Potential of biopesticides and optimising use of conventional insecticides for control of cabbage stem flea beetle (*Psylliodes chrysocephala*).

**Background**

Cabbage stem flea beetle (CSFB, *Psylliodes chrysocephala*) is a widespread pest in the UK. In the UK, adults migrate into emerging oilseed rape crops in late August or early September, initially feeding on the cotyledons and new leaves, before mating and laying eggs in cracks in the soil around young seedlings. Hatched larvae feed in mines within petioles and stems. Damage to autumn sown crops is caused by both adults and larvae and results in seedling death or stunting of survivors, and poor plant vigour, and can result in reduced plant density or complete crop failure (Nicholls & Ellis, 2016).

Following the EU neonicotinoid moratorium on use of neonicotinoids on flowering crops such as oilseed rape beginning in autumn 2013 serious crop losses due to establishment failure have been reported, together with significant yield effects from larval feeding in surviving crops. Most serious losses have occurred in eastern and southern England (Alves et al., 2016). Defra-funded surveys of larval numbers in oilseed rape in autumn and spring have similarly shown that larval numbers have risen substantially in all regions since 2013. On 27 April 2018 the EU voted to ban outdoor use of the neonicotinoid active ingredients clothianidin, imidacloprid and thiamethoxam on all outdoor grown crops.

Until 2013 control of adults was achieved by using insecticides, mainly neonicotinoid seed treatments typically containing either of the active ingredients clothianidin or thiamethoxam. These were often supplemented by pyrethroid sprays when the seed treatments had worn off if flea betles were still active. Larval control, if applied was largely reliant on pyrethroids (Dewar et al; 2016). First records of kdr resistance to pyrethroids in CSFB were reported in northern Germany in 2008 (Heimbach and Müller, 2013). Both kdr resistance and an unknown form of metabolic resistance is currently present in UK populations of CSFB (Nicholls & Ellis, 2016). The risk posed was accentuated by withdrawal of neonicotinoid seed treatments, with anecdotal reports that this has led to use of multiple applications of pyrethroid sprays. Alternatives to pyrethroids for control of adults or larval stages of this pest, such as thiacloprid, pymetrozine, acetamiprid, and flonicamid, have not shown good efficacy in trials (Dewar and Walters, 2016) and future control of this pest is likely to have to rely on a wider range of control methods.

**Project outline**

This project responds to the need for novel approaches to CSFB control by investigating the potential of biopesticides, including entomopathogenic fungi, nematodes and bacteria as well as plant extracts used singly or in combination. The efficacy of these potential controls will be investigated against a background of commercial cost and application technology constraints, together with improved understanding of the temporal patterns of susceptibility to selected biopesticides and insecticide controls.

**Part 1: Identification of effective biopesticides**

Field trials testing the efficacy of entomopathogenic fungi and nematodes as well as plant extracts, such as neem, have already been demonstrated to show considerable promise against another flea beetle pest, the crucifer flea beetle (*Phyllothreta cruciferae*), a pest of spring grown crops, in Canada (Reddy et al., 2014). CSFB is known to be susceptible to strains
of entomopathogenic fungi and bacteria, including Metarhizium brunneum (anisopliae) (Butt et al., 1994) but there is a lack of specific information facilitating assessment of efficacy of candidate (currently and soon to be commercialised) biopesticide options against their adults and larvae. This part of the proposed project is divided into the following Objectives:

1. Screening of biopesticides: A range of biopesticides will be screened for efficacy against CSFB larvae and adults. Biopesticides already commercialised or close to gaining registration include entomopathogenic fungal isolates (e.g. Metarhizium brunneum and Beauveria bassiana), entomopathogenic nematodes (e.g. Steinernema carpocapsae and Heterorhabditis bacteriophora), entomopathogenic bacteria (e.g. Bacillus thuringiensis), plant extracts (e.g. neem). This work will firstly establish LD50 and LT50 values for each biopesticide tested. To investigate further the pests immune responses to particular biopesticides and plant extracts, immune function assays (primarily total hemocyte counts and lysozyme-like assays) will be undertaken on challenged and control beetles to assess the impact of the screened products.

2. Efficacy of biopesticides: The most promising biopesticides identified through the initial screen will be tested under laboratory and semi-field conditions. Replicated experiments will be completed under controlled laboratory and semi-field conditions to which known populations of CSFB can be added. The final stage of these efficacy evaluations will be field testing of selected biopesticide products. Where a suitable Trials Permit from CRD is obtained field testing could link with the AHDB Strategic Arable Farm in the East and West which have indicated an interest in conducting on-farm demonstrations under the theme of “Lower Managed Inputs” and “Environmental Protection”.

Part 2: Combinations of biopesticides

Biopesticides have a number of potential benefits over many conventional pesticides, such as operator safety and lack of toxic residue. However, biopesticides are usually less efficacious than fully effective conventional insecticides and they can be adversely affected by environmental conditions. Improvements to the efficacy of biopesticides can be achieved by combining different agents in an IPM programme, for example by using two different entomopathogens together or by combining an entomopathogen with a partially effective chemical insecticide, e.g. one that some insects in a population may be resistant to, or one used at a reduced dose rate (Chandler, 2015). This part of the project is divided into the following Objectives:

3. Combinations of entomopathogens: Based on the results from Objectives 1 and 2 the most promising biopesticides will be selected. Combinations of biopesticides with distinct modes of action will then be tested against CSFB larvae and adults. For each biopesticide combination efficacy of the first agent will be plotted against that of the second, at a range of relevant doses, in order to determine whether the combination has a synergistic, antagonistic or additive effect compared to the solo treatments.

4. Overcoming insecticide resistance in CSFB populations: adult CSFBs will be collected from field sites where pyrethroid resistance has been reported. Pyrethroid resistance within collected CSFB populations will be confirmed by adapting IRAC test method No. 21 ‘adult-vial-test’. PCR techniques will also be undertaken to screen for the kdr mutation in these populations (Zimmer et al., 2014). Similar to the previous Objective, a graphical plot of the effect of the pyrethroid insecticide versus the effect of the biopesticide at different doses will be completed in order to determine the nature of the interaction (deviation from a straight line relationship indicates a synergetic or antagonistic interaction) and ultimately to determine
whether the biopesticide has the effect of making the CSFB population more susceptible to pyrethroid applications.

5. Cost benefit analysis: a cost-benefit analysis of selected individual and combination applications of control agents against CSFB in oilseed rape fields will be calculated.

Part 3: Temporal patterns in CSFB susceptibility to biopesticides and insecticides

Insect physiology and behaviour is known to be under the control of an endogenous circadian clock. For most pests, insecticide applications coincide with peak activity of the pest insect. This is important as insect activity is likely to increase the chance of the pest coming into contact with the insecticide. In addition, studies of the temporal variations in insect susceptibility (chronotoxicity) to insecticides indicate that insecticide susceptibility corresponds with the onset of a time of increased activity in the insect (Shipp & Otton, 1976). This is linked to the fact that insect circadian clocks control many of the genes encoding detoxification enzymes of xenobiotics, such as insecticides (e.g. Hooven et al., 2009). Given that CSFB is both nocturnal and populations in eastern and southern counties have developed metabolic resistance to pyrethroid insecticides, chronotoxicity may be important in determining the efficacy of novel biopesticides and the currently used pyrethroid insecticides where resistance is apparent. This part of the project is divided into the following Objectives:

6. Temporal variation in CSFB susceptibility to selected biopesticides: populations of CSFB adults with known pyrethroid resistance will be exposed to selected biopesticides (see Objective 1) at regular intervals throughout a 24-hour cycle in order to record susceptibility of the pest at each timepoint. This work will be complemented by immune function assays (see Objective 1) to assess the impact of the screened products at each time point.

7. Temporal variation in CSFB susceptibility to pyrethroid insecticides: this Objective will be completed in a similar way but using pyrethroid insecticide and exploiting the adapted IRAC test method and PCR techniques (see Objective 6) to confirm the presence of target site and/or metabolic pyrethroid resistance in the CSFBs tested.

References


Starting dates

The studentship would commence in September 2019 at Harper Adams University.