Research to quash CSFB

AHDB

from theory to field

66 Finding areas of the genome that control adult flea beetle feeding, and including them in commercial varieties, is the aim. **99**

It's almost a decade since oilseed rape growers lost neonicotinoid seed treatments. *CPM* looks at some of the research into alternative solutions for mitigating the threat of cabbage stem flea beetle attack in the UK's top break crop.

A lot of column inches have been dedicated to the issues facing oilseed rape growers since 2013, when neonicotinoid seed treatments were banned in flowering crops across Europe.

The ban created some emotive debate, with growers feeling helpless as they watched adult cabbage stem flea beetles (CSFB) ravage their newly planted crops each autumn. If seedlings were lucky enough to escape, larvae were the sting in the tail come spring, causing irrecoverable destruction of stems after significant sums had been spent on expensive inputs.

Many called for neonicotinoids to return, while others reduced their area or abandoned the crop altogether as the stakes became far too high. However, with few profitable alternative break crops, many stuck with OSR and one positive from the mess has been the significant improvement in establishment practices, with crops now given the best chance of survival.

Another positive is the speed at which researchers have gathered vital information on the pest and its control, and several ongoing AHDB-funded projects are starting to bear fruit, with genetic tools and effective biopesticides on the horizon.

Research colony

One substantial project that aims to provide breeders with resistance or tolerance traits, is being led by Dr Rachel Wells at the John Innes Centre, where its very own CSFB colony has made this possible.

Rachel says the JIC entomology department's beetle colony's origins can be traced back to 2013 when the significant damage started to occur in commercial crops, with samples taken and returned to its Norwich site.

"I asked the entomology department if it was possible to rear the beetles in-house. We quickly needed to build tools and resources to research the insect's behaviour and interactions with the OSR plant.

"The CSFB research team and JIC entomology have now developed protocols to ensure the colony is maintained and beetles are always available," she explains.

Once the tools and resources were in hand, a PhD project carried out by Jessica Hughes kick-started research that might lead to some genetic solutions to the CSFB problem. Using the beetle colony and many *Brassica napus* lines — representative of the genetic diversity found within the plant family — Jessica set up 'choice chambers' to find out if there were plants that CSFB adults preferred over others.

Some clear differences were identified in

laboratory assays, with some plants much more palatable than others when adult beetles were presented with options. Rachel says that the genetic diversity in the Brassica Diversity Fixed Foundation Set (DFFS) is much wider than the genetics found in the commercial OSR varieties planted in the field today.

This hints that resistance to the pest was lost when breeding for other commercially favourable traits, she says. "Now we can identify feeding preferences; it's about associating specific genetics with adult flea beetle behaviour in the field and understanding the mechanisms underpinning that.



The genetic diversity in the Brassica Diversity Fixed Foundation Set hints that resistance to the pest was lost when breeding for other commercially favourable traits, says Rachel Wells.

Theory to Field

"Ultimately, finding areas of the genome that control adult flea beetle feeding, and including them in commercial varieties, is the aim," explains Rachel.

Last year, a fresh injection of funds is allowing researchers to build on Jessica's PhD, with £1.8M from a BBSRC Industrial Partnership award and AHDB bringing together JIC, Rothamsted Research and several OSR breeders in a collaborative project.

The aim is to facilitate the flow of information between researchers and breeders, in particular CSFB resistance or tolerance traits that can be incorporated in market-ready varieties quickly. From work so far, Rachel says two major regions of the *B. napus* genome have been identified as areas of interest. Within those areas, candidate genes will now be tested to see



Choice chambers were set up to find out if there were plants that CSFB adults preferred over others.

how beetles behave in the presence of plants and why.

Reasons could be physical, like leaf hairs, or perhaps chemical composition of the plants, with Rothamsted Research biochemist Frederic Beaudoin testing for differences in leaf wax composition, for example.

Looking at larvae

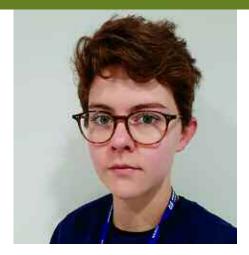
The expertise of Sam Cook, behavioural ecologist at Rothamsted, is also driving the project and while a larger proportion of the work on CSFB research has focussed on adult behaviour, more attention is now being paid to larvae.

At Rothamsted and JIC, some 20 lines provided by breeders are currently in field trials to study larvae behaviour and interactions with plants, with the hope of uncovering interesting differences.

Larval evacuation assays have been carried out by the team over winter, with plants taken and laid on mesh over a water-filled box. As the plants dry out, the larvae look to escape and drop into the water below.

"It's looking at how larval numbers change across the winter. The interesting thing is that in February, there were still first instar [juvenile] larvae inside the plants. This suggests that the adults continued to lay eggs that hatch into the winter," explains Rachel.

It's too early to draw any meaningful



Claire Hoarau is testing a range of biopesticides already available in other crops for activity on CSFB.

conclusions from the larvae work and the experiments are due to be repeated in the 2022-23 cropping year. However, Rachel says that plants outside the *B. napus* species, like white mustard (*Sinapis alba*), are known to be less vulnerable to feeding.

She adds that at present, there has been no link made to a specific chemical compound in *S. alba*, but antibiosis an antagonistic association between two organisms (OSR and larvae), in which one is adversely affected (larvae) — have been observed previously.

With modern breeding methods, it may be possible to transfer this into OSR varieties once mechanisms are understood. There are a further two PhD students at JIC looking at

Genetics will help stop OSR boom or bust

Bayer UK Nordics and Baltics OSR breeder Matthew Clarke says that CSFB resistance in varieties will have a vital role to play in maintaining sustainable domestic OSR production once more.

The last couple of seasons have not seen the high CSFB pressure that encouraged such a dramatic decline of the UK OSR area since 2013. This can be attributed to the lower intensity of OSR cropping in affected areas, but with demand and price at record highs, the area could quickly surge to previous levels and push pest pressure back up again.

"The area has effectively halved in a short number of years, but now the outlook is a bit more positive and there are some nice-looking OSR crops around this year," says Matthew.

"I suspect flea beetle has settled down in the worst affected areas but to prevent a boom-and-bust cycle in the future, it's very important to find a breeding solution that at least partially addresses the problem," he says.

One of consortium of breeders that will

directly benefit from work at JIC, Bayer have already observed that some hybrids are better than others at withstanding pressure from CSFB within its own portfolio.

He cites the example of DK Expedient, which has a growth habit that seems to enable it to grow away from larvae damage in the spring. Matthew adds that it isn't purely about plants withstanding the pest, because growers have shifted agronomic practices in recent years to help avoid key parts of the CSFB lifecycle.

Pre-neonic ban, the trend was planting later in August or early September. Now, it's estimated that about 30% if the area is planted before mid-August and some in July to get plants away before peak adult migration.

"That's quite important for variety choice as well, as you don't want plants that stem extend early in the winter and cause other problems, such as frost damage," he explains.

Matthew is already encouraged by what he sees in the company's own material and from

work being carried out at JIC, with access to genetic markers on the horizon.

In terms of timelines, he says there are two possibilities. Firstly, the project at JIC could start to identify hybrids in existing advanced pipelines that are better than others and suited to a wider drilling window. This means that growers may have commercial varieties with CSFB tolerance in 2-3 years' time.

The second is a longer wait, with strongly positive traits identified at JIC having to be bred into varieties from scratch. Before these can be released, other characteristics need to be brought up to commercially acceptable standards, which takes time, he says.

"In that scenario, we're talking 10 years. In the meantime, we've seen that some of the ideas, like planting huge populations of farm-saved seed, don't always work. You're much better going for robust hybrid genetics and planting in the right soil conditions and at the right time, even if it doesn't appear to be the right calendar date," he notes.

Theory to Field



To prevent a boom-and-bust cycle with OSR in the future, it's very important to find a breeding solution that at least partially addresses the problem, says Matthew Clarke.

 relatives of OSR that could provide genes that might help commercial crops repel CSFB attack.

These could be bred conventionally into commercial lines, or in the future — if the technology is accepted — gene editing or genetic modification techniques could help achieve this quicker and more reliably, she says.

Asked when growers might see commercial varieties with genetics helping to resist or tolerate CSFB attack, Rachel says it would typically take between eight and 15 years to get them to market via National and Recommended List trials. However, she's optimistic that with modern marker technology and speed breeding techniques, it should be at the lower end of the estimate.

"As soon as we find something interesting, we'll pass it on to breeders everyone is working together to push things forward," says Rachel.

Breeding is considered as one of the most important tools in the quest to protect the UK's number one break crop from CSFB, but it should also be used in conjunction with other integrated pest management (IPM) techniques, so another AHDB-funded project is aiming to add to the toolbox.

Harper Adams University researcher Claire Hoarau is testing a range of biopesticides already available in other crops. As part of her work, she's tested several entomopathogens — essentially pathogens that can kill insect pests — and some botanically-derived biopesticide products and fatty acids.

Claire started her work in controlled lab conditions, directly applying each agent to beetles and assessing the results. She achieved good mortality using nematode products from BASF, fungal product Botanigard WP and FliPPER,

a fatty acid-based product from Bayer. Feeding beetles Btt bacteria — well

Biopesticides tested on CSFB in the field

Туре	Product	Active organism/substance
Fungi	Botanigard WP	Beauveria bassina
Bacteria	-	Bacillus thuringiensis sbsp. Tenebrionis (Btt)
Nematode	Nemasys	Steinernema feltiae
Nematode	Nemasys-H	Heterorhabditis bacteriophora
Contact insecticide	FLiPPER	Fatty acids
Contact insecticide	CEU-40640-I-SL	Undisclosed

Source: JIC, 2022

known for its use in GM crops — and azadirachtin achieved poorer mortality results. Based on the lab assays, Claire selected the most promising to take forward into field experiments.

One of the surprises in the initial lab assays was the strong efficacy of FLiPPER, as its current targets, as outlined on the product label, are soft bodied insects like aphids, not hard cuticle bugs like CSFB.

Claire didn't see the lab potential of any product translate into significant results in the field, underlining the difficulty of using biopesticides in broadacre agriculture, rather than in controlled horticultural environments where they've been very successful.

"Due to logistical difficulties, we applied during the day, which isn't the best time to target beetles. Some of the products are also sensitive to UV and other environmental factors, too. We're going to repeat the experiment this autumn and spray in the evening to see if we can achieve better results," explains Claire.

In addition to application timing, Claire will be investigating the potential of adjuvants that might help improve efficacy. These will be tested in the lab to see if they can keep plant leaves wetter for longer, as all the products need moisture to work.

"I don't think any of the products will offer a silver bullet, as that's not how crop protection works now. They are more likely to be used in a cocktail as part of a wider IPM solution."

Research roundup

From Theory to Field is part of AHDB's delivery of knowledge exchange on grower-funded research projects. *CPM* would like to thank AHDB for its support and in providing privileged access to staff and others involved in helping put these articles together. For further info:

AHDB Project 21120219: Varietal resistance to feeding (herbivory) by the cabbage stem flea beetle (CSFB) in oilseed rape is being led by the John Innes Centre



The biopesticide trials have seen good mortality using a fungal biopesticide ©Claire Hoarau.



Nematodes are also being assessed to see if they can be used as a biocontrol for CSFB ©Claire Hoarau.

(JIC), alongside AHDB and industry partners, including DSV, KWS, Limagrain, Bayer, Elsoms Seeds, RAGT and LS Plant Breeding. The total value of the project is £1,886,025.

AHDB Project 21510042: Novel Approaches to control cabbage stem flea beetle (CSFB) (PhD) is being led by Harper Adams University. The total value of the project is £42,150, of which £36,150 came from the Cereals and Oilseeds levy.