Annual Project Report
21/11/2022 to 21/11/2023

Project title	Varietal resistance to feeding (herbivory) by the cabbage stem flea beetle (CSFB) in oilseed rape		
Project number	21120219		
Start date	21/11/2021	**End date**	21/11/2025

Project aim and objectives

This project aims to determine the genetic controls and mechanisms underlying variation in feeding by adult cabbage stem flea beetle (CSFB) and larval resistance in oilseed rape (*Brassica napus*). Working with breeders, we will help develop crops with resilience/tolerance against CSFB. There are three main objectives:

Objective 1: Characterising the genes associated with adult CSFB feeding and the mechanisms of plant resistance

- We use genome sequence from lines with contrasting CSFB feeding to identify the genetic causes of variation.
- Further experiments will identify the mechanism(s) by which these genes affect CSFB feeding, supporting breeding for resistance.

Objective 2: Understanding the basis of variation underlying reduced CSFB palatability and exploitation for breeding

- We use a mapping population to identify loci associated with a reduction in feeding.
- Gene expression analysis will be used to investigate plant-CSFB interactions and identify responses induced by CSFB feeding.
- Metabolite analysis will be employed to establish a link between causal loci, gene expression and compounds that influence beetle feeding.
- The germplasm and data will be used with breeding companies to select for adult CSFB feeding resistance.

Objective 3: Understanding the basis of variation in larval resistance to CSFB in *B. napus* and exploitation for breeding

- We use diverse *B. napus* and breeding material to identify lines showing the lowest adult emergence and characterise the effect on larval development.
- These data will be used to identify loci associated with this resistance.
- This will be combined with gene expression, metabolite analysis and candidate gene studies to develop our understanding of plant-CSFB larvae interactions.
- Resulting knowledge will be shared with breeding companies to accelerate breeding for resistance in OSR.

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Key messages emerging from the project

1. Methodologies for rearing CSFB and screening germplasm for CSFB damage have been optimised, providing the ability to screen large germplasm collections for adult CSFB susceptibility. These have been successfully transferred to industry.
2. Previous observed variation in adult CSFB feeding within a diverse collection has been confirmed both in controlled and field conditions. Material has been developed for further study.
3. Variation exists in levels of adult CSFB feeding within current varieties and breeder's material.
4. Gene expression analysis has identified plant responses to beetle feeding differ from those observed for mechanical damage suggesting a distinct response to flea beetle damage. Responses include plant hormonal responses and downregulation of growth typically observed during pathogen infection.
5. Metabolite analysis on cotyledonary tissues shows a wide range of sugars, waxes and secondary metabolites present. Any roles in herbivory have yet to be confirmed.
6. Controlled environment larval screens suffer from high levels of variability for larvae successfully progressing to adulthood. New methodologies are being tested for screening for larval resistance in controlled environments.
7. Preliminary analysis of data from field trials suggests variation in larval numbers between lines. Further trials have been established to confirm observations.

Summary of results from the reporting year

BASF has joined the project consortium as a further breeding partner.

<u>Enabling technologies: Optimisation of CSFB rearing, screening for adult feeding, and transfer of skills to industry</u>

Methodologies for rearing and screening germplasm for CSFB damage have been transferred to industry partners. Previous measurements of adult feeding were performed on percentage area eaten. For key lines within the project, further replication has been performed looking at physical area eaten to consider differences in seedling size. This can now be accounted for within studies.

<u>Objective 1: Sequencing of parental lines showing variation in adult CSFB feeding</u>

Variation in adult feeding previously observed between the two lines identified in Objective 1 was previously shown to be reproducible in both laboratory settings and in the field. Oilseed rape shows variation in genome organisation, gene copy number and sequence variation. To identify causal genetic variation linked to differences in feeding it is useful to have genome sequence data from the

exact plant material being used for studies. We have further refined our high-quality genome assemblies produced for the two lines showing extreme variation in CSFB palatability. Data has been transferred to industry partners. Comparison of the two genomes identified considerable genetic variation between the two lines. Initial studies of candidate genes also identify copy number variation.

- Development of assays within Arabidopsis, the model plant, for screening potential candidate genes controlling adult feeding and testing of mutants

Arabidopsis is a small model plant with a simple genome for which many resources exist to study gene function. Analysis of selected mutant lines by associated PhD student, Jessica Hughes, has shown mutants within candidate gene on A02/C02 and a related family member show increased levels of feeding, suggesting gene function supports resistance. Feeding assays in Arabidopsis and image analysis have been further optimised to allow higher throughput screening for further testing of candidates. Seed is currently being bulked to allow rigorous testing of these initial observations.

- Machine learning for the scoring of adult damage

Estimation of adult damage on brassica cotyledons is difficult and subjective. Current analysis systems used within the team are semi-automated and so throughput could be increased by further computational tools. Discussions with the Turing SciVision group have suggested several machine learning approaches that could be applied to data. Images have been shared and investigation of machine learning based scoring approaches are underway.

Objective 2: Benchmarking the adult feeding tolerance trait against current commercial material

Results show variation in levels of adult feeding in controlled trials within material provided by our breeding partners. Results of individual lines have been transferred to specific breeding partners to develop an understanding of where their material sits within the distribution of material tested. This suggests that there is likely to have been selection for resistance for adult feeding in recent breeding history. Original data was based on percentage eaten; reanalysis is underway to update this to assess area eaten. Adult feeding assays performed in the field on breeder's material have also been recorded for both 2021,2022 and 2023 field seasons. Data analysis is still in progress.

- Population production for the genetic mapping of adult CSFB feeding

About 250 doubled haploid (DH) lines were produced form a cross between a high and low palatable line. However, bulking failed to produce the seed quantities required for trialling. 100 lines have been selected for further work and these are currently being bulked at JIC. During this time, material will

also be genotyped and genetic maps developed. Extra seed will allow trialling in 2024-25 and distribution to breeding partners as a population developed from a diverse cross.

- Gene expression/metabolite profiling for determining chemical association with adult feeding

Gene expression experiments have been performed to determine the effects of beetle feeding on gene expression and induction of plant defences, when compared to mechanical damage and an undamaged control. This analysis was performed at three timepoints (2, 4 and 8 hours) for both our resistant and susceptible lines. Principle component analysis of the RNA-seq data showed clear clustering by treatment suggesting differences were present. Differential gene expression analysis identified the number of genes showing differences in expression increased across the time-period with around 2,500 genes being common to all timepoints in the resistant line. Clustering of these genes produced some clear classes of gene expression behaviour. Genes could be classed as a) being upregulated/downregulated with mechanical damage and feeding, thus showing a general response to damage; b) being specifically upregulated to damage in the resistant line only; c) showing a specific response to beetle feeding in both lines and d) showing a specific response to beetle in either resistant or susceptible lines. This analysis suggests that some of the responses observed by the plant are specific to beetle feeding and there is variation in response between resistant and susceptible lines.

Gene ontology (GO) term analysis, which looks at genes according to their function and biological process, identified these genes were involved in a number of plant responses including a) response to salicylic acid, an important plant hormone that is best known for mediating host responses upon pathogen infection, b) indole containing metabolic process (defence compound synthesis) and c) response to chitin, a primary component of cell walls in fungi and the exoskeletons of arthropods, such as crustaceans and insects. Investigations are now underway to follow up these results.

Reactive oxygen species (ROS) are involved in the signal transduction process associated with plant growth and defence. ROS is produced by brassica on application of a chitin solution. We therefore hypothesised that beetles may elicit a measurable ROS response, and this may differ between resistant and susceptible lines. Previously measurement of ROS in response to beetle extract had been non-successful (Thursfield, PhD thesis 2023) however experimentation was not combined with mechanical damage to mimic beetle feeding damage. Further work is ongoing in this area.

Cotyledon surface compounds and internal metabolites may influence feeding by CSFB. Protocols have been developed for the quantification of surface compounds such as waxes, phenolics, sugar alcohols (polyols), and internal compounds such as sugars, glucosinolates and polyphenols (e.g., phenolic acids, flavonoids). These compounds have previously not been measured on cotyledonary tissue. Material from the 96 lines of the diversity set previously screened to identify adult beetle feeding has been grown for quantification of these compounds. Screening is currently ongoing but measurements on a small number of replicates show excellent variation across the panel. These data will be correlated with beetle feeding data and used for genome wide association analysis to identify loci associated with metabolite production.

Objective 3: Phenotypic analysis of variation in larval infestation in breeding material

In 2021/22, a field trial of 20 varieties (x 5 replicates) was performed at JIC and Rothamsted Research (RRes) research sites. Material was assessed for establishment, feeding damage, fresh weight, dry weight, vigour, flowering, yield, seed size and oil content. Larval evacuation experiments in December and February were used to assess variation in larval numbers between lines at JIC. At RRes, analyses were performed monthly to determine differences in larval number and development between lines. Statistical analysis identified variation in larval numbers exists between lines, however large variation existed between sites. Analysis of monthly data identified significant differences between the number of larvae between varieties, but these differences were not consistent across months. However, varieties that often have less larvae than the mean between varieties can be identified.

Replicated field trials established for 2022/23 suffered from poor establishment at the JIC site due to unknown underlying soil conditions. Establishment was successful at RRes and assessed for the same variables as in 21/22. To gain further data, we are repeating this larval experiment for the 23/24 season.

- Phenotypic analysis of variation in larval success in diverse brassica material

Previous observations of differences in adult emergence from diverse brassica lines could not be produced in replicated trials. This was believed to be due to variation in hatch rate of eggs for plant infestation. Improved methodology involving the addition of one day old larvae was developed and material taken through to adult emergence for one replicate of the diversity set. However, large variation in adult emergence was observed within the control lines of *B. napus* and *S.alba*. Material is currently being assessed for growth of larvae within two weeks of infestation with one day old larvae

(similar to methodology published by Doring and Ulber, 2020) to produce a more rapid larval assay system and determine if this provides a better metric for assessing larval resistance mechanisms.

- Effect of climate perturbation on field larval infestation

Mathiasen *et al.* (2015) showed significant effects of temperature on egg laying, egg hatching and embryonic development, with warmer temperatures >4°C being more favourable for egg production, development rate and hatching percentage. Modelling data (Ortega-Ramos et al, 2023) showed a high level of importance of daily temperature between December and February, with higher temperatures related to higher larval numbers in the spring. Observations from field environmental perturbation trials by heating trial plots with soil warming cables (O'Neill et al., 2019) identified that plots heated across the October period appeared to suffer from larger amounts of larval damage. To test the hypothesis that warmer field temperatures over the winter period results in greater numbers and more developed CSFB, plots will be heated during the December and January period, predicted to be critical for spring larval numbers by Ortega-Ramos *et al.*, to compare larval numbers.

- Machine learning for larval counting and assessing larval stage

During the 2021/22 field trials assessment of larval numbers resulted in the counting and staging of about 60,000 larvae, an intensive, skilled and time-consuming process. To address this, images have been taken of 30 larvae at L1, L2 and L3 instar stage and shared with the Turing institute to assess for the potential of machine learning for larval counting and developmental staging.

Key issues to be addressed in the next year
Results and statistical analyses are expected for all the replicated experiments above for reporting to consortium partners.

Lead partner	JIC
Scientific partners	Rothamsted Research
Industry partners	Elsoms, Limagrain, RAGT, Bayer, LS Plant Breeding, KWS, DSV, BASF, AHDB
Government sponsor	BBSRC

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Has your project featured in any of the following in the last year?	
Events	***Press articles***
AHDB project meeting, Jan 23 Engage Crop Solutions visit to JIC, Jan23 BR2CSFB consortium update meeting, Feb23 Apex agronomy visit, March 23 Visit from BASF policy team, March 23 BASF CSFB focus meeting, March23 JIC field station open day, March23 Visit from Sakata (UK and Japan representatives), March 23 Visit from Erik Van Der Biezen, BASF April 23 Morley Innovation Day, Jun23 Indigro farmer academy visit to RRes – Jun23 Breeders Day, Jun23 Groundswell Jul23 BBSRC visit (Debbie Harding), Aug 23 Farming Agricultural Network visit, Sept 23 Visit from Animal and Plant Health and Welfare directorate at Defra Nov 23 Visit from NovoNordish Nov23	Eastern Daily Press John Innes Centre seeks crop resistance to flea beetle pests, Feb23 Arable Farming (Farmers Guardian) Research in Action: Oilseed rape genetics might beat CSFB, May 23
Conference presentations, papers or posters	***Scientific papers***
Frontier Eastern Regional Agronomy Conference Dec 22 Frontier Annual Norfolk Growers Conference 22 – invited speaker Feb 23 University of Herts Life Science and Medical Research Conference - Invited speaker Jun23 Ento23 – Invited speaker Sept23 16th International Rapeseed Congress – invited speaker Sept23 European Congress of Entomology – Heraklion, Greece. Oct23	
Other	
Lab rearing and field-collection of the Cabbage Stem Flea Beetle (*Psylliodes chrysocephala*) – Technical standard produced. Provision of CSFB to commercial partner – Jan 23 Royal Society of Biology Plant Health Undergraduate Studentship – Jul23	