ENIGMA I:

Sustainable Wireworm IPM

A collaborative industry project

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Thank you to all of our partners

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WIREWORM – KNOWLEDGE AND CAPABILITY GAPS IN THE UK

The pattern of damage has changed over recent years:

- The amount of damage has increased.
- Damage now occurs in crops on land where grass has never been grown.
- Occurs earlier in the season, making it impossible to avoid by lifting root crops early.

Monitoring is pivotal to IPM in any crop system but there are vast improvements to be made:

- Sampling protocols need to be improved.
- It is difficult/impossible to visually identify some larvae to species.
- Impossible to visually identify small larvae of any species.
- We need to be able to predict where and when they will cause damage.

We needed a greater understanding of the effects of:

- Climate change
- Changing crop rotation practices
- The impacts of cover crops .

Very little research had been done on wireworm since the interwar period.

"We need a better understanding of which species are causing damage in which crops and the parameters which influence this."

PROJECT AIMS

Milestone
1. Project hub website released to project partners
2. Sample collection, extraction and identification of adults and wireworms as far as is possible using existing keys
3. DNA barcoding wireworms
4. DNA meta-barcoding wireworms
5. DNA barcoding gut contents and frass from damaged crops
6. Literature reviews to identify gaps in information required for modelling and decision support for 5 species of greatest concern
7. Glasshouse studies with cover crops and life history studies in lab cultures and from field samples to fill gaps in information required for modelling and decision support for the 5 species of greatest concern
8. Climex modelling completed for the 5 species of greatest concern
9. Production of guidelines and decision support material

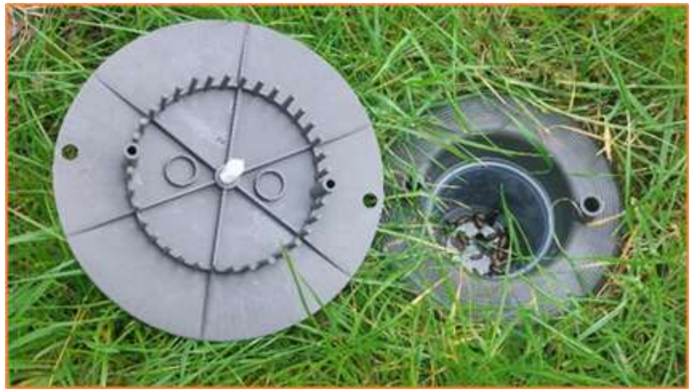
MILESTONE 2 - Sample collection, extraction and identification of adults and wireworms as far as is possible using existing keys

Samples collected for barcoding and adult monitoring via pheromone trapping.

- Larvae and adults of click beetle pest species collected by Martyn Cox and Bruno Ngala were identified by Fera's Entomology diagnostician Rowan Howe as far as taxonomically possible, then passed to the Molecular Biology Team for sequencing.

- Project partners monitored *Agriotes lineatus, A. obscurus* and *A. sputator* using pheromone lures in Vernon pitfall traps.


Agrypnus murinus




Adrastus sp.


Athos sp.


Agriotes lineatus


Agriotes obscurus

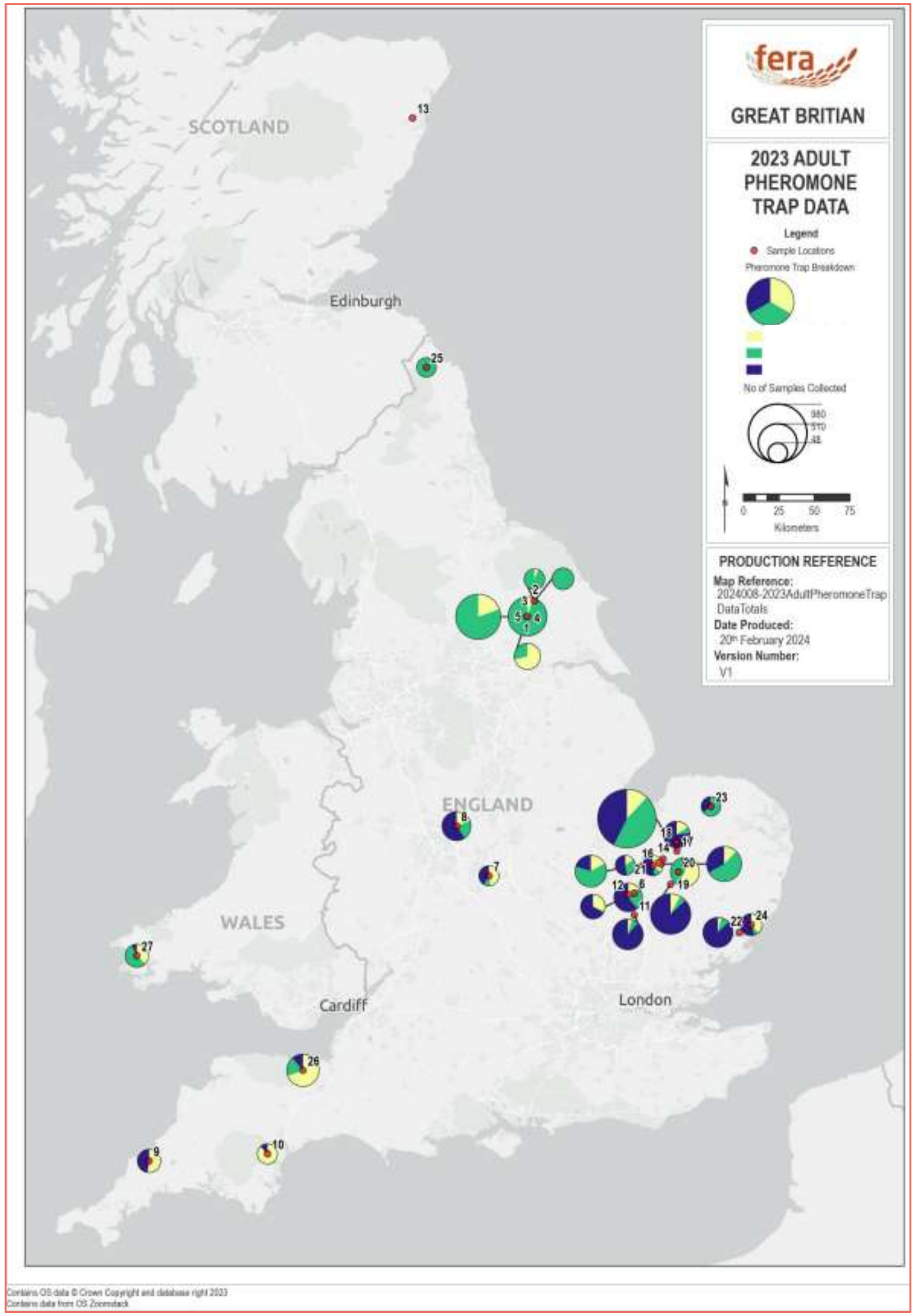

Agriotes sputator

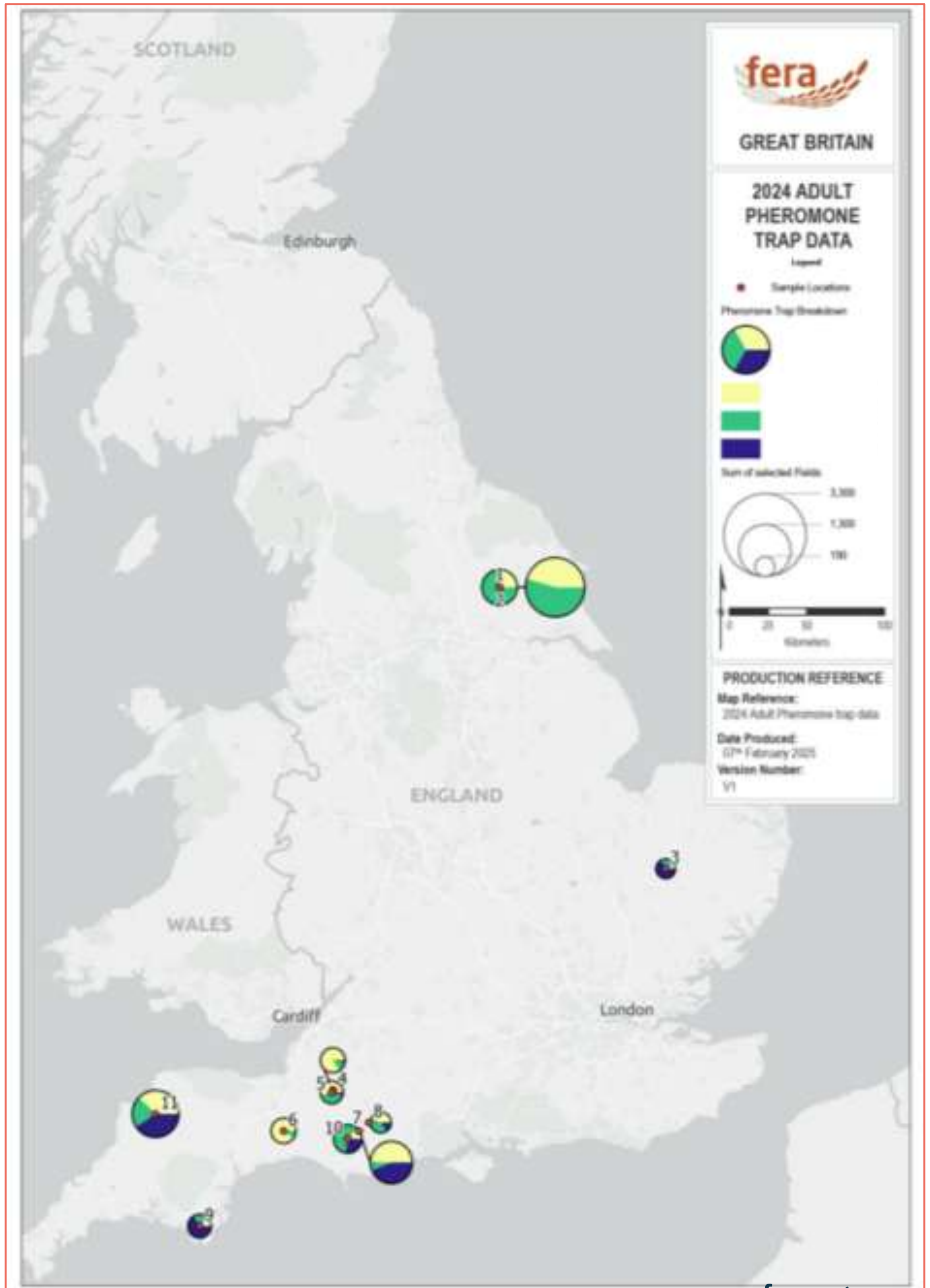
Adult and larval *Agriotes* species were also collected for life history and cover crop studies.

- Bait ball trapping was used to collect larvae for cover crop studies.
- Pheromone traps predominantly capture males.
- Forage traps were used to collect females for life history and cover crop studies studies.
- Modified beetle flight traps were tested but these did not catch any beetles.

Adult *Agriotes* spp. monitoring from pheromone traps located across Great Britain between 2023 and 2024

- 2023 – 6,703 adult *Agriotes* identified
- 2024 – 5,857 adult Agriotes identified





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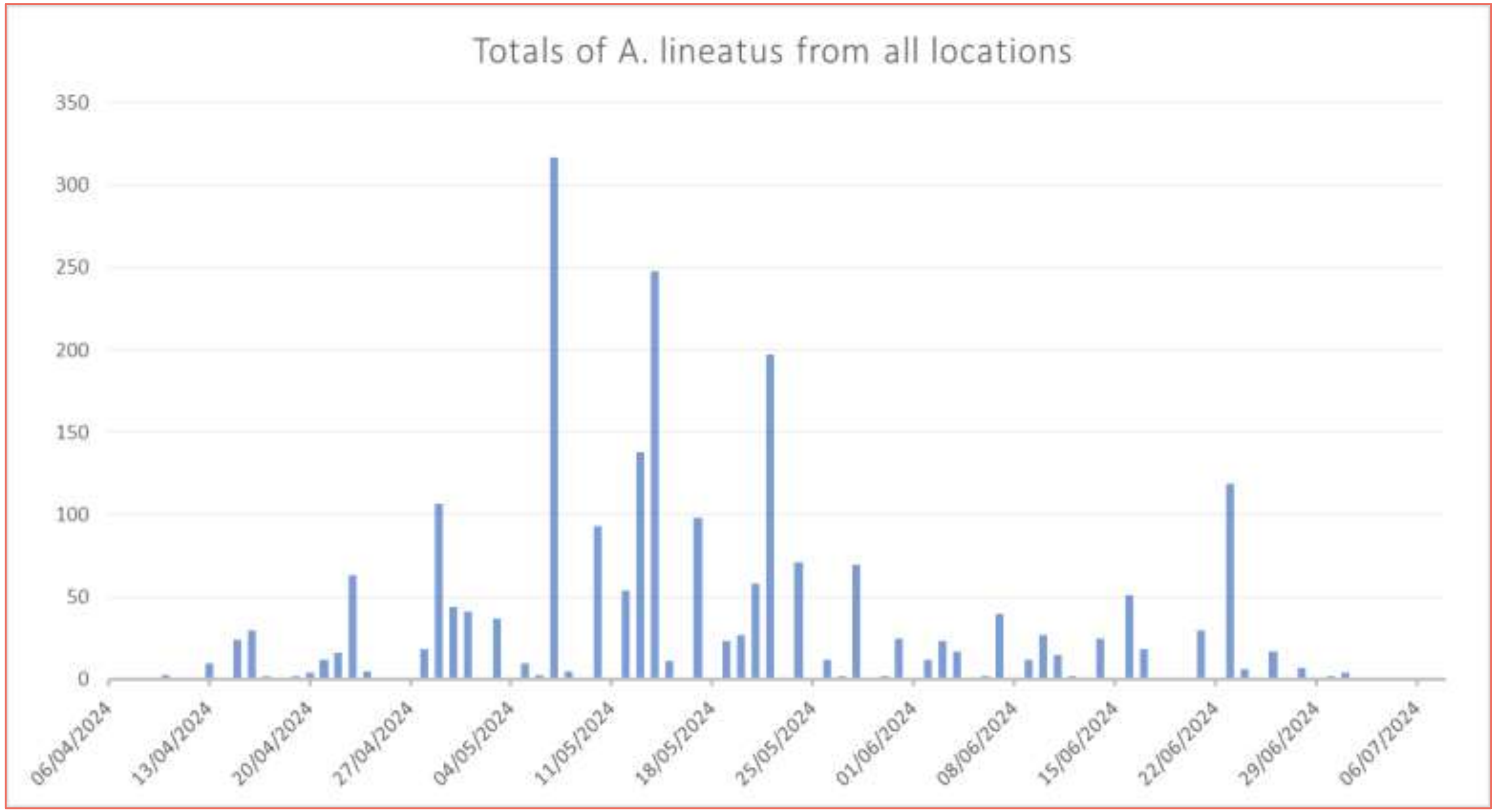
Combined data - indicates emergence of *Agriotes* species in April and peak activity of all three species later in May.



MILESTONE 6 – Literature review to identify gaps in information required for modelling and decision support for species of greatest concern

We have identified the six species of greatest concern for the UK as:

1. *Agriotes lineatus*
2. *Agriotes sputator*
3. *Agriotes obscurus*
4. *Agrypnus murinus*
5. *Athous haemorrhoidalis*
6. *Hemicrepidius niger*

The aim was to develop an understanding of the relationship between the effects of temperature and moisture on the development of wireworm larvae for two purposes:

- To allow us to understand which crop the adult females had previously oviposited into
- To allow us to predict life cycle stages to provide information for decision-making

MILESTONE 6 – Literature review to identify gaps in information required for modelling and decision support for species of greatest concern

- Large gaps in our knowledge about life cycle and the relationship between life cycle and soil temperature, including timing of larval instars (stages).
- *A. obscurus* has been more extensively studied than the other species of interest.
- Information on the number of degree days required to complete development of each life stage.
- Some information on the upper and lower thermotolerance levels.
- Some information on soil moisture and its influence on activity and feeding but not specifically on wireworm development.
- Agreement within the literature that these species likely to have broadly similar life cycles, however, some differences in their requirements likely.
- Conclusion: Life history studies under controlled environmental conditions would be required to determine the parameters needed for accurate Climex modelling.

Stage egg/ larval instar/ pupa	Mean ± SD days to complete	Range (days)	Mean heat sum > 9°C
Egg	22.5	17 -25	248
L1	17.3 ± 1.36	15 -18	190
L2	25.5 ± 10.11	18 - 48	281
L3	46.9 ± 15.73	33 - 89	516
L4	65.8 ± 17.64	46 - 113	723
L5	83.3 ± 16.82	45 -109	917
L6	100.9 ± 34.48	24 - 153	1110
L7	98.3 ± 43.39	34 - 153	1082
L8	67.7 ± 35.21	29 - 137	744
L9	83.5 ± 31.65	37 -120	919
L10	107.2 ± 19.49	80 - 133	1179
L11	144.3 ±7.50	133 - 148	1587
Total (L1 – L11)		494 - 1221	9248
Pupa	15.4 ± 2.15	13 -20	169.5

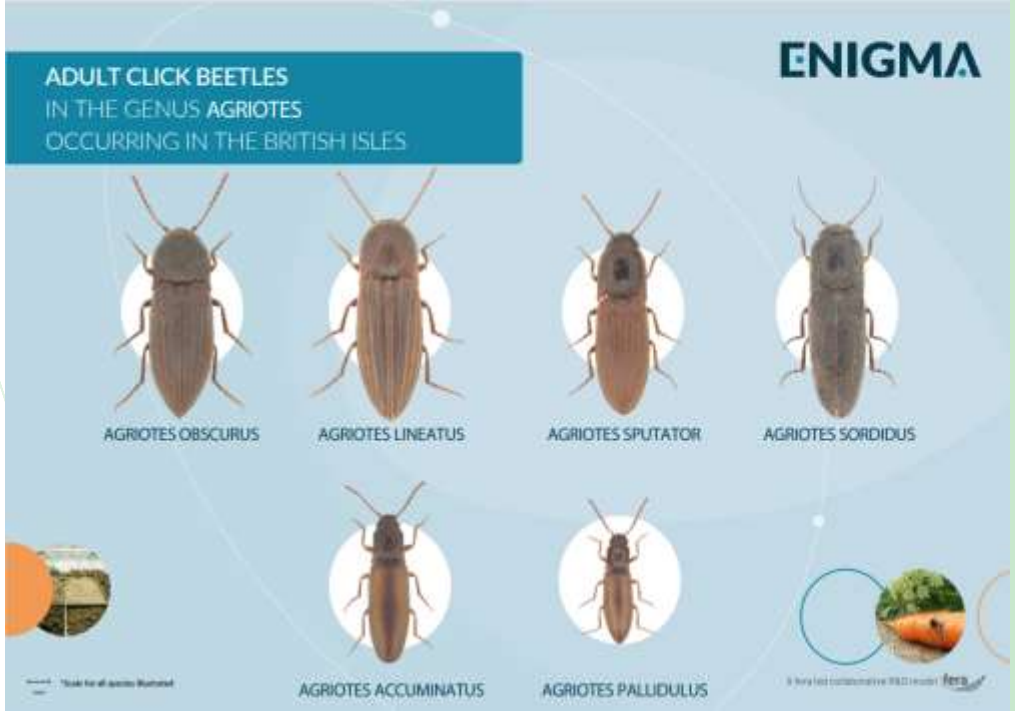
Data from Sufyan *et al.* (2014). The mean duration (number of days ± standard deviation of each developmental stage, and average day degrees above a base of 9°C (heat sum) to complete each developmental stages for *A. obscurus* reared at a constant 20°C.

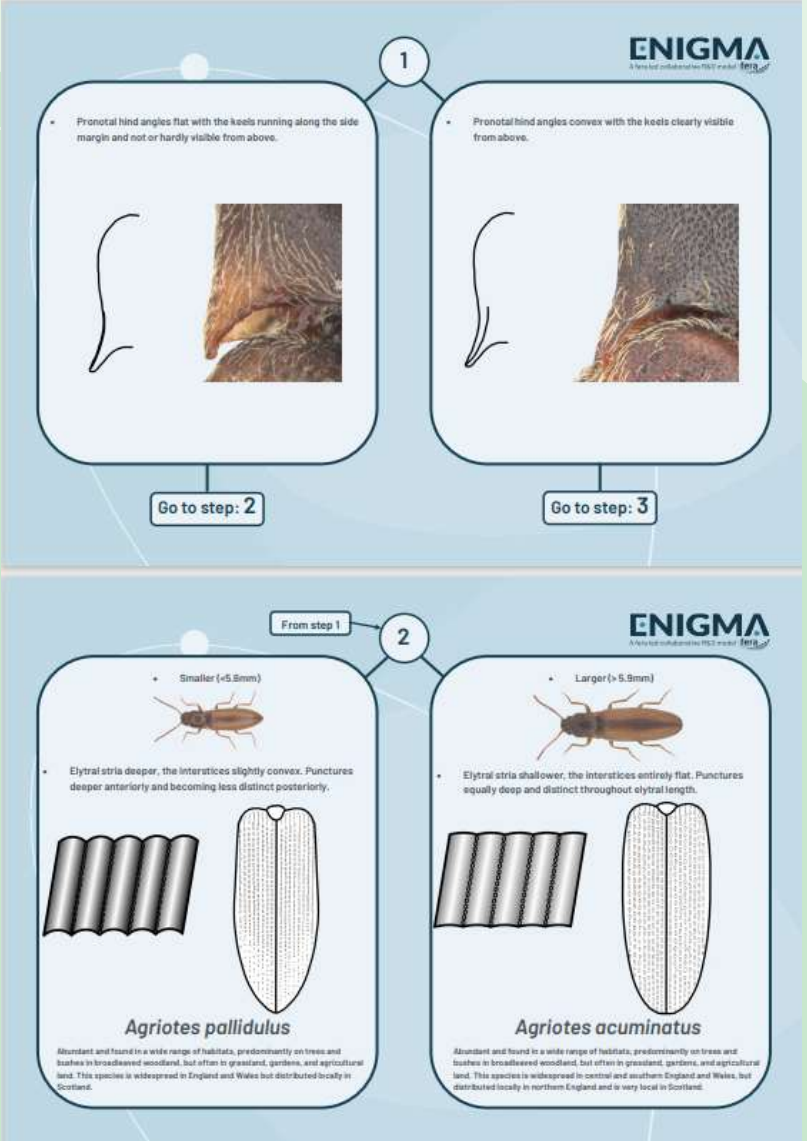
Sufyan *et al.* (2014) Bulletin of Insectology 67(2): 227-235.

Visual key and workshop for agronomists

Generated high resolution photographs which were used to produce a visual key and poster to aid identification.

Workshop on identification of click beetles and wireworms
- Identify wireworms as far as possible using a hand lens.
- Identification to species of click beetles of greatest concern.
- Discussion about monitoring and IPM measures.





MILESTONE 3 – DNA barcoding wireworms

Table – Progress made for DNA barcoding

Species	Country	Total number of DNA samples	Adults		Larvae	
			COI (Folmer)	COI (Staudacher)	COI (Folmer)	COI (Staudacher)
Agriotes lineatus	UK/France	17	1	3	2	7
Agriotes obscurus	UK/France	34	4	22	7	8
Agriotes pallidulus	France	1	1	1		
Agriotes sordidus	France	1	1	1		
Agriotes sputator	UK/France	14	7	8	3	6
Agrypnus murinus	UK	3	3	3		
Athous campyloides	UK/France	4	2	2	2	2
Athous haemorrhoidalis	UK	5	5	5	1	
Athous bicolor	UK	2	-	-	2	2
Hemicrepidius hirtus	France	1	1	-		
Hemicrepidius niger	UK/France	8	4	4	4	2
Melanotus villosus	UK	1			1	1
Ctenioрus sulphuripes (Tenebrionidae)	UK	1			1	1
Limonius poneli/minutus	UK	1	1			
Dalopius marginatus	UK	1	1			
Adrastus pallens	UK	1	1			
Total sequences generated		**95**	**32**	**49**	**23**	**29**

All sequences generated from each species were shared with Dr. Bruno Ngala and Dr. Jeremy Cigna from INOV3PT (France). Dr. Jeremy Cigna also shared two sequences of *Selatosomus latus* and *Agriotes pilosellus*.

DNA barcodes for 16 species.

[1] *Staudacher, K., Pitterl, P., Furlan, L., Cate, P.C. and Traugott, M., 2011. PCR-based species identification of Agriotes larvae. Bulletin of Entomological Research, 101(2), pp.201-210.*

MILESTONE 7 – Life history study

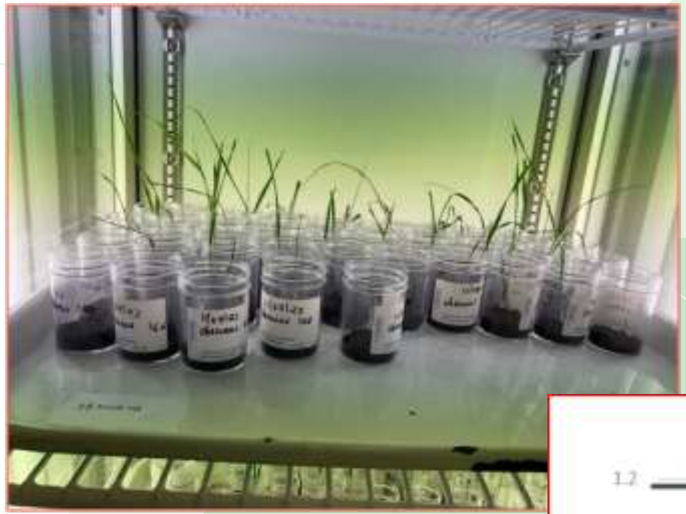
- We collected 6 species of adult click beetles: *Agriotes lineatus*, *A. sputator*, *A. obscurus*, *Athous haemorrhoidalis*, *Hemicrepidius niger* and *Agrypnus murinus.*
- The beetles were put into breeding boxes and monitored for eggs.
- Five of these species produced eggs which then hatched into wireworms.
- Individual wireworms of each of the five species were placed in pots of compost with a germinated wheat seedling for food at temperatures of:
- 7°C, 12°C, 17°C, 22°C, 27°C.

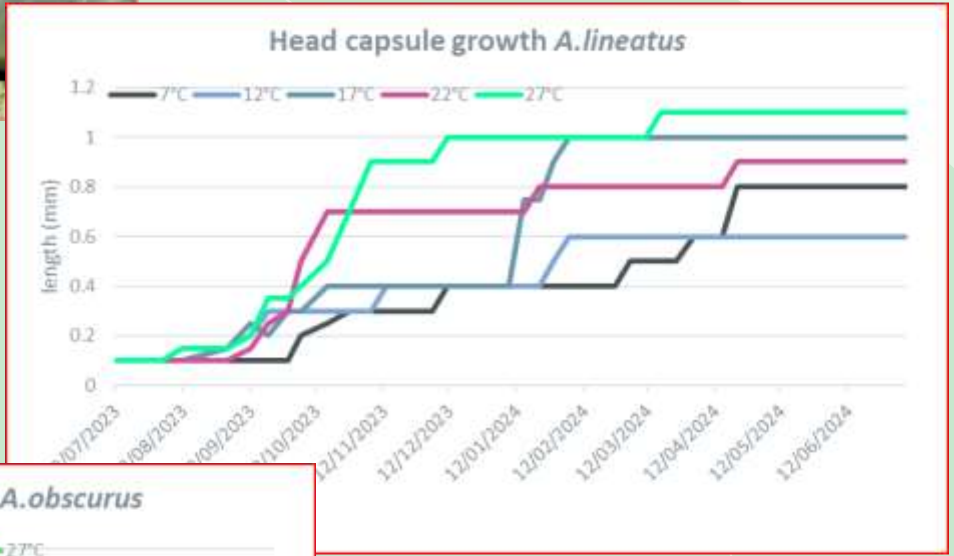
Weight, length and size of head capsule were measured every 7 days.

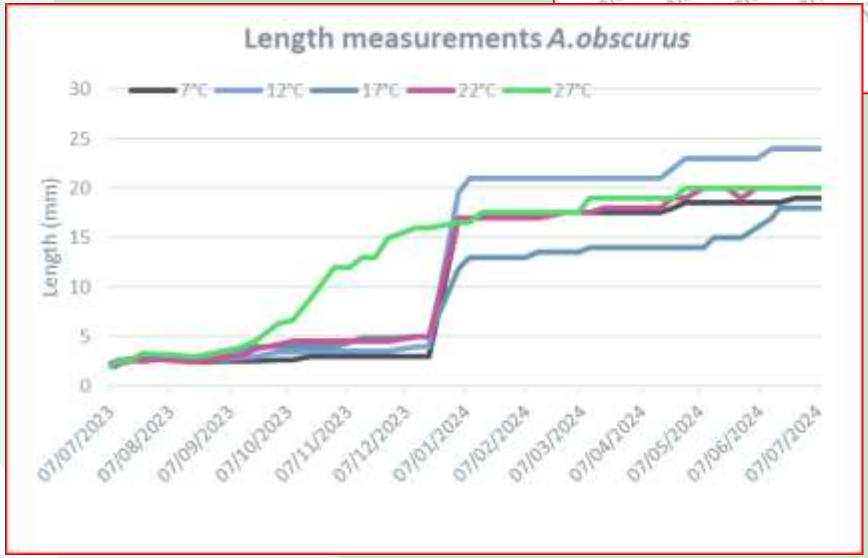
Life history studies

Results

- The rate of development of the larvae was strongly linked to temperature.
- Development data at 5 temperatures for *A. lineatus* and *A. obscurus* although none of the larvae pupated during the project even at 27 °C.
- This data along with the fecundity data has been used for modelling and decision support for wireworm control and management.







MILESTONE 7 – Life history - fecundity

- Collected click beetles from traps, sex and pair up in breeding pots
- Ten pairs each of *A. obscurus* and *A. lineatus* at 12°C and 22°C
- Checked daily to monitor:
    - Time from pair to egg-laying
    - Number of eggs per female
    - Time from egg-laying to hatch
- They can produce a <u>lot</u> of eggs!
- We set up more pairs of beetles and kept these at 27°C and 17°C for weekly observations.

MILESTONE 7 – **Life history study**

Results:

- At 22°C and 27°C, both *A. obscurus* and *A. lineatus* produce hundreds of eggs which will hatch within ~3-4 weeks from emergence and mating.
- Fewer eggs were produced by both species at the lower temperature.
- Time taken to first oviposition generally increased with a drop in temperature
- Little difference between species in time taken to first oviposition.
- Both species need more than double the time taken at 17 °C to hatch at 12°C.
- Both species were observed to have created balls of compost to contain their eggs (up to ~50 in one ball), but generally at the higher temperatures. This could be to moderate environmental conditions for their development, such as temperature and humidity. Eggs found at 12°C were mainly found alone or with one or two nearby and scattered loose in the compost.

Cover crop studies

- 2 cover crops were tested in a glasshouse trial with two follow-on crops – Maris peer potatoes and iceberg lettuce.
- Volcanic basalt rock dust was also tested in combination with the cover crops.

- There was damage in the follow-on crop of potatoes with all the crop/soil types.
- Potato damage and number of holes was significantly reduced when cover crop 1 was macerated into the soil ($P < 0.01$).
- Cover crop 2 had no effect on reducing potato damage.
- There was no reduction in wireworm numbers or their development with either of the two cover crops.
- The cover crops had no effect on preventing damage to the follow-on crop of lettuce.

- Further work is required to assess whether these results can be replicated in the field in the UK.

Crop	% Potatoes damaged by wireworms	Total number of holes caused by wireworms
Cover crop 1	22	152
Cover crop 2	65	310
Wheat	50	234
Soil	65	361

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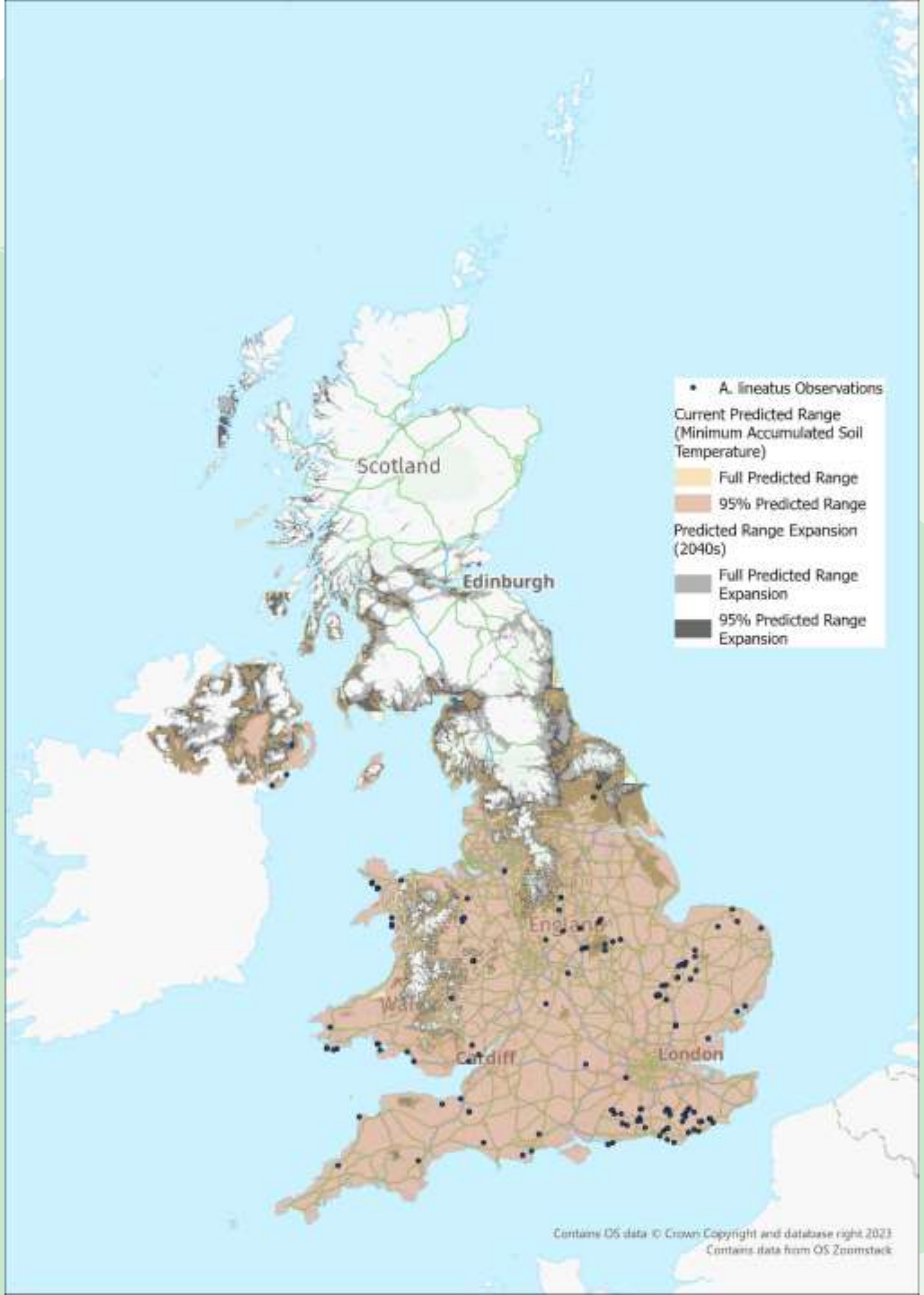
MILESTONE 8 - Climate modelling completed for the species of greatest concern

Current range and range expansion maps for *Agriotes lineatus* and *A. sputator*, *A. obscurus*, *Agrypnus murinus* and *Athous haemorrhoidalis have been produced.*

Experimental data from the life history studies has been used to create temperature-larval stage population relationships for the three *Agriotes* species.

These relationships have been used to create models to predict the proportion of the population in each larval stage through the year.



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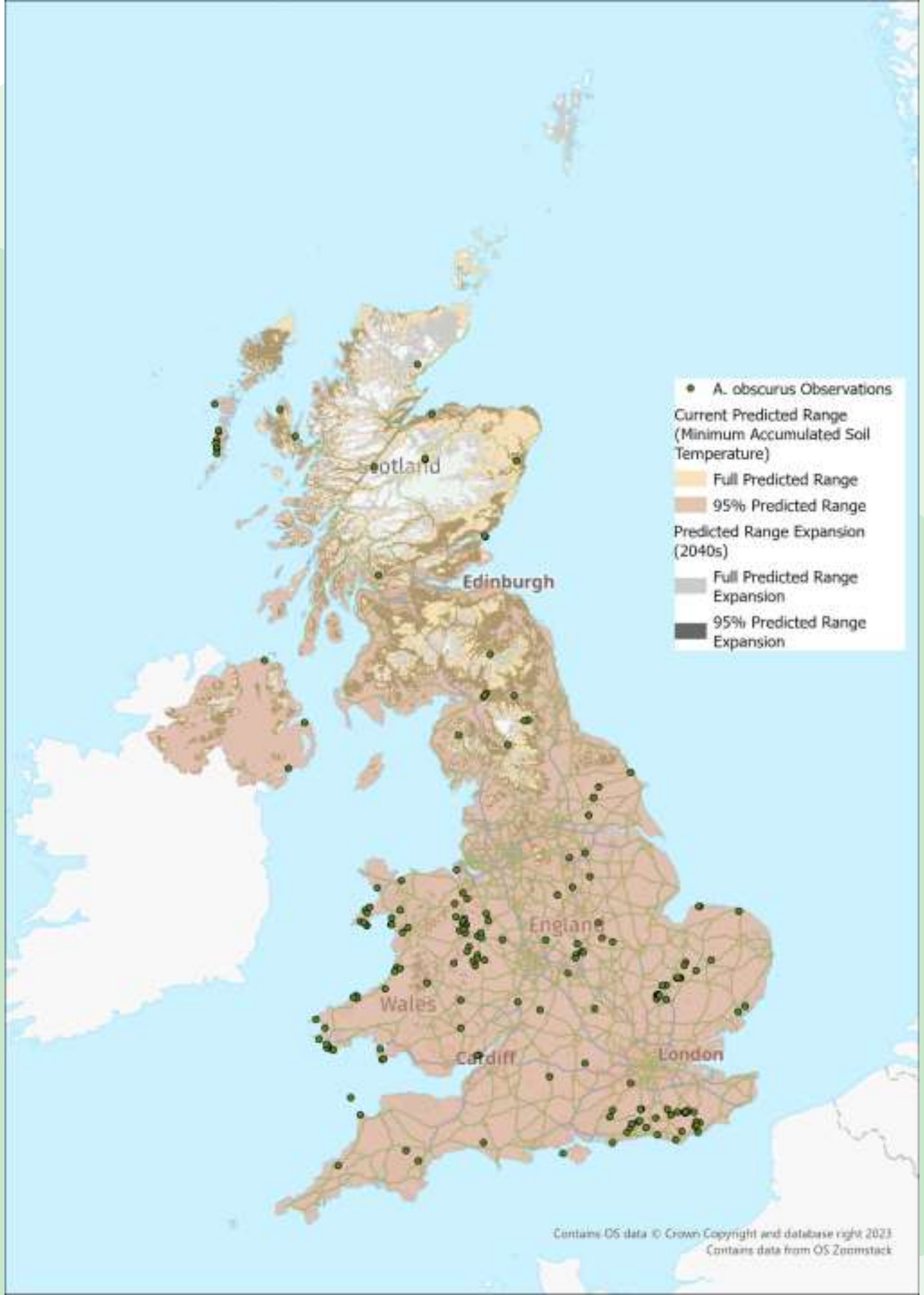
Predicted current range and expanded range for 2040s for *Agriotes obscurus* based on annual accumulated soil temperature predictions.

Note: The darker brown areas indicate where the 95% Predicted Range Expansion overlaps with the Full Predicted Range.



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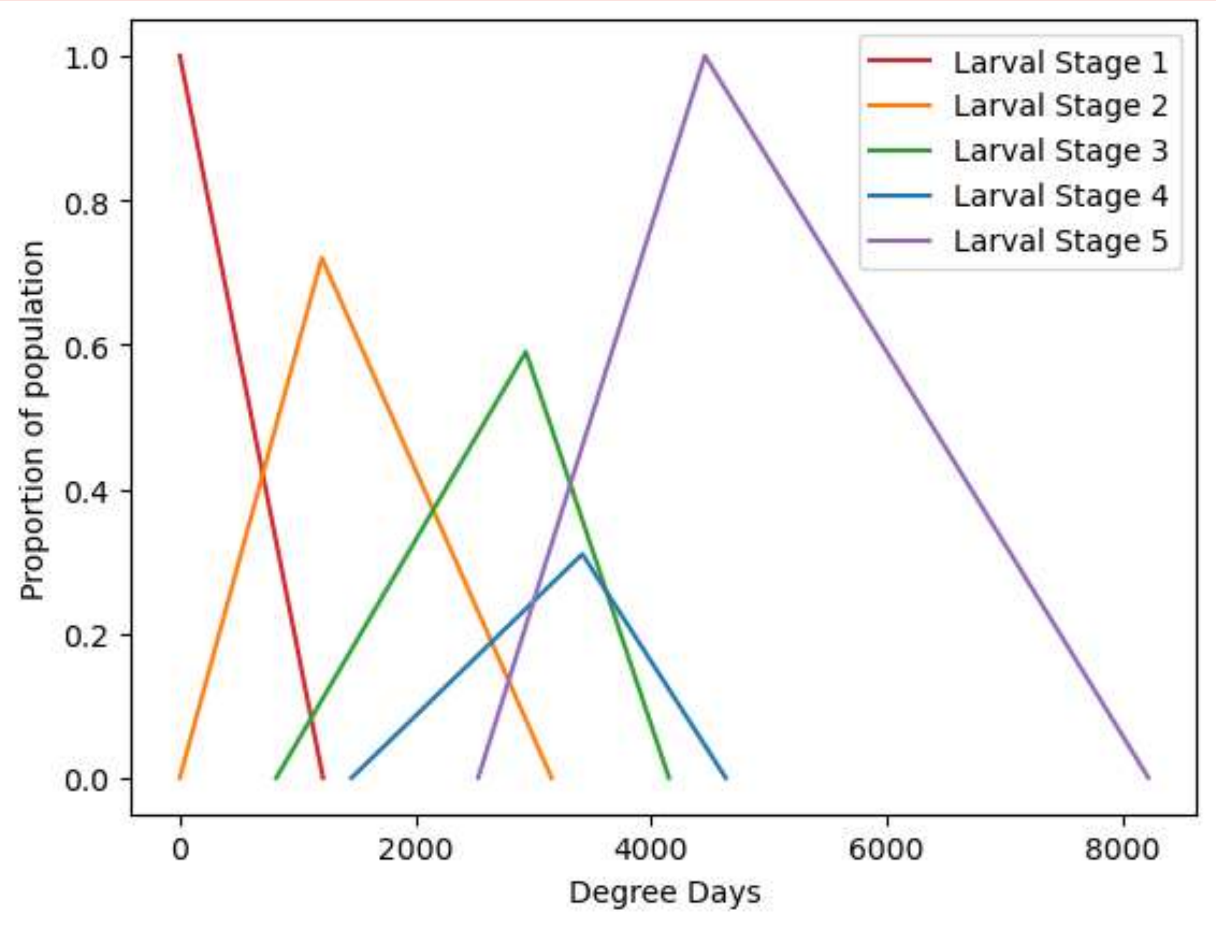
Larval Stage Modelling

- Life history studies - Proportions of population at each stage mapped against degree days
- For each larval stage a triangular relationship was created to predict proportion of population from accumulated degree days
- Hatching date defined *a priori* (7th July)
- For each 1km grid cell in UK
    - Accumulated degree days soil temperature calculated from hatching date for three-year window
    - Daily Larval population composition calculated based on accumulated degree days from hatching date
    - Day of year larval composition from combined three-year larval composition estimates

Degree Day - Larval Stage Relationships

Example - *Agriotes obscurus*



MILESTONE 9 - Production of guidelines and decision support material

Wireworm surveys

- Conducted surveys for the monitoring sites in 2023 and 2024.
- Elements such as soil type, whether there is a hedgerow or a meadow in an adjacent field and what has been grown in the field in the last 4 years.
- Patterns and risk factors may be identified and used to assist in reducing the impact of wireworm in future seasons.
- 18 complete surveys were submitted and 27 factors identified.

Initial investigation:

- Selected two variables which were expected to be important based on what we know about the biology of the species
  a. the type of margin – grass, grass-and-floral, no margin
  b. is there a meadow in an adjacent field

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ENIGMA: WIREWORM SURVEY

Thank you for taking part in the ENIGMA I - Wireworm IPM Project: click beetle monitoring in 2023! Please may we ask you to complete this survey - it should take less than 10 minutes. By fulfilling this very short task you will be getting much more value from the work you put in monitoring the click beetles. We are using the monitoring results together with responses to this short questionnaire to identify risk factors and any outbreak patterns for different species of wireworm. Questions should be directed to Larissa.collins@fera.co.uk.

Section 1. About the field:

1. What type of soil does the field have?
   Choose an item.
2. What is the pH of the soil?
   ☐
3. Is the field:
   ☐ Flat
   ☐ Sloping
4. What is the direction of exposure?
   ☐

Simplified from Furlan et al. 2017 - Risk assessment of maize damage by wireworms (Coleoptera: Elateridae) as the first step in implementing IPM and in reducing the environmental impact of soil insecticides.

Conclusions from survey

The factors surveyed had a significant influence on the numbers of the three *Agriotes* species monitored.

1. The factors interacted differently with each of the three species monitored.
2. Additional data would allow better separation of confounding factors.
3. Further work on correlating adult numbers to wireworms developing in the field would allow risk assessments based on characteristics of the field and local vegetation to be more accurate.

We identified the top 10 factors which have the most influence on the numbers of click beetles.

Our project partners can use these for decision-making on field selection for susceptible crops and to inform decisions on rotation in the lead-up to a susceptible crop being sown or planted.

ENIGMA SUSTAINABLE WIREWORM IPM

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Nematology


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Wireworm IPM 'The Sequel'

Outline

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1. **Identify the Pest**
    - Further familiarisation and training on click beetle and wireworm identification.
    - Access to Fera's identification services – visual and DNA barcoding.

2. **Monitor Pest Activity**
    - Send out traps so that partners can monitor for adults themselves, Fera can confirm their ID. Possibility of on-site training in monitoring with bait balls for wireworms and pheromone traps for adults if that would be useful.
    - Provision of bait balls to monitor for wireworms. And other technology if it becomes available.
    - Assess results to test whether high numbers of adults relate to high numbers of progeny in that field. Relate this relationship to local conditions and crop rotation.
    - Produce improved monitoring method/guidelines with timings and ideal placements.
    - Improve or refine the DNA metabarcoding so that it is a robust method to determine if there is wireworm activity in a particular field and to identify any wireworms present.

3. **Determine action thresholds**
    - In fields, place a predetermined number of adults in a cage over large, buried plant pots. Assess hatching success in that location and soil type.
    - The soil will be collected and returned to Fera and processed using nematology's soil extraction procedure. The number of larvae and stage of development will be recorded.
    - The soil will also be sent for DNA metabarcoding to assess whether other click beetle species are in the soil and to use this opportunity to quality check this method using real samples.

4. Explore treatment options

Cover crops have potential to reduce wireworm numbers and crop damage, but this has not been quantified. Some questions are:

- Which cover crops for which wireworm species, and after which crop?
- What timing would be best to have maximum effect on wireworms?
  - Monitor for wireworm numbers and for other beneficial predatory insects e.g. carabid beetles, in fields of pre-existing cover crops, and fields where there are no cover crops. We would then link this to crop history/soil data etc.
  - Assess whether beneficial insects are more numerous in cover crops and if this enhances wireworm/click beetle control.

Predation by beneficial insects - Use molecular techniques to check whether predatory carabid beetles are eating adult click beetles, eggs and or wireworms. And whether any carabid species do this more than others. An action for control could be to enhance habitats for carabid beetles in particular areas of fields and field margins to encourage those species.

Investigate environmental actions and seed mixes to assess their effect, if any, on wireworm and predatory beetle numbers.

- Monitor numbers of wireworms and other beneficial insects in fields of pre-existing SFI's, and fields where there are no SFI's crops. Compare this data to cover crop data. Link this to crop history, soil type, etc.
- Assess whether beneficial insects are more numerous in environmentally enhanced areas or cover crops, and if this improves wireworm/click beetle control.
- Assess environmentally enhanced areas and seed mix plant species in glasshouse experiments to ascertain their effect on wireworm development. From these results, propose which mix would be best/worst for wireworms and when they should be planted in crop rotations.

4. Continued

Test control agents against different species of wireworms in the greenhouse. Three will be selected from the following list:

- Biological control agents such as ATTRACAP®. This contains a yeast which produces CO_2 when in contact with soil moisture. This attracts the wireworms to the granules which then infect the wireworms with entomopathogenic fungi.
- Grasslanz product (MaxQ) which contains endophytes (fungi) in their stems.
- Entomopathogenic nematodes such as Koppert's products: Casea (*Steinernema carpocapsae*) and Capyphor (*Heterorhabditis bacteriophora*).
- We would compare the efficacy of these products for different species. We would also compare these results with:
  - Bare soil to compare with tilling which may starve the wireworms.
  - Trap crops (e.g. rows of wheat between main crop).

5. Evaluate Results and produce tailored guidelines

- Risk assessment guidelines and IPM options will be produced for each field.
- Use the Climex modelling results achieved in Enigma I to also feed into IPM options.
- Go back round the loop and assess whether improvements could be made at each step.

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