). In our study, sample sizes are smaller (337 larvae for visual identification and 64 larvae for molecular identification); however, consistent numbers of larvae are being identified as *T. oleracea*; 5%, and 11%, with equal numbers coming from cereal and grassland fields. This indicates that in Ireland, there is a greater prevalence of *T. oleracea* present within agricultural settings. Both Humphreys *et al.* (1993) and Benefer *et al.* (2017) reported visual sightings of *T. oleracea* adults; therefore, it has been suggested that there are species-specific oviposition preferences and/or differences in dispersal ability (Blackshaw *et al.*, 1996; Benefer *et al.*, 2016, 2017).

Accurate species identification is a vital point of information when planning a control and management strategy. As mentioned previously, *T. paludosa* larvae are soil dwelling for up to 11 mo. They pass through their first two instars relatively quickly, therefore leaving a narrow window for control, as it is the first larval instar that is most vulnerable (Oestergaard *et al.*, 2006). To target this stage, any control measures should be executed in the early autumn. While *T. oleracea* occur in lower numbers, it is important to monitor their populations over time. In other areas around the world, such as North America, they are causing economic damage to the turf grass and sod production industry (Rao *et al.*, 2006; Peck & Olmstead, 2009; Peck *et al.*, 2010). In contrast to *T. paludosa*, any control measures for *T. oleracea* should

be implemented in both the autumn and summer, to combat their bivoltine lifecycle.

From the survey results, the higher population average in grassland (467,658/ha) compared with tillage (47,497/ha) indicates that Irish grasslands are much more vulnerable to leatherjacket infestations than Irish cereal fields. This could be due to the advantageous use of cultivation techniques such as ploughing before the main oviposition period, which reduces populations. When comparing the mean leatherjacket populations within Irish grassland with those found within the 17-yr grassland survey of Northern Ireland (Blackshaw, 1983), the average population of 887,958/ha (from range 0 to 3,408,750/ha) in 2019 is higher than any of the average populations reported in Blackshaw's study, which range from 297,175 to 779,006/ha. This is also seen in the percentage of fields above the given threshold of 1 million/ha (40% in 2019). However, grassland populations are lower in 2021, with 97.8% of the fields surveyed having larval populations of less than a million per/ha. Year-on-year fluctuations have been reported (Mayor & Davies, 1976) with authors also noting marked differences between different parts of the country (UK). In Ireland, from the heat map (Figure 2) it is clear that larval populations are highest along the western seaboard. As mentioned by Blackshaw & Coll (1999), it was evident that damage was exacerbated at these locations due to predatory gulls feeding on the larvae. There was anecdotal evidence from farmers that seagulls were disturbing the sward by ripping up sods of turf. In south-west England between the years 1963 and 1970, the mean leatherjacket populations ranged from 80,000 to 1,027,000/ha (Mayor & Davies, 1976). While chemical control was still available then, these peaks and troughs in larval populations show the natural fluctuations over time, which are often dictated by the weather in mid-late September, which is the oviposition period (Mayor & Davies, 1976).

The latest economic threshold for grasslands, of 1 million larvae/ha, was published in 1984 by Blackshaw. This threshold was originally based on the value of herbage DM, the cost of insecticide and the cost of fertiliser application. However, since then, there has been a withdrawal of the broad-spectrum insecticide, chlorpyrifos, which was used for the active management of these larval pests. Furthermore, the price of nitrogen fertiliser has increased dramatically in Ireland, with prices increasing by 149% in 1 yr (Agricultural Price Indices March 2022 – CSO – Central Statistics Office, 2022). This highlights the need for economic thresholds to be revisited annually; however, across a range of pest groups, they typically remain static. These contributing factors suggest the need for a revision of leatherjacket threshold limits; however, having a threshold relies on having associated effective control measures, which are currently lacking for leatherjackets in Ireland.

With the prohibition of chlorpyrifos from Europe, alternative non-chemical controls for use within agriculture are in high demand. Blackshaw (2009) discussed leatherjacket management on organic farms and stated two objectives for pest management in organic rotations: (i) ensuring larval populations are managed through the crop rotation and do not reach damage thresholds when a susceptible crop is grown and (ii) taking necessary intervention measures against large pest populations present in the immediate crop. Some suggested control approaches for organic cereal farmers include limiting the fertility building phase of a rotation to a maximum of 2 yr and use of autumn applications of biological control agents preceding a spring-sown arable crop (Blackshaw, 2009). While biological control options, such as the entomopathogenic nematodes, *S. feltiae* and *S. carpocapsae*, have been deemed to be effective (80% mortality through autumn application [Oestergaard *et al.*, 2006]), they have been researched within laboratory conditions, and often application is not suited to all soil and sward types. Furthermore, the suggestion of an annual autumnal prophylactic application at farm scale (Blackshaw, 2009) is likely to be prohibitively costly. Blackshaw's review (2009) into leatherjacket management in organic systems focused on cereals after grass leys. Organic options for permanent pastures have been largely overlooked. While Blackshaw does mention restricting grass-clover swards to a maximum of 2 yr, from an Irish agricultural perspective, this is a very popular and common sward mix, which could lead to increased leatherjacket populations. More research is therefore needed into feasible, alternative sward mixes for permanent pastures.

Conclusion

In conclusion, since the predominant *Tipula* species feeding on arable and pasture crops has been identified as *T. paludosa*, the first step in developing an effective IPM strategy against leatherjackets in Ireland is achieved. Given the variation of grassland populations between the two sampling years, the effectiveness or relevance of the existing threshold could not be determined. However, as previously stated, an effective economic threshold is only effective when an appropriate active control product is available, which is currently not the case in Ireland. Therefore, it may be necessary to re-evaluate how we manage persistent, soil-based insect pests such as leatherjackets and focus more heavily on continuous active management and population suppression through rotation and soil cultivation. This should not be underestimated as the registration of any insecticide product for use as a soil-based insecticide is unlikely under the European regulatory regime. Our study highlights the lack of management options for grasslands specifically, and emphasises the need for

further research into viable and effective pest management techniques. Those which complement current European policy such as sward cultivars and mixes, soil microbial community analysis and potential biocontrol options, are of utmost importance.

Acknowledgements

The authors acknowledge the research funding received from Teagasc, Agriculture and Food Development Authority. The farmer involvement, including that of the Danú Farming Group, is greatly appreciated, as well as the technical help from Liam Shepard and the interns of the Entomology team at Teagasc Oakpark.

References

- Ahmed, M. 1968. A study of the Tipulidae (Diptera) of agricultural importance in south-east Scotland. PhD thesis, University of Edinburgh.
- Anon. 1994. Leatherjackets and their control. SAC Technical Note T361. 1HF. SAC, West Mains Road, Edinburgh.
- Benefer, C.M., D'Ahmed, K.S., Blackshaw, R.P., Sint, H.M. and Murray, P.J. 2016. The distribution of soil insects across three spatial scales in agricultural grassland. *Frontiers in Ecology and Evolution* **4**: 41.
- Benefer, C.M., D'Ahmed, K.S., Murray, P.J. and Blackshaw, R.P. 2017. Molecular identification and distribution of leatherjackets (Diptera: Tipulidae) in UK agricultural grassland. *Agricultural and Forest Entomology* **19**: 400–407.
- Blackshaw, R.P. 1983. The annual leatherjacket survey in Northern Ireland, 1965–82, and some factors affecting populations. *Plant Pathology* **32**: 345–349.
- Blackshaw, R.P. 1984. The impact of low numbers of leatherjackets on grass yield. *Grass and Forage Science* **39**: 339–344.
- Blackshaw, R.P. 2009. A comparison of management options for leatherjacket populations in organic crop rotations using mathematical models. *Agricultural and Forest Entomology* **11**: 197–203.
- Blackshaw, R.P. and Perry, J.N. 1994. Predicting leatherjacket population frequencies in Northern Ireland. *Annals of Applied Biology* **124**: 213–219.
- Blackshaw, R.P. and Coll, C. 1999. Economically important leatherjackets of grassland and cereals: biology, impact and control. *Integrated Pest Management Reviews* **4**: 145–162.
- Blackshaw, R.P. and Petrovskii, S.V. 2007. Limitation and regulation of ecological populations: a meta-analysis of *Tipula paludosa* field data. *Mathematical Modelling of Natural Phenomena* **2**: 46–62.
- Blackshaw, R.P., Coll, C., Humphreys, I.C. and Stewart, R.M. 1996. The epidemiology of a New Leatherjacket Pest (*Tipula oleracea*) of Winter Cereals in Northern Britain. HGCA Project Report 120 [WWW document]. Available online: http://cereals.ahdb.org.uk/media/369259/project_report_120.pdf [Accessed 15 June 2022].
- Blasdale, P. 1974. A method of turf sampling and extraction of leatherjackets. *Plant Pathology* **23**: 14–16.
- Brereton, A.J. 1981. Potential grass production under grazing. In: "Plant Physiology and Herbage Production", (ed. C.E. Wright), British Grassland Society Occasional Symposium No. 13, Pages 31–35.
- Cohen, M. 1953. Survey of leatherjacket populations in England and Wales 1946–51. *Plant Pathology* **2**: 80–82.
- CSO.ie. 2022. Agricultural Price Indices March 2022 - CSO - Central Statistics Office. Available online: <https://www.cso.ie/en/releasesandpublications/ep/p-api/agriculturalpriceindicesmarch2022/> [Accessed 15 June 2022].
- Evans, K.A. 2003. Wheat bulb fly and other pests of cereal. Technical Note 552. Scottish Agricultural College, London.
- French, N. 1969. Assessment of leatherjacket damage to grassland and economic aspects of control. *Proceedings of the 5th British Insecticide and Fungicide Conference*, pages 511–521.
- Golightly, W.H. 1967. Control of *Tipula* spp. larvae. *Plant Pathology* **16**: 33–35.
- Humphreys, I.C., Blackshaw, R.P., Stewart, R.M. and Coll, C. 1993. Differentiation between larvae of *Tipula paludosa* and *Tipula oleracea* (Diptera: Tipulidae) using isoelectric focusing, and their occurrence in grassland in northern Britain. *Annals of Applied Biology* **122**: 1–8.
- Königer, J., Panagos, P., Jones, A., Briones, M.J.I. and Orgiazzi, A. 2022. In defence of soil biodiversity: towards an inclusive protection in the European Union. *Biological Conservation* **268**: 109475.
- Lange, B. 1963. Der heutige Stand der Tipula – Bekämpfung in Hinblick auf die Befallslage 1962/63. *Anzeiger für Schadlinsekunde Pflanzenschutz Umweltschutz* **36**: 88–93.
- Maercks, H. 1953. U ber den Massenwechsel von *Tipula paludosa* Meig., in den Jahren 1918–1953 und seine Abhängigkeit von der Witterung. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* **4**: 129–133.
- Mayor, J.G. and Davies, M.H. 1976. A survey of leatherjacket populations in south-west England, 1963–1974. *Plant Pathology* **25**: 121–128.
- McCracken, D.I., Foster, G.N. and Kelly, A. 1995. Factors affecting the size of leatherjacket (Diptera: Tipulidae) populations in pastures in the west of Scotland. *Applied Soil Ecology* **2**: 203–213.
- Newbold, J.W. 1981. The control of leatherjackets in grassland by winter pesticide applications. *Proceedings of Crop Protection in Northern Britain*, pages 207–211.
- Oestergaard, J., Belau, C., Strauch, O., Ester, A., van Rozen, K. and Ehlers, R.U. 2006. Biological control of *Tipula paludosa* (Diptera: Nematocera) using entomopathogenic nematodes (*Steinernema* spp.) and *Bacillus thuringiensis* subsp. *israelensis*. *Biological Control* **39**: 525–531.
- Peck, D.C. and Olmstead, D. 2009. Invasive *Tipula* (Diptera: Tipulidae) in turfgrass of the northeast United States: geographic

- distribution and local incidence three years after detection. *Journal of Economic Entomology* **102**: 652–658.
- Peck, D.C., Olmstead, D.L. and Petersen, M.J. 2010. Pest status of invasive crane flies in New York turfgrass and the repercussions for regional plant protection. *Journal of Integrated Pest Management* **1**: E1–E8.
- Peters, A. and Ehlers, R.U. 1994. Susceptibility of leatherjackets (*Tipula paludosa* and *Tipula oleracea*; Tipulidae; Nematocera) to the entomopathogenic nematode *Steinernema feltiae*. *Journal of Invertebrate Pathology* **63**: 163–171.
- Ramirez-Gonzalez, R., Yu, D.W., Bruce, C., Heavens, D., Caccamo, M. and Emerson, B.C. 2013. PyroClean: denoising pyrosequences from protein-coding amplicons for the recovery of interspecific and intraspecific genetic variation. *Public Library of Science One*, 8(3), p.e57615.
- Rao, S., Liston, A., Crampton, L. and Takeyasu, J. 2006. Identification of larvae of exotic *Tipula paludosa* (Diptera: Tipulidae) and *T. oleracea* in North America using mitochondrial cytb sequences. *Annals of the Entomological Society of America* **99**: 33–40.
- Rayner, J.M. 1969. Chemical control of leatherjackets (larvae of *Tipula* spp.) in spring cereals with special reference to alternatives to persistent organochlorine insecticides. *Proceedings of the 5th British Insecticide and Fungicide Conference*, pages 71–77.
- Strickland, A.H. 1965. Pest control and productivity in British Agriculture. *Journal of the Royal Society of Arts* **113**: 62–81.
- Smith, K.G. 1989. An introduction to the immature stages of British flies: Diptera larvae, with notes on eggs, puparia and pupae (Vol. 10, No. 14). *Royal Entomological Society*.
- van der Putten, W.H., Ramirez, K.S., Poesen, J., Winding, A., Lemanceau, P., Lisa, L., Simek, M., Moora, M., Setälä, H., Zaitsev, A. and Economou-Eliopoulos, M. 2018. Opportunities for Soil Sustainability in Europe. Available online: https://easac.eu/fileadmin/PDF_s/reports_statements/Soil_Sustainability/EASAC_Policy_Report_on_Soil_Sustainability_September_2018.pdf.
- Veerman, C., Pinto Correia, T., Bastioli, C., Biro, B., Bouma, J. and Ciencielä, E. 2020. Caring for Soil is Caring for Life. EU Soil Health and Food Mission Board: Brussels, Belgium. Available online: https://ec-europa-eu.ezproxy.is.ed.ac.uk/info/publications/caring-soil-caring-life_en.
- White, J.H. 1963. Observations on leatherjacket population assessment, forecasting and control measures. *Proceedings of the 2nd British Insecticide and Fungicide Conference*, pages 51–58.