

## Annual Project Report 21/11/2021 to 21/11/2022

<b>Project title</b>	Varietal resistance to feeding (herbivory) by the cabbage stem flea beetle (CSFB) in oilseed rape		
<b>Project number</b>	21120219		
<b>Start date</b>	21/11/2021	<b>End date</b>	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 against CSFB. There are three main objectives:

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

- Using genome sequence from lines with contrasting CSFB feeding, we aim to identify the genetic causes of variation
- Further experiments will identify the mechanism(s) by which these genes affect CSFB feeding and, thus, support breeding for resistance

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

- Using a mapping population, we aim to identify loci associated with 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**

- Using diverse *B. napus* and breeding material, we aim 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
- Knowledge will be shared with breeding companies to accelerate breeding for resistance in OSR

The results described in this summary report are interim and relate to one year. In all cases, the reports refer to projects that extend over a number of years.

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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 and larval CSFB susceptibility.
2. Previously observed variation in adult CSFB feeding within a diverse collection has been confirmed both in controlled and field conditions.
3. Variation exists in levels of adult CSFB feeding within current varieties and breeders' material.
4. Previously observed differences in larval success/adult emergence between extreme diverse lines could not be reproduced.
5. Preliminary analysis of data from field trials suggests variation in larval numbers between lines.

### Summary of results from the reporting year

#### Enabling technologies

##### **Optimisation of CSFB rearing, screening for adult feeding, and transfer of skills to industry**

Methodologies for rearing and screening germplasm for CSFB damage have been optimised. These have greatly improved the ability to screen varieties or seed treatments for resistance to feeding. Protocols have been developed to transfer skills to breeding company partners. Training courses for plant breeders to develop entomology skills for breeding selection have been made available.

#### Objective 1

##### **Sequencing of parental lines showing variation in adult CSFB feeding**

Variation in adult feeding (previously observed between the two lines identified in Objective 1) is reproducible in laboratory and field settings (confirming differences exist between lines). 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. High quality genome assemblies have been produced for the two lines showing extreme variation in CSFB palatability. We will use these genome sequences to determine the exact differences between the two lines in our regions of interest, which may be linked to beetle feeding.

##### **Development of assays within Arabidopsis, the model plant for screening potential candidate genes controlling adult feeding**

Arabidopsis is a small model plant with a simple genome for which many resources exist to study gene function. We have developed feeding assays in Arabidopsis and screened mutants for two candidate genes for adult CSFB feeding. We are currently optimising image analysis to allow higher throughput screening for further testing of candidates.

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### **Objective 2**

#### **Benchmarking the adult feeding tolerance trait against current commercial material**

Preliminary results show variation in levels of adult feeding in controlled trials within material provided by our breeding partners. This suggests that there is likely to have been selection for resistance for adult feeding in recent breeding history. Further analysis is required to confirm results. Adult feeding assays performed in the field on breeder's material have also been recorded for both 2021 and 2022 field seasons (data analysis underway).

#### **Population production for the genetic mapping of adult CSFB feeding**

A doubled haploid population has been produced between a high and low palatable line. This is currently being bulked to allow trialling in the field for adult feeding in 2023.

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

Gene expression experiments have been performed to determine the effects of beetle feeding and induction of plant defences. Sequencing is ongoing. Protocols have been successfully tested for the quantification of waxes, sugars and phenolic compounds that may influence adult CSFB feeding. These can now be used to assay germplasm for association with feeding.

### **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 is ongoing. However, preliminary analysis of data from larval field trials suggested variation in larval numbers exists between lines. Replicated field trials have been established for 2022/23 to investigate this further.

#### **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. Improved methodology has been developed to allow screening of the panel and experimentation is ongoing.

### **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.

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<b>Lead partner</b>	JIC
<b>Scientific partners</b>	RRes
<b>Industry partners</b>	Elsoms, Limagrain, RAGT, Bayer, LS Plant Breeding, KWS, DSV, AHDB
<b>Government sponsor</b>	BBSRC

<b>Has your project featured in any of the following in the last year?</b>	
<b>Events</b>	<b>Press articles</b>
New Investigator Webinar BBSRC 21 Sense About Science Evidence Week 21 JIC Field Research Station Drop in 21 JIC Breeders Day 21 National Farmers Union Development Programme JIC visit 22 Oilseed Rape Genetic Improvement Network (OREGIN) 22 Institute Partnership meeting with BBSRC Nov 22 IOBC-WPRS Working Group "Integrated Control in Oilseed Crops, ICOC", May 2022 – talk Growndswell, June 2022 Cereals, June 2022 Visit from Unilever Regenerative Agriculture team to Rothamsted Research, November 2022	Initial press release featured in: Farming UK Farmers Weekly Farmers Guardian Eastern Daily press  Chemistry and Industry (Vol86 March22) – Oilseed rape chaos Crop Protection Magazine June 22 Theory to Field – Research to quash CSFB
<b>Conference presentations, papers or posters</b>	<b>Scientific papers</b>
DSV European Breeders meeting 21 – invited speaker Frontier Winter Agronomy Conference 21 – invited speaker BCPC Pests and Beneficial Review 21 – invited speaker Oilseed Rape Genetic Improvement Network (OREGIN) 22 – Invited speaker	

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<p>Agritech East REAP conference 2022 – invited speaker Cereals 2022 – Poster and demo plot Groundswell 2022 Poster NIAB CSFB day 2022 – poster International Plant Health Conference, September 2022 – poster AAB Biocontrol and IPM conference, November 2022 – poster + short talk</p>	
<p><b>Other</b></p>	
<p>JIC website 508 page views, 278 engagements (includes likes, retweets etc) BBC Farming today with Anna Hill x2 interviews CSFB rearing SOP made available to commercial partners on request CSFB training made available to commercial partners in collaboration with the BR2CSFB project BR2CSFB consortium meeting “Future Explores” at the Science Museum of London – engagement activity with general public</p>	

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