

Final Project Summary

Project title	Investigating pyrethroid resistance in UK cabbage stem flea beetle populations and developing a PCR-based assay for detecting turnip yellows virus in aphids.		
Project number	RD-214-0019	Final Project Report	PR552
Start date	01 July 2014	End date	31 December 2014
AHDB Cereals & Oilseeds funding	£8,000	Total cost	£8,000

What was the challenge/demand for the work?
<p>Part A. Cabbage stem flea beetle (CSFB), <i>Psylliodes chrysocephala</i>, is a major pest of winter oilseed rape (OSR) in several European countries, particularly attacking young emerging plants in autumn. Up until December 2013, seedlings were protected by neonicotinoid seed treatments. However, an EU imposed restriction currently in place for these compounds when applied to OSR seed has left growers with only one alternative of applying a pyrethroid spray. Recent reports of knock-down resistance (kdr) to pyrethroids in CSFB in Germany in the last few years demonstrate that this pest has evolved resistance which would potentially undermine this alternative control strategy. Furthermore, there have been growing numbers of reports from UK OSR growers that pyrethroids are failing against CSFB suggesting that resistance is now present in this country.</p> <p>Part B. The transmission of turnip yellows virus (TuYV) by the peach–potato aphid, <i>Myzus persicae</i>, is also a major concern for OSR growers in respect of the neonicotinoid seed treatment restriction. It is already estimated that 60% of UK OSR is affected by TuYV with average yield losses in untreated crops of 15–30%. The loss of the neonicotinoid seed treatment (which is currently very effective for controlling UK populations of <i>M. persicae</i>), combined with existing strong resistance to alternative compounds (pyrethroids and pirimicarb) will undoubtedly result in a significantly increased threat of TuYV transmission for OSR in 2014. At present, it is difficult to monitor both resistance status (kdr/super-kdr and MACE) and likelihood of virus transmission in <i>M. persicae</i> populations in real time. Although rapid, high throughput PCR-based tests are available for detecting resistance mutations in aphid samples, a similar DNA-based assay is not currently available for detecting TuYV in the same samples. Instead, TuYV and other plant viruses are generally monitored retrospectively at the end of the season using more labour intensive enzyme-linked immunosorbent assays (ELISA).</p>

How did the project address this?
Part A tested 18 English CSFB samples, (collected in Bedfordshire, Cambridgeshire, Essex,

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Hertfordshire, Norfolk, Suffolk and Yorkshire) taken from oilseed rape in 2014 for the presence of the *kdr* mutation. These samples were also screened with lambda-cyhalothrin in bioassays to measure resistance levels.

Part B developed a rapid PCR-based diagnostic assay for TuYV that can be run alongside the insecticide resistance assays that gives a more complete 'real-time' picture of both resistance status and the virus transmission potential of *M. persicae* populations collected from OSR.

What outputs has the project delivered?

Part A

- A bioassay method was developed for quickly testing CSFB adults for resistance to pyrethroids.
- This was used to test the 18 English CSFB samples which were shown to contain mobile (resistant) beetles after exposure to doses equivalent to the field rate (7.5 g ai/ha) or above (10 g ai/ha) of lambda-cyhalothrin.
- This demonstrates widespread, strong resistance in this pest in this country in 2014 (figure 1).
- The *kdr* resistance mutation (L1014F), known to confer pyrethroid resistance in other pests, such as aphids and pollen beetles, was found, respectively, in the heterozygous and homozygous form in 17% and over 50% of the beetles tested.
- However, resistance in the bioassays did not compare well with *kdr* genotype, as some *kdr*-susceptible (SS) adults were scored as being fully mobile after being treated with 100% lambda-cyhalothrin.
- This suggested CSFB were carrying at least one other form of resistance, a hypothesis that was verified in bioassays pre-treating beetles with PBO (a synergist), which circumvents the presence of metabolic-based resistance.
- Further research is needed to identify this mechanism which could be based on the over-expression of a P450, as seen in pollen beetles.
- In the meantime, using *kdr* frequencies alone will not accurately describe pyrethroid resistance in CSFB in the UK and bioassays on live beetles will continue to be needed to diagnose resistance phenotype in this pest.

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Figure 1. Origin of English cabbage flea samples containing mobile (resistant) & susceptible beetles when exposed to a dose equivalent to field rate in the lambda-cyhalothrin bioassays.

Part B

- We also developed a rapid PCR-based taqman assay for the detection of TuYV in individual *Myzus persicae*.
- The full assay protocol from collection of aphids through to scoring of the assay (for a plate of 96 samples) can be completed in 5 hours.
- Additionally, the same aphids can be simultaneously tested for a range of mutations (MACE, kdr/ super-kdr and nicR) that are known to confer resistance, respectively, to pirimicarb, pyrethroids and neonicotinoids in this species.
- A preliminary analysis of *M. persicae* samples collected from the Insect Survey suction traps at Kirton and Wellesbourne in June/July 2015 indicated high infection levels with TuYV, ranging between 70% (Wellesbourne) and 80% (Kirton).
- Oilseed rape growers and agronomists have been advised through a range of KT activities (presentations, press articles, Resistance Alerts; details are available in the Final Project Report) that control-busting pyrethroid resistance is present in CSFB in England.
- Growers have been advised not to use repeat applications of these compounds if they do not work initially as this will only exacerbate the situation by selecting for resistant beetles.

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- Furthermore, repeat pyrethroid applications will kill off any beneficial insects in the crop, such as predatory beetles and spiders, which would otherwise reduce the pest population.

Who will benefit from this project and why?

- Oilseed rape growers and agronomists will benefit from our findings as they have been made aware of pyrethroid resistance in CSFB in England.
- They will also benefit in the future from the availability of a fast molecular diagnostic for detecting TuYV in individual *M. persicae* that can be run alongside the diagnostics for known insecticide resistance mechanisms.
- The data gained in this project represent a significant return on the £8,000 funding from AHDB Cereals & Oilseeds.

If the challenge has not been specifically met, state why and how this could be overcome

We met all of our objectives.

Lead partner	Rothamsted Research
Scientific partners	None
Industry partners	None
Government sponsor	No

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