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

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Abstract

The cabbage stem flea beetle (CSFB) *Psylliodes chrysocephala* Linnaeus is the most important pest of oilseed rape (*Brassica napus*) crops in Europe. Control has become more difficult since the European Union ban in 2013 on the use of neonicotinoid seed treatments. This situation is made more challenging by the development of resistance to pyrethroid insecticides, the only remaining conventional synthetic insecticides with which to control CSFB.

The purpose of this paper is to review the potential of biological alternatives to the use of synthetic pesticides for the control of the CSFB. Only a small number of studies have investigated biological control agents against CSFB itself. More research has, however, been published on two other, closely related chrysomelid pests of brassica crops that have similar life cycles, namely the crucifer flea beetle *Phyllotreta cruciferae* and the striped flea beetle *Phyllotreta striolata*, which enable us to extrapolate reasonably across to CSFB. The biological control agents investigated include entomopathogenic fungi (EPF) such as *Metarhizium anisopliae* and *Beauveria bassiana*, entomopathogenic nematodes (EPN) such as *Steinernema feltiae* and *Steinernema carpocapsae*, parasitoids such as *Microctonus brassicae* and predators such as the ground beetle *Trechus quadristriatus*. Results vary depending on the setting (laboratory versus field), but several biological control agents investigated resulted in CSFB mortality greater than 50% under laboratory conditions. The biological control of the CSFB shows potential as a viable alternative to the use of conventional synthetic insecticides. Nonetheless, many research gaps remain, as current research has focused largely on crucifer flea beetle and striped flea beetle, with comparatively few studies investigating the potential of biological controls against the CSFB. The research published to date on CSFB has been limited to a small number of species of EPN and EPF with comparatively little work investigating the potential of parasitoids and predators. More field studies using EPF are required, while in contrast laboratory studies are underrepresented for EPN.

Further research is required, testing existing and new strains of fungi and nematodes, exploring the potential of endophytic fungi, enhancing the formulation and application of biological control for use in inundative strategies, and investigating the potential of conservation biological control. Effective biological control agents should ultimately be

combined with cultural control methods in Integrated Pest Management (IPM) systems for the sustainable management of this pest.

1. Introduction

Oilseed rape (*Brassica napus* Linnaeus) is an important crop, with more than 35 million hectares grown globally in 2020, mainly in Europe, Canada, China and India (FAOSTAT, 2023). In Europe, almost 9 million hectares were grown in 2020, which represented 25% of the global area grown (FAOSTAT, 2023). Oilseed rape is grown for its oil extracted from the seeds, as animal feed, and as a break crop to prevent the build-up of pathogens and pests associated with the other crops in the rotation, typically cereals (I. H. Williams, 2010; Nicholls, 2016a). Globally, oilseed rape is the third-largest source of vegetable oil and the second-largest source of protein meal (AHDB, 2020). In the UK, which is typical of other European countries, oilseed rape crop has been the third most widely grown crop behind wheat and barley, and the fourth most productive arable crop behind wheat, barley and oats (Defra, 2022).

Cabbage stem flea beetle (CSFB), *Psylliodes chrysocephala* Linnaeus (Coleoptera: Chrysomelidae), is the main stem-mining pest of winter oilseed rape crops in central and northern European countries (Alford and Gould, 1976a; Bromand, 1990; Winfield, 1992a; Garbe, Gladders and Lane, 2000; Alford, Nilsson and Ulber, 2003; Ferguson *et al.*, 2003a; Nicholls, 2016a). It can also damage other overwintering brassica crops (Newton, 1929; Roebuck, 1936) including turnip, mustard and cabbage (Ahuja, Rohloff and Bones, 2011). It is native to Europe, North Asia and North Africa (Bonnemaison, 1965; Cox, 1998; Gruev and Döberl, 2006; I. H. Williams, 2010). It is invasive in North America (Gruev and Döberl, 2006) although it is less important than the indigenous crucifer flea beetle (*Phyllotreta cruciferae* Goeze) and striped flea beetle (*Phyllotreta striolata* Fabricius) (Coleoptera: Chrysomelidae) (Lamb, 1984; Bracken and Bucher, 1986; Weiss *et al.*, 1991; Palaniswamy and Lamb, 1992) which have a similar life cycle but which cause damage primarily through adult feeding on foliage of spring-sown oilseed rape crops (Lamb, 1989). The striped flea beetle is also a pest of brassicaceous crops in south-China (Yan *et al.*, 2013a).

Management of CSFB is based heavily around the use of synthetic chemical insecticides, but even with routine insecticide applications economic losses caused by CSFB are often significant. For example, in England, CSFB damage to winter oilseed rape crops resulted in losses of around £23 million in 2013, representing 3.5% of the national crop (Nicholls, 2016a). Losses have increased further following withdrawal of authorization of neonicotinoid pesticide seed treatments in 2013, which has prompted farmers to reduce the crop area grown. To assess the effect of this ban on oilseed rape cultivation, Scott and Bilsborrow (2019) surveyed more than 200 farms across England in the 2014/2015 and 2015/2016 growing seasons. They

observed that the area of oilseed rape grown decreased in both seasons compared to the years before the withdrawal of neonicotinoids, with CSFB cited by growers as one of the main reasons for this decrease, alongside crop rotation and a fall in commodity price. In the UK, the crop area declined from a peak of 712,671 hectares in 2012, with yields up to 3.6 tons/ha between 2011 and 2016 and an estimated crop value of more than £800 million per year (Nicholls, 2016a), to 342,372 ha in 2023 (Defra, 2023), and yields on average of 3.7 tons/ha in 2022 (Defra, 2022). CSFB is especially difficult to manage now without a systemic seed treatment as the larvae burrow into the plant and therefore are out of reach of contact-acting foliar insecticides. Foliar insecticides can be applied against the adults, but control is difficult when the plant canopy is dense in the spring, reducing the efficacy of spray applications (Ebbe-Nyman, 1952).

Seed treatments based on the active ingredient cyantraniliprole (Lumiposa, Corteva Agriscience), a ryanoid insecticide that impairs insect muscle function, has proven effective against several pests of winter oilseed rape. This insecticide led to 65% control against CSFB in field trials compared to untreated plots (von Nieuwenhoven, 2017). It is advertised as safe for pollinators and other non-target organisms, but – as with all insecticides – it will need to be used judiciously to prevent or delay the evolution of heritable resistance in CSFB populations. Pressures to develop farming systems that include reduced chemical inputs, and which can help reverse declines in non-pest insect biodiversity, are also becoming increasingly urgent, as part of the general drive to make food production more sustainable (Benton *et al.*, 2019). These factors all point to the need for a range of alternative, environmentally benign methods for CSFB control, to be used as part of an Integrated Pest Management (IPM) approach. One of the many definitions of IPM is “a decision-based process involving coordinated use of multiple tactics for optimizing the control of all classes of pests (insects, pathogens, weeds, vertebrates) in an ecologically and economically sound manner” (Prokopy, 2003).

In this review, I investigate the potential of biologically based controls, with a focus on the use of entomopathogenic fungi (EPF) and nematodes (EPN), as sustainable biological control agents of CSFB, that can be used as part of an IPM program. The paper reviews the prospects for biologically based control of CSFB, including studies on CSFB itself and other, closely related pests of oilseed rape. Consideration is given to the likely effectiveness of these agents given the CSFB lifecycle, pesticide resistant populations, and the need to integrate these biological control agents into management programs. As well as reviewing available information, I highlight current gaps in research and barriers to the adoption of entomopathogens for the control of CSFB in oilseed rape crops.

2. Cabbage stem flea beetle: description, life cycle and damage

The first study on the biology and incidence of CSFB in the UK was completed by Williams and Carden (1961). CSFB was already known to sporadically attack brassica crops in the country, but severe attacks on brassica seed crops were reported in the winter of 1949-50, prompting further studies of its biology (Williams and Carden, 1961a; Graham and Alford, 1981a). Since this time, CSFB has become a major pest in the UK and elsewhere (Green, 2007, 2008; Holland and Oakley, 2007). In 2014 and 2015, 76% and 70% respectively, of oilseed rape crops were affected by CSFB in the UK (Alves, Wynn and Stopps, 2016; Nicholls, 2016a).

CSFB is a univoltine species in the UK and other northern temperate countries (Williams and Carden, 1961a). The adult is small, 4-5 mm in length, and has a shiny black-blue cuticle, with punctate elytra, large hind femurs that enable it to jump and ten-segmented antennae, typical of *Psylliodes* genus (Ebbe-Nyman, 1952). Young adults begin to emerge in late spring-early summer (late May-early June) (Figure 1) after eight to twelve weeks pupating in the soil (Kaufmann, 1941; Williams and Carden, 1961a; I. H. Williams, 2010). As soon as they emerge, adults feed on mature leaves, stems and pods of oilseed rape and other brassicaceous species for about a month (Kaufmann, 1941; Alford, 1979; Såringer, 1984; I. H. Williams, 2010). The adults then enter aestivation from late June to mid-August (Ebbe-Nyman, 1952; Cox, 1998) in sheltered places such as cracks and crevices in the soil and vegetation, as well as in hedgerows and woodlands (Kaufmann, 1941; Williams and Carden, 1961a; I. H. Williams, 2010).

Adults emerge again in mid-to-late-August, with best conditions for flight above 16°C (Bonnemaison, 1965). Adults are capable of dispersing over distances of two to three miles and migrate to winter oilseed rape crops at the seedling stage of the crop from late August to early September onwards (Alford, 1979). There, adults feed on cotyledons, stems and first true leaves (Kaufmann, 1941; Ebbe-Nyman, 1952).

Mating begins soon after females become sexually mature and can continue through the winter (Kaufmann, 1941). Mated females lay their eggs in groups in the soil at the base of plants (Kaufmann, 1941). Each female can lay around a thousand eggs in her lifetime (Kaufmann, 1941) and is still able to lay viable eggs for up to eight months following mating (Mathiasen *et al.*, 2015).

The hatching of CSFB eggs starts in late September (Alford, 1979; I. H. Williams, 2010). Once a plant is located, the larvae climb onto it and penetrate the base of the first healthy petiole they encounter (Kaufmann, 1941). The third-instar larvae move from the petioles to the main stem and growing points of the plant, mainly in March and early April (Ebbe-Nyman, 1952; Christer Nilsson, 1990; White, 2015). First and second instar larva are sparsely haired,

creamy-white and covered with black dots; the head, neck plate and anal plate are black, with two horn-like structures on the anal plate. Third instar larva can be up to 8 mm in length, with a creamy-white body with nearly transparent black dots, with head, neck plate and anal plate brown in color (Kaufmann, 1941; Ebbe-Nyman, 1952). Once ready to pupate, usually late April, the larvae leave the plants and bury themselves in the soil to a depth of around 2-4 cm (Kaufmann, 1941).

Adult CSFB populations decline rapidly during the winter. Some individuals are able to survive as adults into a second year by burying themselves just below the soil surface, re-emerging again only when conditions are more favorable. Females can then lay eggs through winter and spring (Kaufmann, 1941).

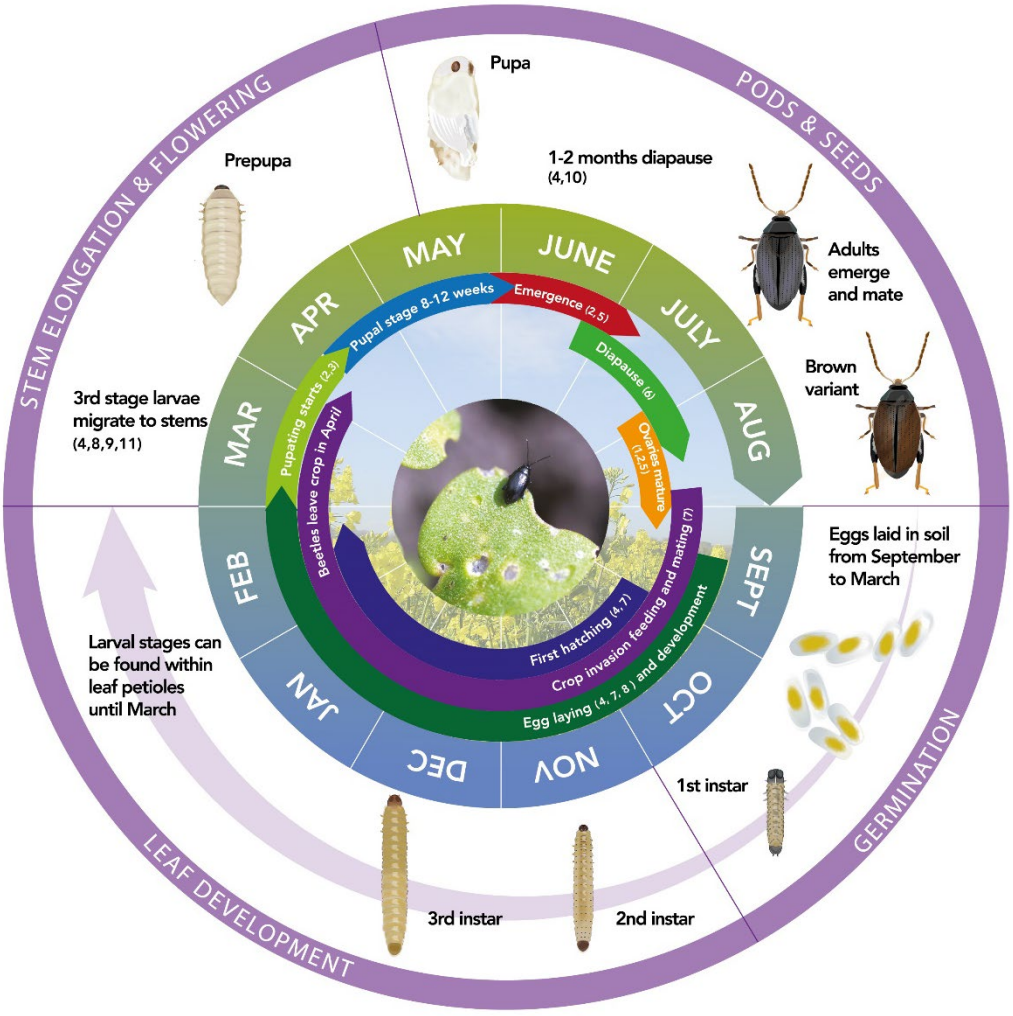

Figure 1. Life cycle of the cabbage stem flea beetle, relative to oilseed rape development (Source: Penny Greeves). Figures in brackets represent the following references: 1: Börner and Blunck, 1920; 2: Kaufmann, 1941; 3: Godan, 1951; 4: Ebbe-Nyman, 1952; 5: Williams and Carden, 1961; 6: Bonnemaison, 1965; 7: Alford, 1979; 8: Alford et al., 2003; 9: Nilsson, 1990; 10: Cox, 1998; 11: White, 2015.

Adult CSFB feeding on oilseed rape seedlings cause damage known as ‘shot holing’ of cotyledons and first true leaves (Alford, Nilsson and Ulber, 2003). In winter-sown oilseed rape crops, if the weather is dry and the crop has been sown early in the autumn, damage can be severe and lead to the death of seedlings if the growing point is eaten (Leach *et al.*, 1994a).

Larval feeding is characterized by the formation of tunnels in petioles and stems. It is often considered the main form of damage caused by CSFB in oilseed rape crops (Williams, 2004). The larval cohort that is the most damaging is the one laid in early autumn that attacks young seedlings of winter oilseed rape. Larvae developing from eggs laid in spring contribute to the total population but are thought to have only a limited impact on mature plants (Bonnemaison, 1965). Direct damage to severely affected plants includes stem wilting, delayed flowering, reduced plant survival through winter or even total plant collapse (Williams and Carden, 1961a; Graham and Alford, 1981a; Christer Nilsson, 1990; Winfield, 1992a; Nilsson, 2002a). Tall plants are also more prone to lodging when the stem has been hollowed out by mature larvae (Pickering *et al.*, 2020).

3. Sustainability challenges with conventional synthetic insecticide treatments

From the early 1990s onwards, the standard approach to controlling oilseed rape pests was the routine use of systemic synthetic neonicotinoid insecticides as seed dressings (Williams, 2010), which were effective in controlling CSFB, together with the use of pyrethroid sprays that were often applied as an ‘insurance’ treatment without considering pest control thresholds (Ulber, Klukowski and Williams, 2010; I. H. Williams, 2010).

On 1st December 2013, the European Union banned the use of neonicotinoid insecticides as seed coatings in many crops (including oilseed rape) following concerns about risks to bees and other pollinators (European Commission, 2013a). The ban was confirmed in April 2018 in accordance with the precautionary principle, and the use of three neonicotinoids (clothianidin, imidacloprid and thiamethoxam) withdrawn from use in flowering crops.

Since then, only pyrethroid insecticides have been available to control CSFB, thereby, increasing the selection pressure for resistance in CSFB to this group of insecticides (Højland *et al.*, 2015a). Overuse of pyrethroid insecticides also threatens biological diversity in and around the fields, for example by killing natural enemies and thus compromising biological control (I. H. Williams, 2010).

The first reports of pyrethroid insecticides failing to control the CSFB in oilseed rape were from Germany in 2008, and it was confirmed that individuals collected there had a decreased susceptibility to lambda-cyhalothrin in adult laboratory bioassays (Heimbach and Müller, 2013).

In a recent study by Willis et al. (2020) in the UK, some populations of CSFB with 100% of resistant beetles to the pyrethroid insecticide lambda-cyhalothrin were recorded for the first time. Willis *et al*. (2020) also found that over the two years of monitoring in this study (2018 and 2019), the overall percentage of highly resistant CSFB increased from 33% to 56%, mostly in the Southeast of England.

It is, therefore, necessary to find effective and sustainable alternatives to pyrethroid insecticides, such as biopesticides and biological control agents that can be used as part of IPM programs.

As the use of synthetic chemical insecticides has been the standard approach for CSFB management for 30 years, and because these insecticides were very effective in controlling CSFB populations, for most of this time, there has been little work to develop alternate methods of control that may be used as part of an IPM program.

4. Biologically based agents as alternative control methods of CSFB

Biopesticide are biologically based pest control agents that are manufactured from living microorganisms or natural products (Chandler *et al.*, 2011). For the purpose of regulation, government agencies tend to classify biopesticides into three different categories: 1) microorganisms; 2) biochemicals, which include for example natural insecticidal compounds produced by plants; and 3) semiochemicals, such as insect pheromones (Health and Safety Executive, 2023).

4.1. Entomopathogens

Entomopathogens contribute to the natural regulation of many populations of arthropods. According to Hokkanen et al. (2003), in the case of oilseed rape pests such as CSFB, entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPN) are considered to be the organisms with the greatest potential for successful control.

Chandler (2017) and Shapiro-Ilan *et al*. (2017) have extensively reviewed the use of EPF and EPN, respectively, as biocontrol agents. Some advantages are that these pathogens have the potential to reproduce in the pest or in its environment, leading to a degree of self-sustaining control. Using entomopathogens instead of conventional insecticides can prevent the development of pesticide resistance in pest populations (Chandler, 2017; Shapiro-Ilan, Hazir and Glazer, 2017). Both EPF and EPN are safe to non-target organisms such as bees, parasitoids and predators, are considered safe to humans, and easy to mass produce (Chandler, 2017; Shapiro-Ilan, Hazir and Glazer, 2017). EPF and EPN can be applied with existing spray equipment, though with adaptations needed depending on the size of the crop,

the cropping system, and if the product is required to be applied to the soil or onto plant surfaces. Further research is, however, needed to optimize the use of application equipment in order to improve the dispersal into the crop and survival of these entomopathogens (Chandler, 2017; Shapiro-Ilan, Hazir and Glazer, 2017).

Temperature, humidity, UV radiation, soil macro- and microfauna, rainfall, soil type and texture, organic matter level etc. are all factors that can influence the persistence of the pathogens in the environment, and consequently their efficacy as a biocontrol agent (Chandler, 2017; Shapiro-Ilan, Hazir and Glazer, 2017). There are however ways to protect the pathogens against some of these factors, such as UV radiation or low humidity, with oil-based formulation and the addition of sunscreens for EPF, and with polymer gels or surfactants to increase persistence and plant surface coverage for EPN. The timing of application, such as applying the pathogens in the morning or evening to prevent their exposure to UV radiation, is also an important factor (Chandler, 2017; Shapiro-Ilan, Hazir and Glazer, 2017).

4.1.1. Entomopathogenic fungi (EPF)

Between 700 and 1000 species of fungi are known to infect arthropods (Lacey, 2017), but only a few have been used as commercial biopesticides for the management of crop pests. These species are naturally present in agricultural soils, but spore numbers in nature are often too low to result in effective control of a pest population outbreak (Vänninen, Husberg and Hokkanen, 1989; Vänninen, 1996; Zec-Vojinovic *et al.*, 2006). However, some species of EPF can be effective when applied in an inundative strategy (Reddy *et al.*, 2014b).

Most research on EPF biopesticides has focused on species belonging to the *Metarhizium* and *Isaria* (Hypocreales: Clavicipitaceae) genera as well as species from the *Beauveria* and *Akhantomyces (Lecanicillium)* (Hypocreales: Cordycipitaceae) genera (Khachatourians and Qazi, 2008; de Barros, Fronza and Bertholdo-Vargas, 2015). Species in the *Metarhizium* genus have been recorded as being capable of killing more than 300 arthropod species and *Beauveria* species are known to be able to infect more than 200 species (de Barros, Fronza and Bertholdo-Vargas, 2015). Two EPF species in particular, *Metarhizium anisopliae s.l. (brunneum)* (Metchnikov) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin, have been studied for their potential against CSFB, as well as *Phyllotreta spp.* flea beetles (Butt *et al.*, 1992; Miranpuri and Khachatourians, 1995; Reddy *et al.*, 2014b).

In the UK and EU, there are currently five commercial biopesticide products based on four strains of entomopathogenic fungi: *Metarhizium anisopliae (brunneum)* strain F52, *Beauveria bassiana* strains ATCC-74040 and GHA, and *Akhantomyces (Lecanicillium) muscarius* strain Ve-6 (European Commission, 2021; Health and Safety Executive, 2021). *Metarhizium anisopliae (brunneum)* is used to control the larval stage of the vine weevil, authorized in protected horticultural crops; *B. bassiana*, strain GHA is used to control whiteflies, authorized

in horticultural crops in permanent protection with full enclosure; *B. bassiana*, strain ATCC-74040 is used to control whiteflies and thrips, authorized in all edible and ornamental protected crops; *A. muscarius* strain Ve-6 is used to control thrips and whiteflies in protected horticultural and ornamental crops. In the EU only, additional strains are authorized for use in biopesticide products: *B. bassiana* strain 147 used against the moth *Paysandisia archon* and the palm weevil *Rhynchophorus ferrugineus*; *B. bassiana* strain NPP111B005 used against the banana weevil and the palm weevil; *Isaria fumosorosea* Apopka strain 97 and strain Fe9901 used against the greenhouse whitefly (European Commission, 2021).

Metarhizium anisopliae (brunneum) and *B. bassiana* have been tested in the following two laboratory studies against adult CSFB. Butt et al. (1992) tested six isolates of *M. anisopliae* at a concentration of 1 x 10^7 spores/ml in a laboratory bioassay, by submerging the beetles in fungal spore suspensions. They selected isolate V90 (ARSEF 819) for use in further bioassays, and this isolate was found to be highly virulent, causing 100% mortality after 14 days at a concentration of 1 x 10^7 spores/ml. They concluded that V90 could be a potentially useful control of CSFB. Despite these promising results, in a subsequent study Butt et al. (1994) reported that the isolate became attenuated after repeated laboratory cultivation and was considered unstable (Tillemans, Butt and Wilding, 1992) and so unsuitable for use as a commercialized biocontrol agent. Research was done to find other suitable isolates, based on an evaluation of 34 additional isolates of *M. anisopliae* and 15 isolates of *B. bassiana* at a concentration of 1 x 10^7 spores/ml (Butt *et al.*, 1994). Of these, 14 isolates of *M. anisopliae* caused over 50% mortality, but none of the *B. bassiana* isolates were as effective (the maximum mortality observed for *B. bassiana* was 47%). The authors selected two isolates (V208 and V245) of *M. anisopliae*, that led respectively to 88% and 73% mortality. Fungal development was observed on over 70% of dead insects within 2-5 days of exposure to the fungus for both isolates.

The only other published laboratory study investigating the use of EPF against flea beetles was completed by Miranpuri and Khachatourians (1995) who sprayed 14 isolates of *B. bassiana* against adult crucifer flea beetles at a dose of 1 x 10^8 spores/ml. Fifty to 90% of crucifer flea beetles were killed and subsequently showed fungal development on cadavers within seven days of inoculation. Among those isolates, GK 2016 and SG 8702 were found to be the most effective.

Isolates of *M. anisopliae* and *B. bassiana* have also been tested under field conditions: Menzler-Hokkanen et al. unpublished (cited in Hokkanen et al. (2003)) reported that there was a reduced emergence of adult *Phyllotreta* spp. flea beetles after a spray application (41% reduction compared to untreated control) and soil incorporation (34% reduction) with *M. anisopliae* (strain/isolate unidentified) in turnip rape (*Brassica rapa*) fields in Finland. (Antwi, Olson and Carey, 2007; Antwi, Olson and Knodel, 2007) tested Botanigard ES, a commercial

formulation of *B. bassiana*, under both laboratory and field conditions against the adult crucifer flea beetle; in the laboratory study only low mortality (<40%) was recorded and in the field study, leaf damage was high where this treatment was applied. It was therefore concluded that Botanigard ES was not effective against this pest.

Combinations of EPF have also been tested under field conditions. Reddy *et al*. (2014) combined Botanigard 22WP and a commercial formulation of *M. anisopliae* F52, Met52 on canola crops to control the adult crucifer flea beetle. In this study, two spray applications strategies with EPF were used: treatment 1) one application of Botanigard 22WP at 15 days after sowing and one application of Met52 at 30 days after sowing; treatment 2) two applications of Botanigard 22WP at 15 and 30 days after sowing and two applications of Met52 at 45 and 60 days after sowing. Treatment 2 significantly reduced feeding damage (percentage of leaf damage) from >25% in the untreated control to 7.5% in the experimental treatment, and in one location resulted in similar or higher yields compared to the conventional synthetic pesticide seed treatment of Gaucho (imidacloprid) (from 2 tons/ha in the untreated control to 3.4 tons/ha in treatment 2 and 2.5 tons/ha in imidacloprid-treated plots). The improved efficacy of the EPF when repeated applications were made (treatment 2) may have been due to the target insects receiving a higher total dose of spores, either through direct spray contact or through acquisition of spores from plant surfaces. Environmental factors such as UV radiation from sunlight are known to cause reductions of spore viability over time on plant surfaces (Ignoffo and Garcia, 1992; Jaronski, 2010), hence repeated applications may be a way to ensure that an effective dose remains on the plant surface for sufficient time for infection to take place. Persistence can also be enhanced through improvements of the formulation of EPF products, such as the addition of UV protectants (Jackson, Dunlap and Jaronski, 2010).

To my knowledge, there are no published studies investigating the effect of EPF on flea beetle larvae.

4.1.2. Entomopathogenic nematodes (EPN)

EPNs are not covered by biopesticide legislation (as metazoans their use is governed by the same legislation that applies to the regulation of other ‘macro’ biological control agents such as arthropod predators and parasitoids). Despite this, EPNs are used in very similar ways to EPF, and have similar strengths and weaknesses.

There are around 30 families of EPN (Nickle, 1972; Poinar, 1975, 1983, 1990; Lacey, 1997). The families Steinernematidae and Heterorhabditidae (order Rhabditida) are the most studied for their potential as biocontrol agents (Lacey *et al.*, 2001).

EPNs are commonly used as short-term inundative biological control agents (Grewal, Ehlers and Shapiro-Ilan, 2005). There are currently twelve EPN products available for use in

Europe, all based on four species as follows: 1) *Steinernema feltiae* (Filipjev) is used to control sciarid fly larvae, leafminer larvae, thrips, cranefly larvae, various weevil larvae and various lepidopteran larvae. These products are used in protected greenhouse horticultural crops, turfgrass and mushroom crops. 2) *Steinernema carpocapsae* (Weiser) is used to control cranefly larvae, large pine weevil larvae, various lepidopteran larvae and shore fly larvae. These products are used in forestry, horticultural, turfgrass and top fruit crops. 3) *Steinernema kraussei* (Steiner) is used to control vine weevil larvae and pupae in soft fruit and ornamental crops. 4) *Heterorhabditis bacteriophora* is used to control vine weevil larvae and pupae, garden chafer larvae, flea beetles *Phyllotreta* spp., common swift moth larvae, true weevil and snout weevil (BASF, 2021; Koppert UK, 2021). As these four species of nematode are often used to infect the larvae of coleopteran species, they could potentially infect larval soil-dwelling stages and pupae of CSFB.

Several studies have investigated the potential of EPN as alternative control agents of CSFB, crucifer flea beetle and striped flea beetle to the use of conventional synthetic insecticides (Knodel, 2017).

Morris (1987) investigated *S. feltiae* against adult crucifer flea beetles in caged canola micro plots, sprayed at a rate of $1.25x10^6$ IJ/m^2 of soil. They then released into the plots wild adult beetles collected from a nearby field, then applied EPN again a month after introducing the beetles. The plants were removed after a couple of months, and the authors recorded the numbers of new generation adults that emerged from the soil. They did not find differences compared to the water control, and therefore concluded that this species of EPN is not effective against this species of flea beetle. The authors suggested that the low performance of the nematode to infect the larval stages of the crucifer flea beetle might be due to the relatively small size of the larva, making it difficult for the nematode to enter the host.

More recent studies have reported encouraging results, presented here in chronological order of publication.

In China, Li and Wang (1990) used *S. feltiae* against the striped flea beetle in laboratory and field trials, and observed between 87 and 100% of parasitized larvae in the lab, and between 77 and 94% in the field. The authors concluded that this nematode may be an effective control agent of the striped flea beetle. Wei and Wang (1993) found that soil treated with 100 *S. carpocapsae* nematodes/cm^2 reduced larval populations from 38 to 84%, with the most affected instar being the third instar larvae. Hou *et al.* (2001) applied 1.75×10^9 *S. carpocapsae* nematodes/hm^2, which caused 71% of larvae infected by EPN.

In Japan, Kakizaki (2004) tested *S. carpocapsae* (strain not indicated) against the striped flea beetle in Japanese radish fields with a drench treatment of 2.5-5 $x10^5$ nematodes/m^2.Damage to roots was 3-5 times lower than in controls, and the root damage 2-

3 times lower again when the EPN was combined with a seed treatment of tefluthrin (pyrethroid).

In Finland, an unpublished study by Hokkanen *et al.* (2001) (briefly mentioned in Hokkanen *et al.* (2003) reported that *S. feltiae* (strain not indicated) applied at a rate of 1 million nematodes/m^2 reduced adult CSFB emergence in oilseed rape fields by 56%.

Hokkanen et al. (2006) observed a reduction in the recorded numbers of adult *Phyllotreta* spp. of 41.5% when applying *S. feltiae* (strain not indicated) to oilseed rape fields at a rate of 1 million nematodes/m^2. However, the effect against CSFB was highly variable with reductions of 60% and 73% recorded at two Finnish sites but no reduction recorded at the third site in this study, without any explanation suggested by the authors. Hokkanen (2008) mentions a study by Menzler-Hokkanen and Hokkanen (2005) that tested *S. feltiae* (strain not indicated) applied to oilseed rape fields at a rate of 1 million nematodes/m^2 against *Phyllotreta* spp. adults and observed a reduction of 50.1% in numbers of flea beetle.

In Slovenia, Trdan *et al*. (2008) tested commercial formulations of *S. feltiae*, *S. carpocapsae*, *H. bacteriophora*, and *H. megidis* against various *Phyllotreta* spp. adults under laboratory conditions. They applied EPN at concentrations of 2,000, 10,000 or 20,000 nematodes/ml to batches of 10 adult beetles, at three temperatures: 15, 20 and 25°C. They found that for all nematode treatments, mortality was greater than in the control treatment but that *S. feltiae* and *H. bacteriophora* were the most effective species (mortality was 77% at 2,000 *S. feltiae* nematodes/ml at 20°C, while the same concentration of *H. bacteriophora* at 25°C resulted in 100% mortality). The authors of this study noted that EPNs were more effective at 20°C than at 15°C, which could be an important factor in northern Europe oilseed rape crops, especially when plants are at their most vulnerable growth stages in the autumn. They concluded that temperature seemed more important than dose, which can be explained by the fact that in theory, only one infective juvenile is required to kill a host Shapiro-Ilan et al. (2017). In China, Xu *et al*. (2010) compared four isolates: *Steinernema carpocapsae* (all strain)*, S. pakistanense* (94-1) and *Heterorhabditis indica* (212-2 and LN2) in laboratory experiments on striped flea beetle larvae and pupae. They investigated the effect of temperatures (range between 15 and 35°C) and nematodes concentrations and found that the highest mortality of third instar larvae was reached at 25°C (above 80% for all four isolates) and the lowest at 15°C (below 10% mortality). As concentration increased at a constant temperature (25°C), third instar larval mortalities increased from 30 to 100% for both *H. indica* isolates, and from 1% to 80% for both *Steinernema* isolates. At a constant temperature (25°C) and nematode concentration (1000 IJ/ml) for the first instar larvae, highest mortalities were obtained with *H. indica* 212-2 at 30% mortality, for the second instar with *S. carpocapsae* and *H. indica* LN2 at 60% mortality, and for the third instar and pupae all isolates caused more than 80% mortality.

In China again, Yan *et al.* (2013) compared *S. carpocapsae* and *Heterorhabditis indica* LN2 with a water control, and a botanical biopesticide, rotenone. Soil-dwelling striped flea beetle larvae, adults on cabbages in the field and leaf damage were monitored. Both EPN species reduced soil-dwelling flea beetle larval populations in the field (from between 5 and 7 individuals per soil sample in the control, to fewer than 3 individuals per sample in treatments), decreased leaf damage (highest reduction rate with *S. carpocapsae* at 67%) and increased yields (increase of 56.1% and 51.1% for *S. carpocapsae* and *H. indica* respectively). The EPN applications were also more effective than rotenone that reduced leaf damage by 14.5% and increased yield by 13.8%. Both EPN species at both concentrations were equally effective.

In the USA, Reddy *et al.* (2014) tested a commercial formulation of *S. carpocapsae* (Millenium), against the crucifer flea beetle. Canola fields were sprayed with two treatment regimens: 1) two applications at 15 and 30 days after sowing, and 2) four applications at 15, 30, 45 and 60 days after sowing. Both treatments significantly reduced adult feeding damage (% of leaf eaten) compared to untreated plots, with no significant differences between the two treatment regimens (around 12% leaf injury in treated plots compared to 27% in control plots). However, the EPN treatments were not as effective as a combined application of EPF *M. anisopliae (brunneum)* and *B. bassiana* (7.5%) done as part of the same study (see section 4.1.1.), nor did EPN treatments increase yields compared to the controls.

In the USA again, Antwi and Reddy (2016) tested the susceptibility of crucifer flea beetle adults to commercial formulations of several species of EPN including *S. feltiae* (Scanmask) and *S. carpocapsae* (Ecomask), applied to canola fields using foliar applications. EPN were tested in each of four different treatments: 1) as a single species at a rate of 300,000 nematodes/m^2; 2) combined with a second EPN species; 3) formulated with a polymer gel that protects nematodes from UV radiation and desiccation (Barricade); 4) combined with Gaucho (imidacloprid). The authors monitored leaf damage (percentage of leaf area eaten) and yield. EPN applied as a single species or combined with a second species, without Barricade, were not effective in reducing feeding damage or improving yields compared to the control. This may be because of the negative impacts of UV radiations and desiccation on the nematodes applied to leaf surfaces, which is known to be a significant impediment to activity (Shapiro-Ilan, Gouge and Koppenhöfer, 2002) and would explain why combining *S. feltiae* (as Scanmask) with 1% Barricade resulted in significantly higher yields in two of the three study sites (increases of 1020.8kg/ha and 670.2kg/ha). Feeding damage was lower in plots where Gaucho or Gaucho + Scanmask were applied, with similar reductions occurring for both treatments. It was concluded that 1% Barricade could be used together with Scanmask to complement the use of Gaucho as a seed treatment when the period of protection offered by this insecticide is exceeded.

At the same study sites, Briar *et al.* (2018) reported that a commercial formulation of *S. feltiae*, Steinernema-System, together with 1% Barricade gave a level of control of the crucifer flea beetle that was almost as high as that provided by the conventional synthetic insecticide Gaucho in terms of leaf injury and yield at one location, and comparable results in terms of yield in the other location, around 60 miles away. The authors suggested the difference in performance in the two locations to be due to the variation in weather conditions. *Steinernema feltiae* + 1% Barricade resulted in significantly lower levels of leaf damage compared to the untreated control at one location (11.8% of leaf area damaged in the treated plot compared to 21.4% in the control), and so the authors concluded that this combination could be a valuable alternative to Gaucho.

In China, Yan *et al.* (2018) investigated the same isolates as Xu *et al*. (2010, see above) in a field experiment on striped flea beetle larvae and adults. They completed two experiments with different species of Brassicaceae, *Brassica campestris* where all four nematodes were applied, and *B. juncea* where only *S. pakistanense* 94-1 and *H. indica* 212-2 were applied. In the experiment with *B. campestris*, EPN treatments did not have any effect on soil-dwelling beetle population, while in the field of *B. juncea* treatments with EPN lead to lower numbers of soil-dwelling pests. For the adult numbers and yield, no significant differences were found between the EPN treatments in both experiments.

In Thailand, Noosidum *et al.* (2021) investigated three EPN species, *Steinernema siamkayai, S. carpocapsae* and *Heterorhabditis indica* against striped flea beetle larvae, pupae and adult in both laboratory and Chinese radish field experiments. In the laboratory, EPNs were applied at different concentrations and dead beetles counted every day for five days. EPN treatments killed all stages of the pest: 1) third instar larvae: after 48h, the highest mortality of 80-85% was observed at the two highest doses of 100 and 200 *S. siamkayai* nematodes/insect. After 72h, 94-100% mortality was observed with 100 and 200 nematodes/insect with *S. siamkayai* and *S. carpocapsae*. 2) pupae: after 96h, the highest mortality rate of 69-74% was recorded for *S. siamkayai* and *S. carpocapsae* at 200 nematodes/insect. 3) adults: after 120h, the highest mortality recorded was 83% with 200 *S. carpocapsae* nematodes/insect, with other species following closely behind with 77% for *S. siamkayai* and 63% for *H. indica*. In the field, no significant differences were recorded between EPN treatments in terms of adult beetle numbers/plant, though those numbers declined between 20 days after planting and 40 days after planting. However, all three EPN treatments significantly reduced damage on radish roots compared to control treatments (range of 0.30-0.95 cm length of damage in EPN treatments, versus 2.35-3.5 cm length of damage in control treatments. EPN treatments also increased the weight of radish roots compared to control treatments (range of 203-269g in EPN treatments, versus 171-181g in control treatments). In

terms of root diameter, *S. carpocapsae* led to the highest values compared to other treatments, but no difference was detected in terms of root length.

4.1.3. Entomopathogenic bacteria

The genus *Bacillus* includes several entomopathogenic species. The most widely used (representing 50% of microbial control agents sold worldwide (Lacey *et al.*, 2015)) is *Bacillus thuringiensis* (*Bt*) Berliner (Bacillales: Bacillaceae), which is commonly found in soils and on plant surfaces and used to control insect pests through inundative applications as foliar sprays (Chandler *et al.*, 2010). Different subspecies and strains exist, targeting mostly species in the orders Lepidoptera, Coleoptera and Diptera (Glare, Jurat-Fuentes and O'Callaghan, 2017).

Two *Bt* subspecies are known to kill coleopteran insects, although these are not authorized for use in biopesticide products in the UK or the EU: *Bt* subsp. *tenebrionis* (*Btt*) (Krieg *et al.*, 1983) was up to 100% effective against the larvae of the white-spotted rose beetle *Oxythyrea funesta* (Poda) (Coleoptera: Cetoniidae) in a study by Robert *et al*. (1994); and subsp. *san diego* (Herrnstadt *et al.*, 1986), which was shown to be effective against the larvae of the Colorado potato beetle *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) and is commercially sold in the USA (Zehnder and Gelernter, 1989).

Hokkanen *et al*. (2003) considered commercially available formulations of *Bt* against coleopteran oilseed rape pests and concluded that there was little prospect for this product to be made available for the control of CSFB, as *Bt* toxins available at the time were not effective against this pest. A recent study tested three different commercial formulations against CSFB adults (*Btt* strain SA-10, and two Btt strains undisclosed). These treatments did not result in beetle mortalities of more than 40% and recorded beetle mortalities were not significantly different from controls (C. S. V. Price, Campbell and Pope, 2023). These results could be explained by the fact that the individuals tested were adults instead of larvae, which is the usual target of *Bt* formulations, or that the strains used were not appropriate for this particular species. As the larvae feed inside the plant and as such are out of reach of foliar applied insecticides, it would not have been possible to test these products on this life stage whilst feeding on the host plant.

Some *Btt* strains patented in the United States are reported to be effective against various Chrysomelidae species, including the Colorado potato beetle and western corn rootworm (Lambert, Jansens and Peferoen, 1994). The strains BTSO2584B and BTSO2584C were tested against larvae of these species by dipping host plant leaves in bacterial suspension, and larvae stopped feeding after one day and died after a few days, with mortality ranging between 87 and 100% for the Colorado potato beetle, and between 71 and 100% for the western corn rootworm. Similarly, Payne *et al.* (2000) patented several *Btt* strains reported to

be effective against coleopteran pests such as the crucifer flea beetle *Phyllotreta cruciferae*. Here feeding-damage bioassays were completed using adult beetles allowed to feed on leaves treated with bacterial suspensions. Adult beetles showed reduced feeding activity when exposed to *Btt* strains PS140E2, PS28O2 and MR513. The same researchers also patented genes from strain PS140E2 that encode for the bacterial delta-endotoxin 140E2, with the objective of using these in genetically modified crops.

A Chinese study reported that *Bacillus firmus* Bredemann and Werner was pathogenic to the striped flea beetle (Huang *et al.* (1992), cited in Hokkanen *et al.* (2003)), a bacterial species most often used for the control of nematodes (Keren-Zur *et al.*, 2000; Mendoza, Kiewnick and Sikora, 2008; Terefe, Tefera and Sakhuja, 2009; d'Errico *et al.*, 2019). There are no other records of this bacteria species killing other insect pests.

4.1.4. Entomopathogenic viruses

There are over 1000 reported viruses that are pathogenic to insects and infect more than 20 insect families (Grzywacz, 2017). The most commonly used entomopathogenic viruses for biological pest control are located within the Baculoviridae, which contains more than 600 member species (Grzywacz, 2017).

Winstanley and Rovesti (1993) published a list of insect pest species that showed potential for control by viruses, and the only coleopteran species cited was *Oryctes rhinoceros* Linnaeus (Scarabaeidae), susceptible to the *O. rhinoceros* virus (OrV) (Vlak *et al.*, 2008), applied by releasing virus-inoculated adults at breeding sites which then transferred infection to larvae, providing multiyear suppression of beetle populations via reductions in adult beetle lifespan and fecundity (Zelazny, Lolong and Pattang, 1992). Baculoviruses have high host specificity, with infections being confined to individual insect species or genera. Given that no baculoviruses have yet been reported from CSFB, Hokkanen *et al.* (2003) concluded that viruses have little immediate prospects of being exploited for the biocontrol of this pest. Indeed, notwithstanding the limited number of coleopteran specific viruses, all larval stages of *Brassica* feeding flea beetles take place inside the plant and out of reach of existing viral formulations. Releasing virus-inoculated adult CSFB in the fields to target larvae in the same way as with *O. rhinoceros* described above is unlikely to be effective, as adults do not come into contact with larvae.

4.2. Parasitoids and predators

Parasitoids and predators are widely used in classical, conservation and augmentative approaches to biological control. Work investigating the role of these organisms for control of CSFB has, however, focused on conservation biological control. Despite this sustained interest in parasitoids and predators most studies have reported that these natural enemies have little

effect on populations of CSFB. Despite this, promising results have been reported for some species, such as the parasitoid *Microctonus brassicae* (Jordan *et al.*, 2020) (Table 1).

Table 1. Parasitoid and predator species used as biocontrol agents against various stages of cabbage stem flea beetle (CSFB).

Parasitoid/predator species	**Targeted pest**	**Level of control**	**Reference**
Tersilochus tripartitus (Brischke, Ichneumonidae)	CSFB larvae	61% parasitism in France	Alford, 2000
Tersilochus microgaster (Szépligeti)	CSFB larvae	0-57% in Germany, 11% in the UK	Ulber et al., 2010b
Aneuclis melanaria (Holmgren, Ichneumonidae)	CSFB larvae	0.2-1.5% in France	Jourdheuil, 1960
Microctonus brassicae (Haeselbarth, Braconidae)	CSFB adult	44% in laboratory	Jordan et al., 2020
Microctonus vittatae (Muesebeck, Braconidae)	Crucifer flea beetle and striped flea beetle adult	3-15% (crucifer) and 15-53% (striped) in the US	Wylie, 1982
Townselitus bicolor (Wesmael, Braconidae)	Crucifer flea beetle and striped flea beetle adult	50% in Europe	Sommer 1981 (in Dosdall and Mason, 2010)
Trechus quadristriatus (Shrank, Carabidae)	CSFB eggs	6 eggs/24h in laboratory	Warner et al. 2003

The effectiveness of conservation biological control based on parasitoids and predators is affected by the impact that other agronomic activities have on these natural enemy populations. In a full review of the sublethal and lethal effects of insecticides on parasitoid of

oilseed rape pests, Ulber *et al.* (2010a) concluded that pyrethroids, the most widely class of insecticide authorized for oilseed rape crops, are lethal to natural enemies, significantly lowering the overall level of parasitism in treated crops. Sublethal effects, such as avoidance of treated leaves and a decreased oviposition rate on those leaves, have also been observed in laboratory studies (Ulber, Klukowski and Williams, 2010). For the conservation of parasitoid population, the authors of the review suggested adapting the choice of insecticide and rate applied (Ulber, Klukowski and Williams, 2010). For example, the pyrethroid tau-fluvalinate has been proven to be less harmful than another pyrethroid, lambda-cyhalothrin, to natural enemies (Ulber, Klukowski and Williams, 2010). Applying pyrethroids at half the recommended rate is also effective in protecting population of parasitoids in crops (Ulber, Klukowski and Williams, 2010). They also recommended regulatory testing of insecticide effects on parasitoids by research groups and agrochemical companies, applying insecticides outside the activity period of parasitoids and to area of high pest density only (Ulber, Klukowski and Williams, 2010). Another way to preserve parasitoid populations is the push-pull strategy, which consists in attracting the pests and their natural enemies in a trap crop grown alongside the main crop; in the case of oilseed rape, Barari et al. (2005) found that using turnip rape (*Brassica rapa)* as a trap crop was effective in reducing the abundance of CSFB in the oilseed rape crop.

In their review of ground beetles as predators of oilseed rape pests, Williams *et al.* (2010) identified crop management practices that are detrimental to ground beetles as well as approaches that can help enhance their population in oilseed rape. Large fields, use of conventional tillage, bare soils and pesticide applications have been shown to negatively affect ground beetle populations. Instead, minimum tillage should replace ploughing and provision of field margins and beetle banks within fields offer habitats for overwintering populations as well as shelter from farming operations (Williams *et al*., 2010).

4.3. Botanical biopesticides

Botanical biopesticides may affect insect herbivores in different ways, including both lethal and sublethal effects. Sublethal impacts can include reduced growth, fertility, reproduction, oviposition and feeding (Mordue and Blackwell, 1993; Nisbet, 2000). An advantage of botanical biopesticides is that they are less persistent than conventional synthetic insecticides (Smith, 1989), can be applied as powders, aqueous solutions, oils, emulsions, etc. (Isman, Miresmailli and Machial, 2011).

One of the most studied plant extracts is the tetranortriterpenoid (limonoid class) azadirachtin, produced by the neem tree (*Azadirachta indica* A. Juss., Meliaceae) native to India (Schmutterer, 1990). It can be mixed with other biopesticides such as microbial

organisms (Koppenhöfer and Kaya, 2000; Yan *et al.*, 2013a) and in doing so, it helps to enhance the level of control achieved, but the mechanisms by which this occurs are not known.

For example, in combination with EPN, Yan *et al.* (2013) found that azadirachtin significantly decreased the emergence of striped flea beetle adults The authors had previously conducted unpublished laboratory assays to confirm that azadirachtin would cause no direct harm to EPN.

5. Conclusions

CSFB is an economically important pest for which there are currently no effective IPM programs combining alternative biological control agents with or without conventional control methods. With existing research indicating the potential of biological control agents for the control of flea beetle pests of *Brassica* crops, there is the opportunity to provide farmers with biological solutions within which to manage this pest. While the results of these studies have been encouraging, biological control agents do not yet feature prominently in management programs for CSFB. To date, no EPF-based product has yet been approved for use in oilseed rape in the UK (Health and Safety Executive, 2021), and adoption of alternative forms of pest control to reduce the use of conventional pesticides remains low in arable crops, even though these approaches are widely used in protected crops (Chandler *et al.*, 2010). At the time of publication, Hokkanen and Menzler-Hokkanen (2017) reported that none of the research investigating the potential of EPF on CSFB had been applied to commercial crops, and growers were still relying on conventional synthetic pesticides. The authors suggested that this was due to a combination of a lack of trust and training in the use of fungal-based biopesticides, the fact that conventional insecticides are cheap and more convenient to apply, and that there is no real incentive for growers to adopt alternative approaches. Besides, biocontrol agents released or applied to the crop are subjected to variable biotic and abiotic factors limiting that may influence efficacy (Shapiro-Ilan *et al.*, 2006; Gul, Saeed and Khan, 2014).

Based on the studies completed so far, EPF and EPN in particular and parasitoids show potential as effective biological control agents of CSFB that may be included within future IPM programs, which may be more prominent in future control strategies due to a lack of effective conventional insecticides and the need for environmentally safe forms of crop protection (see section 3). To achieve this, however, further research is required to improve their efficacy and better understand the factors that determine the level of control reported.

5.1. Improving the efficacy of EPN and EPF within IPM for CSFB

In the case of flea beetles and oilseed rape, there have been few field studies to investigate the efficacy of EPF under conditions that reflect commercial cropping practices. By contrast, EPN have primarily been studied under field conditions, meaning that efficacy has been

determined through indirect measures of adult emergence following nematodes application. In addition, the majority of studies so far completed have focused on the control of the crucifer flea beetle and the striped flea beetle, and some of these studies provided little information on how the biological control agents have been applied and without detailed results. *Bacillus thuringiensis* on the other end does not seem to be a promising control option, as my unpublished results indicate that this biopesticide is not effective against CSFB adults.

Improving the effectiveness of biocontrol agents within an IPM program instead of single treatments to replace conventional insecticides is a key area for future research. For farmer adoption of these approaches, however, biology and ecology must be considered alongside the economics of adopting the use of these controls.

Many of the studies cited in this review have compared entomopathogens as stand-alone treatments with conventional insecticide treatments. While these pioneering studies are a necessary first stage in the evaluation of candidate biological control agents, the most likely way of using entomopathogens in the future will be as part of an IPM program. The way in which different components of an IPM program interact needs to be understood so that they support each other. Indeed, antagonistic, synergistic and additive interactions with other biological control agents and conventional pesticides need to be taken into account and understood. For example, fungicides and nematicides can be lethal to entomopathogens (Chandler, 2017; Shapiro-Ilan, Hazir and Glazer, 2017). In addition, EPF such as *Beauveria bassiana* and *Isaria fumosorosea* are antagonists, while *Metarhizium anisopliae (brunneum)* and the bacteria *Bacillus thuringiensis* are synergists, and parasitoids are neutral or competitors to EPN (Shapiro-Ilan, Hazir and Glazer, 2017). EPF can be used in combination with predators and parasitoids (Labbé *et al.*, 2009) and with *Bacillus thuringiensis tenebrionis* (Wraight and Ramos, 2005). Non-antagonistic interactions have proven to improve the efficacy of EPN as biological control agents (Shapiro-Ilan, Hazir and Glazer, 2017). There is also the option of host plant resistance to combine with biological control agents. There is currently work being done to select less palatable and resistant varieties of oilseed rape to CSFB, but there is no published work at this time.

According to (Chandler (2017) and Shapiro-Ilan *et al.* (2017), future research should include the use of EPF and EPN as conservation control agents, as improved knowledge of their ecology and biology should allow successful conservation biocontrol rather than reliance on inundative applications only. Furthermore, the modification of crop management practices could improve the activity of pathogens naturally present in fields. There is also the selection of new species and strains of entomopathogens that are more effective, or resistant to abiotic factors such as UV radiation, or species-specific to minimize non-target effects. Improving formulations to improve the persistence of pathogens in the field is necessary. As I stated earlier in this paper, there are ways to protect entomopathogens against UV radiation and low

humidity, such as polymer gels, surfactants, sunscreens, and a different time of application. In terms of species specificity, it is important to consider the effects on non-target organisms (Chandler, 2017; Shapiro-Ilan, Hazir and Glazer, 2017).

5.2. Making better use of the attractive biological properties of EPF and EPN

Biopesticides based on entomopathogens have generally been developed according to a chemical pesticide paradigm which emphasizes finding 'winning' candidate strains with fast speed of kill and high efficacy and tends to overlook other attractive properties of entomopathogens as living organisms, such as the ability to provide self-perpetuating control, or to induce plant defenses against insect damage (Waage, 1997). Under this paradigm, there can be unrealistic expectations of chemical-like performance and the potential role of the entomopathogen as a component of holistic IPM systems tends to be ignored (Waage, 1997). As living organisms, the efficacy of EPN and EPF is subject to biotic and abiotic factors, which means that they cannot perform in the same way as conventional insecticides. The impacts of these factors on EPF have been investigated in several studies (see Jaronski, 2010), though most often in the laboratory and not in commercial crop situations, which should be the focus of future work. A good entomopathogen would then be one that is virulent, can be economically mass-produced, has a low impact on the environment, will not lead to the development of resistance (these already apply to several species of EPF and EPN) and can resist environmental conditions enough to play its role as a biological control agent. The product based on the entomopathogen must also deliver the right amounts of infectious agents (spores or infective juveniles) to kill the pest. As a living organism, the fact that they can persist in the crop (under the right environmental conditions) by being transmitted from cadavers to healthy host, is a very attractive feature of entomopathogens. Key parameters that need to be investigated are the lethal dose of infectious agents, the effective dose required to apply on the plants and soil, the ability to deliver the effective dose to the target pest, and how long it persists in the environment. Little information in these areas is available for CSFB and will be a priority for future research.

EPF and EPN for now represent the most promising candidate entomopathogens to include in a IPM program for CSFB and related species of flea beetle. They are most likely to be used as inundative biopesticides, but there is also potential for novel application strategies, such as the use of endophytic EPF, possibly applied as a seed coat or soil inoculum. Endophytic fungi can grow inside the tissues of a healthy plant without inducing any symptoms of illness (Stone, Polishook and White, 2004) and can be used as biocontrol agents against pests such as insects and pathogens (Mejía *et al.*, 2008; Brum *et al.*, 2012; Zhang *et al.*, 2014; Mantzoukas and Eliopoulos, 2020). In the case of EPN, the use of the symbiotic bacteria living in their gut

or the metabolites they produce could also be considered (Shapiro-Ilan, Hazir and Glazer, 2017). For example, Mohan *et al*. (2003) found 100% mortality within 24h of the cabbage white butterfly (*Pieris brassicae*) larval stage after foliar sprays of *Photorhabdus luminescens*, bacteria living in the gut of nematodes *Heterorhabditis* spp.

5.3. Using parasitoids and predators within IPM

More work on the potential of predators and parasitoids against CSFB is required. In their study on the potential of the parasitoid *Microctonus brassicae* against adult CSFB, Jordan *et al. (2020)* concluded that the next research goals would be to determine which of conservation or augmentation biocontrol strategies would be the more pertinent approach, to gather more data on the biology, distribution, field parasitism rate, and to improve the methods of rearing. In the case of conservation biocontrol, several measures can be put in place to mitigate the impact of farming practices that have negative effects on the abundance and activity of parasitoids and predators of CSFB, such as minimum tillage, field margins and applications of insecticide when these beneficials are not active. In their review presentation of the importance of parasitoids against pest of oilseed rape, Ulber (2017) stated that many species of parasitoids were sufficiently abundant and widespread across Europe to be economically important in the control of these pests, but that potential has not been exploited yet and there is a need to improve the strategies of conservation biocontrol of parasitoids to improve their efficacy in the fields.

5.4. Creating an IPM program

Several studies have attempted to combine biocontrol agents together, such as two species of EPF (Reddy *et al.*, 2014b), two species of EPN (Antwi and Reddy, 2016), EPN with a conventional insecticide (Antwi and Reddy, 2016), or azadirachtin with EPN (Yan *et al.*, 2013a). However, the rationale of these choices of combinations seems arbitrary and not based on an understanding of the ways these biocontrol agents interact, as the authors do not always give any explanation about why these biocontrol agents would work well together. Rather, combinations of biocontrol agents should be done according to a proper strategy to identify and optimize positive interactions between the different components, as parts of an IPM program (Stenberg, 2017).

Only a few pioneering studies have been done on the biocontrol of CSFB and other related flea beetle species, that show that there is a potential to develop an IPM program based on multiple, complimentary components. An IPM pyramid details the different actions to undertake to control pests starting from the bottom (prevention), then progressing towards the top (chemical control) if prevention techniques and biological control were not enough to control

the pest. Non-chemical agronomic practices that could be included in an IPM program have been extensively reviewed by Blake *et al.* (2021), Ortega-Ramos *et al*. (2021) and Pickering *et al.* (2020). These studies identified the most promising means of controlling CSFB while limiting the use of chemicals, such as a modified sow date (earlier or later), decreased seed rate, increased seedbed moisture, leaving long stubble before drilling oilseed rape, resistant cultivars, the use of volunteer oilseed rape as trap crop, and defoliation of oilseed rape in winter. As said above in Antwi and Reddy (2016) study, it is also possible to include conventional chemical insecticides in an IPM approach to benefit from their effect while limiting their excessive use by alternating with biocontrol agents or as combination, as a strategy of pesticide resistance management to support the arms race between crop breeders and pest species (Stenberg, 2017). Indeed, as entomopathogens are slower acting than conventional insecticides, it may be better to use them as a preventive treatment when CSFB populations are still low in the crop, for example late August/early September before the main migration of young adults into the crops. Then, as a solution to achieve sufficient control without relying excessively on conventional insecticides, it would be interesting for the farmer to apply conventional insecticides only if pest populations passed an action threshold, which would reduce the total amount of conventional insecticide used. A possible IPM pyramid gathering all these components is illustrated in Figure 2.

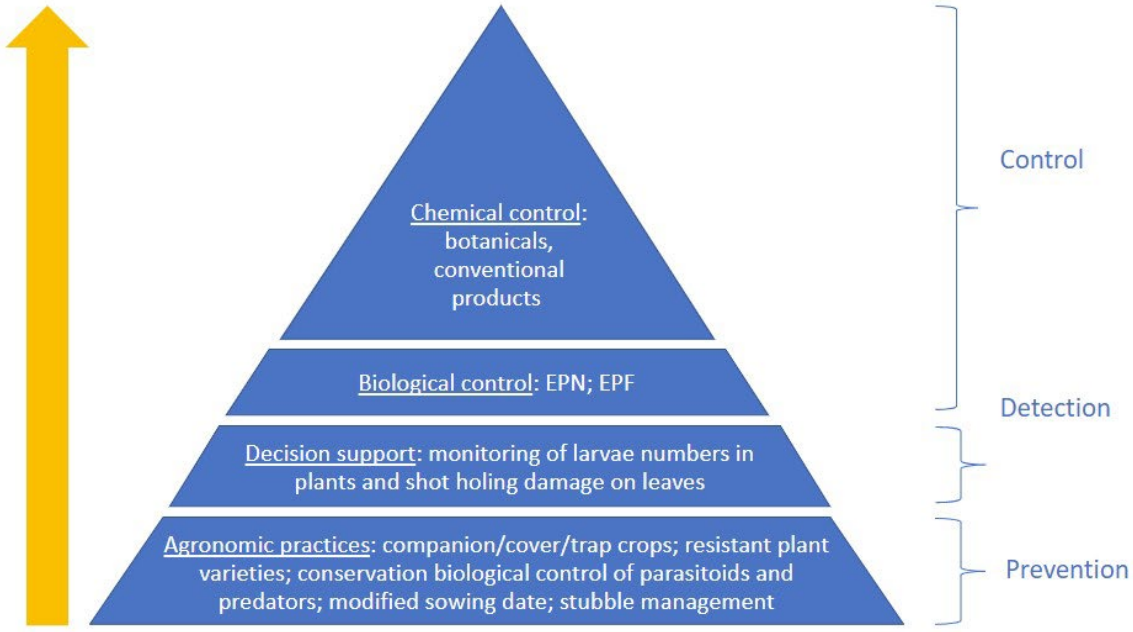

Figure 2. Integrated Pest Management pyramid for the control of cabbage stem flea beetle in oilseed rape (EPN: entomopathogenic nematodes; EPF: entomopathogenic fungi).

In summary, below are recommendations for future works:

• There is a real need for more studies in which the target pest is CSFB instead of related species of flea beetles.

• It would be interesting to understand why parasitism levels by parasitoids varied between countries (Ulber *et al.*, 2010), and to do more studies on the impact of parasitoids and predators against CSFB populations in the fields in terms of predation and parasitism rates. Indeed, as concluded by Jordan *et al.* (2020), whether the best approach is conservation or inundative biocontrol is still to be determined;

• Endophytic strains of entomopathogenic fungi to control the larval stage of CSFB need to be studied.

• The selection of oilseed rape varieties that are resistant or less palatable to CSFB should be explored further.

• Future research could also focus on selecting entomopathogen strains that are more resistant to environmental factors, and on increasing their efficacy and reliability in the fields.

• Pathogen byproducts, such as the bacteria living in the gut of EPN and actively kill the insect host, could be tested against CSFB.

• Laboratory studies need to be done with EPN as published studies only report field studies, and more field studies need to be done with EPF against CSFB, as the studies published focused on other species of flea beetles.

• The various biopesticides identified need to be tested in combinations as part of an IPM program rather than simply stand-alone treatments as conventional insecticides.

6. Aims and objectives

This thesis aims are to investigate the potential of biopesticides to fight the cabbage stem flea beetle in oilseed rape crops.

The objectives are the following:

- Screen selected biopesticides effect on adult CSFB survival under laboratory conditions. Biopesticides include entomopathogenic nematodes, fungi, and bacteria; physically acting products such as fatty acids; and botanical insecticides such as azadirachtin; and study the potential of adjuvants to improve the effect of selected biopesticides.
- After laboratory screenings, test selected biopesticides under oilseed rape field conditions and record feeding damage on leaves by adult CSFB and larval density.

Chapter 2: Rearing and maintaining cabbage stem flea beetles *Psylliodes chrysocephala* under laboratory conditions

Abstract

Cabbage stem flea beetle (CSFB) is an economically important pest of oilseed rape in the UK. Before the ban of neonicotinoid seed treatments by the European Union in 2013, this class of insecticide was used by oilseed rape growers to control CSFB. Since then, the use of foliar applications of pyrethroid insecticides has increased and this has led to the development of widespread resistance in UK CSFB populations and elsewhere in Europe. Research is ongoing to find alternative solutions to control CSFB. Some of this research requires that cultures of this species of insect are maintained under laboratory conditions before use in experiments. The objective of this chapter is to evaluate different laboratory rearing techniques as well as the efficacy of methods to maintain field collected CSFB adults.

Over three years I used various methods to rear and maintain CSFB under laboratory conditions, each method had its pros and cons. Collecting CSFB adults at oilseed rape harvest in July or early August allowed for thousands of CSFB to be collected quickly, however, typically a small number of adults in each collection was parasitised and this often led to a rapid decline in populations as large numbers of CSFB adults became parasitised under laboratory conditions over the following few months. Rearing CSFB adults from eggs laid by field collected adults under laboratory conditions ensured that the adults were clean of parasitoids, however, the process was time consuming, and the number of adult beetles obtained low. Field collecting CSFB larvae and rearing larvae through to adults under laboratory conditions was more effective than attempting to rear adults from eggs. I concluded that combining the collection of CSFB adults in the summer and the collection of plants infested with third instar larvae in the later winter/early spring is the most effective way of ensuring a continuous supply of CSFB adults and larvae for use in laboratory experiments.

1. Introduction

Cabbage stem flea beetle (CSFB, *Psylliodes chrysocephala* Linnaeus) (Coleoptera: Chrysomelidae) is the most economically important pest species of oilseed rape (*Brassica napus*), which they invade during crop establishment as adults (Alford and Gould, 1976b; Winfield, 1992b; Ferguson *et al.*, 2003b; Nicholls, 2016b; Ortega-Ramos *et al.*, 2021). Adult feeding in late summer/early autumn can lead to plants death (Leach *et al.*, 1994b), and larvae

feeding inside the plants over winter can lead to total plant collapse in the case of heavy infestations (Bonnemaison and Jourdheuil, 1954; Williams and Carden, 1961b; Graham and Alford, 1981b; C. Nilsson, 1990; Winfield, 1992b; Nilsson, 2002b; Ingrid H. Williams, 2010).

CSFB were controlled by neonicotinoid insecticides applied as a seed dressing prior to their ban by the European Union in 2013 (European Commission, 2013b). Since then, the only class of synthetic insecticides authorised to control CSFB is pyrethroids applied as a foliar treatment. The overreliance on the use of pyrethroids has negatively affected non-target organisms such as pollinators and natural enemies (Ingrid H. Williams, 2010) and has resulted in the development of widespread resistance of CSFB to these insecticides (Højland *et al.*, 2015b; Willis *et al.*, 2020). One notable consequence is that the production of oilseed rape in the UK has decreased over the years since the ban of neonicotinoids, from 756,000 hectares in 2012 to 307,000 hectares in 2021, largely due to an inability to effectively control CSFB (Scott and Bilsborrow, 2019; Defra, 2022).

There is an intense research effort in the UK and in many other European countries to find alternative solutions to control CSFB, including my own project investigating the potential of biopesticides against CSFB, under laboratory and field conditions. Laboratory studies necessitate maintaining and/or rearing of CSFB adults and/or larvae in the laboratory for easy access to test subjects throughout the year. As this is a univoltine species, each development stage can only be collected from the field at certain times (Ingrid H. Williams, 2010).

1.1. Maintaining field collected CSFB adults

Previous studies have employed a range of methods for maintaining flea beetle cultures under laboratory conditions. This includes studies on species closely related to CSFB. For example, Miranpuri and Khachatourians (1995) collected adult crucifer flea beetle (*Phyllotreta cruciferae* Goeze) with an aspirator from canola crops and maintained them at 21-25°C in mesh cages (25 x 25 x 38 cm) that also contained 1l beakers or jars in which canola plants were placed as food. (Xu *et al.*, 2010) collected adults of the striped flea beetle (*Phyllotreta striolata* Fabricius) from vegetable fields and kept them in the laboratory at 25 ± 1°C, 70 ± 10% RH, and a 14:10 h L:D photoperiod, with leaves of Chinese cabbage (*Brassica rapa chinensis*) as a source of food. (Noosidum, Mangtab and Lewis, 2021) collected adults of *Phyllotreta striolata* from a brassica field and kept them under laboratory conditions at 25 ± 2◦C; 75 ± 5% relative humidity (RH) with a photoperiod of 12h:12 h L:D photoperiod with Chinese radish (*Raphanus sativus* var. *longipinnatus*) as source of food.

There are several examples where CSFB adults have been collected from the field and then kept in the laboratory but each of these studies has varied in the host plants used, temperatures and/or photoperiod. For example, (Butt *et al.*, 1992, 1994, 1995), collected CSFB

adults and maintained these on Chinese cabbage at 14°C in 16:8 h L:D photoperiod. (Ibrahim *et al.*, 1999) maintained CSFB for one month on Chinese cabbage plants at 15°C and 16:8 h L:D photoperiod before using them for experiments. (Beran *et al.*, 2018) reared CSFB on *Brassica rapa* at 24°C, 75% relative humidity, 16:8 h L:D photoperiod. In (Willis *et al.*, 2020) CSFB were maintained on Chinese cabbage in mesh cages at 15 ± 1 ◦C, 65% relative humidity, 12:12 h L:D photoperiod. (Jordan *et al.*, 2020) maintained CSFB on potted oilseed rape plants or Chinese cabbage within micro-perforated bags (38 x 90 cm) in controlled environment rooms at L16 (22°C Day): D8 (20 °C) photo-thermoperiod. (Hovorka, 2022) maintained CSFB on potted oilseed rape or kohlrabi (*Brassica oleracea* var. *gongylodes*) at an L16 (22°C): D8 (20°C) photo-thermoperiod.

1.2. Egg-laying activity and egg development

Alford (1979) kept CSFB adults under laboratory conditions at different temperatures, then recorded the egg-laying activity of females and monitored egg development. The author observed that mature eggs were first laid by females about 12-14 days after they began feeding on oilseed rape. They reported that egg development is significantly impacted by temperature: eggs took an average of 37 days to hatch at 10°C, but 70 days or more when temperatures were below 6°C, with development stopping completely at 3.2°C. They calculated that an accumulated 240-day degrees above 3.2°C from the date of egg laying is necessary for the egg to hatch. These figures are similar to those recorded in the field by Johnen and Meier (2000) who completed field observations over 8 years and concluded that an accumulated 200-day degrees above 4°C is necessary for eggs to hatch.

Såringer (1984) kept CSFB adult cultures (containers of 20 adults, 10 males and 10 females) in different environmental conditions to observe oviposition. This work was completed in the laboratory using constant temperatures of 28, 23 and 18°C temperatures, short-day (13 h) and long-day (17 h) light conditions. Additional ‘control’ cultures were kept under field conditions where temperatures varied. They found that optimal oviposition takes place at temperatures between 4 and 12°C and that with increasing temperatures the number of eggs laid per female decreased.

Vig (2003) kept their cultures of adult CSFB in large Petri dishes (dimensions not indicated) covered with moistened filter paper. CSFB adults were provided regularly with fresh oilseed rape leaves at constant temperatures of 10°C ± 1.7°C and 18°C ± 0.7°C and 15:9 h L:D photoperiod or in an insectarium under field conditions where temperatures varied. They recorded egg laying between 5 and 10 days after copulation was observed. In the laboratory, they observed that egg development time increased with decreasing temperatures (20.3 ± 2.6 days at 18°C and 35.8 ± 1.4 days at 10°C).

Mathiasen *et al*. (2015) collected adult CSFB at crop harvest. Collected beetles were sexed and one male and one female placed together at 16°C in small plastic containers (16 x 22 x 32 cm) with leaves of Chinese cabbage or oilseed rape as a source of food. The containers were then divided into five random groups (each replicated 20 times) and placed in an incubator at five different constant temperatures (4, 8, 12, 16 and 20°C), each with 12:12 h L:D photoperiod. The number of eggs and mortality were assessed every two days for one month then twice a week until the end of the experiment. The authors observed that female CSFB took longer to start laying eggs when temperatures were low. Frequency of egg laying was regular, except at the lowest temperatures of 4 or 8°C at which the time between egg laying increased. Females laid the highest number of eggs when the temperature was 16°C and the lowest number of eggs at 4°C, and daily oviposition rate increased with temperature. Females continued laying eggs for as long as 8 months even if the male died or was lost and survived significantly longer at the lowest temperature tested of 4°C compared to the highest temperature of 20°C. Egg development time decreased with increasing temperature (average of 180.74 days at 4°C compared to 12.04 days at 20°C). The authors of this study calculated an egg development threshold of 5.1°C and thermal requirements of 184.9 degree-days. Hatching percentage was lower at 4°C compared to the other temperatures tested. They concluded that temperature is an important factor in CSFB reproduction and identified 16°C and 20°C as an optimum temperature range for reproductive success.

1.3. Rearing CSFB adults from larvae collected in the field

Barari *et al*. (2004) reared CSFB larvae to adult stage as part of their study on parasitoids of this crop pest. They collected infested plants in 2002 and placed these in an outdoor insectary for a week, where mature larvae ready to pupate would exit the plants and fall into drawers for collection. The larvae were then transferred to two different containers: 1) pot emergence traps, with a capacity of up to 20 larvae and made of plastic flowerpots capped with a metal frame and black tulle to prevent emerging adults from escaping, connected to a screw-on plastic container that could easily be removed to collect adults; 2) corked tubes, made of glass tubes with cork stoppers, with a capacity of up to 5 larvae. Both types of containers were filled with soil collected from an untreated oilseed rape field and the soil was topped with moss in the pot emergence trap to maintain humidity. Larvae were left to bury themselves in the soil, then the containers were kept in the outdoor insectary out of direct sunlight, and the containers were checked weekly for adult emergence and to water the pot emergence trap if the soil was too dry. Between April and July of the same year, they collected a total of 465 mature CSFB larvae exiting the plants in the insectary and placed 348 in pot emergence traps and 117 in corked tubes. Only 13% of these larvae reached adult stage (60

adult CSFB collected), and a majority of these were obtained from corked tubes, first emerging in June, 23 days after placing the larvae in the soil. The authors suggested that corked tubes were more effective than pot emergence traps due to better water regulation of the former. There was still a high larval mortality as only 60 adults were obtained from 465 larvae. The authors observed that the soil might have been too heavy and compacted in some of the containers and weekly watering of pot emergence traps led to a soil that was too wet, making the soil unsuitable for larval and pupal development. They suggested adding 20% of sharp sand may help to resolve this issue.

The present chapter gives an overview of the different methods used to maintain CSFB adults, collect CSFB larvae, and rear them to the adult stage, under laboratory conditions.

2. Material and Methods

2.1. Maintaining field collected CSFB adults in the laboratory

In July 2019 and 2020, adult CSFB were collected at harvest at Apley Estate Farm, in Norton (Shropshire, UK) and from GC Davies & Co, in Shrewsbury (Shropshire, UK) in 2021 from grain stores using a hand-held vacuum. Collected CSFB adults were first kept in ventilated mesh cages (30x30x30cm) at a constant 20°C temperature, 60% RH, 16:8 h L:D photoperiod in a growth chamber (Fitotron® SGR 122, Weiss Technik UK Ltd, UK). CSFB were fed on potted Chinese cabbage (*Brassica rapa chinensis*) grown in John Innes No. 2 compost (Westland Horticulture Ltd, Dungannon, UK) in 2019 and directly on potted oilseed rape (*Brassica napus*) plants at growth stage of 12 (BBCH system (Lancashire *et al.*, 1991)) in 2020 and 2021. CSFB adults were then counted and evenly allocated to new cages (same size as above, approximately 200 beetles per cage) with plants, forming the stock culture. On 4th November 2019, Chinese cabbage plants were replaced by potted oilseed rape (same stage as above. I watered the plants from above when the compost at the top of the pot was dry to the touch. Other studies have used oilseed rape or Chinese cabbage as host plants for their own CSFB cultures (Butt *et al.*, 1992, 1994, 1995; Ibrahim *et al.*, 1999; Mathiasen *et al.*, 2015; Beran *et al.*, 2018; Jordan *et al.*, 2020; Willis *et al.*, 2020; Hovorka, 2022), but environmental conditions vary between studies, from setting the temperature as low as 14-15°C (Butt *et al.*, 1992, 1994, 1995; Ibrahim *et al.*, 1999; Willis *et al.*, 2020) and as high as 24°C (Beran *et al.*, 2018), with some studies having variation between day at 22°C and night at 20°C (Jordan *et al.*, 2020; Hovorka, 2022). As in my own culture, all the studies cited above kept their CSFB at a photoperiod of 16:8 h L:D except for Willis *et al.* (2020) and *Mathiasen et al.* (2015) who set up a photoperiod of 12:12.

Plants were replaced once or twice a month, depending on their overall state. Removed plants were placed in clean empty cages to allow any larvae and pupae to develop to adult stage (see next section).

2.2. Breeding second (F2) and third (F3) generations adults CSFB with no handling of larvae and pupae

As mentioned in the previous section, plants that were removed from the stock culture cages were placed in clean empty cages kept in the same environmental conditions as the stock culture, to allow larvae and pupae to develop into adults without any human intervention apart from watering the host plants when the compost as the top of each pot was dry to the touch. Beran *et al*. (2018) used a similar method to raise F2 adult CSFB, but as the focus of the study was to explain the mechanisms by which CSFB overcome oilseed rape chemical defences, the authors did not report on the numbers of CSFB obtained in this way. F2 adults were counted and transferred to new cages with potted oilseed rape plants as a source of food. To create an F3 population, the plants from this F2 cage were replaced at regular intervals and kept in a separate cage to allow for F3 larvae and pupae to develop.

2.3. Breeding second (F2) and third (F3) generations adult CSFB by transferring larvae and pupae to new plants and compost

Here, instead of leaving the larvae and pupae develop in planta without human intervention, after approximately one month of exposure to the stock culture of CSFB adults, plants were dissected to collect larvae and the compost was searched for pupae. Larvae (all instars) were deposited at the base of fresh oilseed rape plants (same growth stage as above) in separate ventilated mesh cages (30x30x30cm) and left to enter the plants to feed. Pupae were placed into plastic plant pots (12cm diameter, 11.5 cm high) filled with compost (John Innes No. 2), placed within the first few centimetres of compost. Plant pots were placed into ventilated mesh cages (same size as above) and pupae were left to develop into adult CSFB. Water was applied to the compost in the pot when it felt dry to the touch. Cages and pot were kept at a constant 20°C temperature, 60% RH, 16:8 h L:D photoperiod in a growth chamber (Fitotron® SGR 122, Weiss Technik UK Ltd, UK). After adult emergence, CSFB adults were counted and the percentage of larvae and pupae that reached adult stage was calculated.

2.4. Raising larval CSFB in planta from plants collected in fields

2.4.1. Leaving field-collected larvae in original plants

On 25th January 2022, I collected 18 winter oilseed rape plants from a commercial oilseed rape field (Apley Estate Farm, Norton, Shropshire, UK) and transplanted these plants into plastic pots (12 cm high, 10 cm wide at the base and 13 cm wide at the top) filled with John Innes No. 2 compost. I placed the plants in two insect proof mesh cages (50x50cm, Gribblybugs.com), with nine pots per cage, at a constant 20°C temperature, 60% RH, 16:8 h L:D photoperiod in a growth chamber (Fitotron® SGR 122, Weiss Technik UK Ltd, UK). I watered the plants when the compost at the top of the pot was dry to the touch.

On 14th March, adult CSFB started emerging from the compost, and as by then the plants were dead, these were removed, but the pots and compost were kept. I collected the beetles with a pooter as they appeared and transferred them to two smaller mesh ventilated cages (30 x 30 x 30 cm) kept in the same environmental conditions. I fed them with a single oilseed rape plant in a pot per cage. I regularly watered the plant when the compost at the top of the pot was dry to the touch.

On 23rd March, I collected the last adults to emerge. On 14th of April, I poured each of the 18 pots one after the other into a plastic tray and searched the compost by hand for pupae and adults. Once all the pots were checked, I checked the cage itself for adults. The total of CSFB adults collected was counted.

2.4.2. Transferring field-collected larvae to new plants

In January 2021, I collected plants from oilseed rape crop grown on Apley Estate Farm (Norton, Shropshire, UK) and dissected these the laboratory. Similarly, a sample of oilseed rape plants sent by an agronomist working in Wiltshire, UK. The collected larvae were transferred to fresh potted oilseed rape plants at growth stage 12 (BBCH system) in John Innes No. 2, placed in mesh ventilated cages (30 x 30 x 30 cm) and kept at a constant 20°C temperature, 60% RH, 16:8 h L:D photoperiod in a growth chamber (Fitotron® SGR 122, Weiss Technik UK Ltd, UK). The plants were watered when the compost at the top of the pots felt dry to the touch. After emergence, CSFB adults were counted and the percentage of larvae that made it to adult stage was calculated.

The next year, on 4th February 2022, I collected 30 winter oilseed rape plants from Apley Estate Farm again and transplanted them to two square plastic trays (91x91x12 cm), with 15 plants in each. Each plastic tray was kept in a tent cage (base 143cm^2) placed in an unheated polytunnel. Temperature and hygrometry were not monitored. On 11th April one of the cages had been partly destroyed by rats. To avoid further damage, plants in this cage were dissected on 20th and 21st April and the larvae were recovered. The plants in the second cage were

dissected on 29th April. All larvae collected in this way were placed on four fresh oilseed rape plants at similar growth stage and kept in the same conditions as the paragraph above. After emergence, CSFB adults were counted and the percentage of larvae that made it to adult stage was calculated.

3. Results and Discussion

3.1. Maintaining field collected CSFB adults in the laboratory

In 2019, a total of 3,670 beetles were counted on 3rd October. On 11th November, two parasitoid wasps (provisional identification of *Microctonus brassicae* (Haeselbarth)) were recorded in one cage containing CSFB adults alongside 22 dead beetles. Taking into account the removal of some individuals for experiments, the stock culture should have been formed of 3,438 beetles on 9th January 2020, but I counted only 1,890 CSFB, and on 13th March I counted 570 CSFB instead of 1,338. During the first COVID-19 lockdown between March and June 2020 I was not able to monitor the stock culture. When I counted CSFB again, I recorded 186 individuals in the stock culture left out of 570 in March. The last count of CSFB adults was completed on 30th June, when only 44 beetles remained as many had by then reached the end of their lifespan. This might be due to the fact that CSFB adults were kept at 20°C, as Mathiasen *et al*. (2015) showed that CSFB survival decreases markedly when the temperature is set above 16°C. However, the need to accommodate several insect species in the same growth room meant that the temperature was set to 20°C.

In 2020, a total of 3,245 new adult CSFB were collected. This new CSFB stock culture was again infested by parasitoid wasps and 24 wasps were recorded across several cages on 24th September. All parasitoids were removed immediately, and the cages monitored for further parasitoid emergence. Parasitoid infestation within field-collected CSFB was also observed by Jordan *et al*. (2020), which demonstrated the potential of parasitoid to control CSFB. Again, taking into account the removal of some individuals for experiments, on 30th September I counted 1,900 CSFB adults from an expected 2,655 beetles. On 27th October only 730 adult CSFB remained, and on 7th December only 266 remained, and after some of these CSFB adults were taken for use in experiments, only 21 adults were left in the stock culture on 21st January 2021.

In 2021, the number of beetles collected was not determined, but approximately 1,140 were used in experiments. This population was once again infested with parasitoid wasps and numbers decreased rapidly.

3.2. Breeding second (F2) and third (F3) generations adults CSFB with no handling of larvae and pupae

On 18th June 2020, a total of 136 F2 was counted and 45 F3 beetles. The next year, on 22nd January 2021, 25 adult F2 were counted, and this number had increased to 90 individuals by 29th March. These numbers are lower than those obtained the previous year, which can be explained by the fact that the adult CSFB collected in July 2020 were heavily infested with parasitoid wasps, leaving very few breeding adults.

3.3. Breeding second (F2) and third (F3) generations adult CSFB by transferring larvae and pupae to new plants and compost

Numbers of individuals of each growth stage are summarised in Tables 1 and 2. In the first cohort of plants (Table 1), despite 295 larvae and 243 pupae being collected, only 36 adult CSFB were reared in this way (7%), larval and pupal mortality was therefore very high. In the second cohort of plants (Table 2), the number of pupae and larvae collected from the plants was lower (240 larvae and 96 pupae) and only two individuals successfully developed into adults, or 0.5% of the collected larvae and pupae.

Table 1. Number of CSFB larvae and pupae found in plants exposed from 7th October to 4th November 2019 and percentage of individuals that reached adult stage.

Date	New Larvae	New pupae	Total	% adult
26th November	216	172		
27th November	59	54	295 larvae and 243 pupae	
28th November	20	17		
6th December			36 adults	7%

Table 2. Number of CSFB larvae and pupae found in plants exposed from 15th November to 13th December 2019 and percentage of individuals that reached adult stage.

Date	New Larvae	New pupae	Total	% adults
24th January	240	96	240 larvae and 96 pupae	
3rd February			2 adults	0.5%

3.4. Raising larval CSFB in planta from plants collected in fields

Where field-collected larvae were left in their original plants, a total of 326 adults from 18 plants was collected.

Where larvae were transferred to new plants, in 2021 380 third instar larvae were dissected out of the plants in January. On 23rd February, only 40 adult CSFB were collected using a pooter and searching the compost by hand, so only 11% of larvae reached adult stage. In 2022, plant dissection yielded 246 third instar larvae. From 16th May to 3rd June, a total of 217 adult CSFB emerged, so 88% of larvae reached adult stage.

4. Conclusion

The advantages and disadvantages of each of the methods cited above are discussed in Table 3 below. The best approach for maintaining populations of CSFB adults, larvae, and pupae in the laboratory for experimental purposes is in fact to use a combination of methods to obtain the highest number of CSFB adults and larvae and provide a continuous supply of individuals throughout the year.

The first method would be field collecting CSFB adults in the late summer (July-August) during oilseed rape harvest, preferably from heavily infested fields as it decreases the likelihood of the beetles being infested by parasitoid wasps, as host and parasitoid abundances influence each other (Hassell, 2000; Jordan *et al.*, 2020). The issue I had was that the local farm that provided us with CSFB adults was not severely impacted by CSFB, potentially because of the presence of natural enemies, such as parasitoid wasps, which were abundant here and kept pest numbers low, which also meant that a lot of collected adults were parasitised. The second method would be to collect oilseed rape plants from heavily infested fields in spring (March to May) so that plants contain large numbers of mostly third instar larvae (Vig, 2003), and leave the larvae in their original host plants. Third instar larvae are more robust and less likely to die if taken out of the plants for experiments and more likely to reach adult stage. In the case where most of the CSFB adults are rapidly used in experiments, to bridge the gap in CSFB supply until the next collection at oilseed rape harvest, the third method would be to keep a breeding population of CSFB in the lab to rear small numbers of second and third generations of CSFB adults.

Table 3. Pros and cons of the different CSFB rearing methods tested in the study and other studies (F2: second generation; F3: third generation).

Method	Pros	Cons
Collecting CSFB adults in late summer to maintain under laboratory conditions	• Thousands of beetles may be collected that can live up to one year and be used for experiments that do not involve them feeding on plants, as older beetles show reduced feeding activity • Time efficient collecting method and particularly useful if large experiments are planned or if several experiments are to be run at the same time	• Field collected beetles are potentially infested with parasitoids that will thrive under laboratory conditions
Natural F2 and F3 production (left to develop and collected only as adults)	• Healthy adults as they were not subjected to attack by parasitoid wasps in the field	• Slow process (up to six months) to get only relatively low numbers of healthy adults
F2 and F3 production with handling (larvae and pupae are handled)	• Possibility to monitor number of individuals produced • Easy access to larvae and pupae for experiments during plant dissections	• Larvae and pupae are very fragile so can easily be killed during these stages when handled • Once larvae have been taken out of the plants, not many are able to infest a fresh plant if the larvae are too young (1st instar), and cannot survive outside of plants • Only relatively low numbers of healthy adults obtained even when large numbers of larvae were collected
Rearing field-collected larvae in planta until adult stage, left in their original plant host	• Collecting plants from heavily infested fields means there is the potential to collect a lot of larvae • Healthy adults at the end of this process as they were not subjected to attack by parasitoid wasps in the field • Large numbers of adults can be obtained this way relatively quickly (three months)	• Necessitate access to an infested oilseed rape field and being allowed to collect plants

Rearing field-collected larvae in planta until adult stage, plants dissected, and larvae transferred to new plants	• Possibility to monitor the number of individuals produced • Easy access to larvae and pupae for experiments during plant dissections • Collecting plants from heavily infested fields means there is the potential to collect a lot of larvae • Large numbers of adults can be obtained this way relatively quickly (three months) • Healthy adults at the end as they were not subjected to attack by parasitoid wasps in the field	• Necessitate access to an infested oilseed rape field and being allowed to collect plants • Dissecting a lot of plants from the field is time consuming • Larvae and pupae very fragile so can easily be killed during these stages when handled • Once larvae have been taken out of the plants, not many are able to infest a fresh plant in if they are too young (1st instar), and cannot survive out of plants
Barari *et al*. 2004: using various containers for larvae to pupate into, in insectary outside	• Larvae exiting the plants by themselves so low labour required • Adults quickly obtained (first emergence within 23 days of placing the larvae in the soil)	• Necessitate access to an infested oilseed rape field and being allowed to collect plants • Only low numbers of healthy adults obtained even when large numbers of larvae collected

Chapter 3: Assessing the potential of biopesticides to control cabbage stem flea beetle *Psylliodes chrysocephala*[1]

Abstract

Cabbage stem flea beetle (CSFB) is an economically important pest of oilseed rape crops in Europe that was effectively controlled by neonicotinoid insecticide seed treatments until they were banned by the European Union in 2013. Since then, CSFB has been a difficult pest to control effectively, in part due to many populations having developed resistance to pyrethroids, the only authorized insecticides used to control this pest in many countries. Alternative solutions are therefore necessary, such as biopesticides. We tested an entomopathogenic fungus, three entomopathogenic bacteria isolates, two fatty acids and azadirachtin against CSFB adults under laboratory conditions. We also tested the efficacy of the pyrethroid insecticide lambda-cyhalothrin.

Fatty acids were effective with up to 100% CSFB mortality after 24 hours. The entomopathogenic fungus *Beauveria bassiana* resulted in up to 56% mortality 14 days after treatment. Entomopathogenic bacteria formulations and azadirachtin were not effective (< 50% and <40% mortality, respectively). Results from a bioassay using lambda cyhalothrin indicated that the CSFB used in this study were resistant to this insecticide.

Entomopathogenic fungi and fatty acids could potentially be used to control CSFB as part of an Integrated Pest Management (IPM) programme. This study is the first to investigate the efficacy of different biopesticides to control CSFB under laboratory conditions. As such, these biopesticides require further testing to optimise formulation, application methods and to assess impact on non-target organisms. Finally, efficacy under field conditions must be determined to understand the influence of environmental variables.

1. Introduction

Cabbage stem flea beetle (CSFB; *Psylliodes chrysocephala*, Linnaeus, Coleoptera: Chrysomelidae) is the most damaging stem-mining insect pest of oilseed rape crops grown in Europe (Alford, Nilsson and Ulber, 2003; I. H. Williams, 2010). Young adults begin to emerge in late spring-early summer after around 2-3 months pupating in the soil (Williams and Carden, 1961a; I. H. Williams, 2010). After completing a summer diapause, adult CSFB damage young

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seedlings when they invade the crop from early August onwards where they feed, mate and lay eggs (Alford, Nilsson and Ulber, 2003). Larvae hatch from eggs laid in the soil from late September onwards and climb up young oilseed rape plants before boring into leaf petioles, (Alford, Nilsson and Ulber, 2003) and then through the winter and spring larvae move into the main stem of infested plants (White, 2015). CSFB larvae pupate in the soil after completing their development inside the plant (Alford, Nilsson and Ulber, 2003). Adult damage, known as shot-holing (Alford, Nilsson and Ulber, 2003), can kill young plants if feeding pest pressure is high (Leach *et al.*, 1994a). In the spring, stem mining by mature larvae can lead to stem wilting, delayed flowering or even total plant collapse (Williams and Carden, 1961a; Graham and Alford, 1981a). A more detailed description of the CSFB life cycle can be found in recent reviews (Ortega-Ramos *et al.*, 2021; Hoarau *et al.*, 2022a).

Until recently, CSFB was effectively controlled by neonicotinoid insecticides (I. H. Williams, 2010). However, in December 2013 the European Union, concerned about the impact of this class of insecticide on pollinators, banned the use of three neonicotinoids: imidacloprid, thiamethoxam and clothianidin, in all flowering crops (European Commission, 2013a). Since then, only pyrethroid insecticides have been authorised for use in oilseed rape crops against CSFB, but CSFB populations have developed resistance to these insecticides in many European countries such as Denmark, Germany, France and the UK, rendering them ineffective in most situations (Heimbach and Müller, 2013; Zimmer *et al.*, 2014; Højland *et al.*, 2015a; Robert, 2019; Willis *et al.*, 2020; Ruck *et al.*, 2022). In the UK, populations of CSFB where 100% of beetles are resistant to the pyrethroid lambda-cyhalothrin have been recorded recently (Willis *et al.*, 2020). In some areas such as the South East of England where pest pressure has historically been high, the percentage of CSFB classed as being highly resistant to pyrethroids has increased rapidly from 33% in 2018 to 56% in 2019.(Willis *et al.*, 2020). Similarly, in a recent French study, knock-down resistance to pyrethroids, also known as kdr, was found in 94% of CSFB populations studied (Bothorel *et al.*, 2018). Difficulty in effectively controlling CSFB has been closely associated with a reduction of the area of oilseed rape grown in Europe (Ortega-Ramos, Cook and Mauchline, 2022). In the UK, for example, the area of oilseed rape was 756,000 hectares in 2012 before the ban on neonicotinoid seed treatments but had reduced to 307,000 hectares in 2021 (Defra, 2022). A survey of CSFB management completed in the UK in 2020 recorded responses from 220 oilseed rape growers. From this survey, 14% of oilseed rape crops were recorded as having to be redrilled and only 61% of crops were harvested. Furthermore, a wide variation between regions was recorded with 71% of crops harvested in Yorkshire and Humberside compared to just 45% in the East Midlands)(Bayer, 2020). It has also been shown that numbers of larvae found in oilseed rape plants in the UK has increased following the neonicotinoid ban in 2013 (Ortega-Ramos *et al.*, 2023). In Germany, oilseed rape yields have decreased from 4.27t/ha between

2010 and 2015 to 3.57t/ha between 2016 and 2019 (Andert, Ziesemer and Zhang, 2021). In the same German study, growers were asked about their future plans regarding oilseed rape growing. From this survey, growers reported that they anticipated growing less oilseed rape than before, the main reason cited being insect pests in autumn and spring (Andert, Ziesemer and Zhang, 2021).

In addition to the development of resistance in CSFB populations, pyrethroid insecticides are also known to be harmful to non-target organisms, including natural enemies of CSFB, such as parasitoid wasp species (I. H. Williams, 2010). It is therefore necessary to find alternate solutions to reduce the economic and environmental impact of CSFB in oilseed rape crops.

One potential solution for the control of CSFB is the use of biopesticides. Biopesticides are biologically based pest control agents that are manufactured from living microorganisms or natural products (Chandler *et al.*, 2011), such as botanicals, entomopathogens, and physically acting products. Botanical biopesticides are chemical compounds extracted from plants that can have both lethal and sublethal effects (Mordue and Blackwell, 1993; Nisbet, 2000). Widely used examples of botanical biopesticides include pyrethrum, a substance obtained from the flower of *Tanacetum cinerariifolium* (Asteraceae) (Casida and Quistad, 1995) that has the same mode of action and quick knockdown effect as synthetic pyrethroids, but with reduced persistence in the environment (Glynne-Jones, 2001). Another widely used example is azadirachtin, a tetranotriterpenoid obtained from the neem tree (*Azadirachta indica* A. Juss., Meliaceae)(Schmutterer, 1990) that has both lethal (Karnavar, 1987; Mordue and Blackwell, 1993) and sublethal effects, including reduced insect growth, longevity, fertility, reproduction, oviposition and feeding (Mordue and Blackwell, 1993; Nisbet, 2000; Mancebo *et al.*, 2002). In addition, there are a wide range of essential oils components such as limonene (extracted from citrus oil) (Isman, 2020), which may kill the pest but that also has repellent properties (Karr and Coats, 1988).

Entomopathogens are species of bacteria, virus, nematodes or fungi that are pathogenic to insects and can be used as control agents of pest species (Lacey, 2017). Other studies have focused on the potential of entomopathogenic nematodes against CSFB (C. Price, Campbell and Pope, 2023; Godina *et al.*, 2023). Most research on entomopathogenic fungi as biopesticides has focused on species belonging to the *Metarhizium* (Hypocreales: Clavicipitaceae) and *Beauveria* (Hypocreales: Cordycipitaceae) genera. The insect is infected when spores adhere to the insect cuticle and germinate, penetrating through the cuticle using a combination of mechanical pressure and the secretion of enzymes such as proteases and chitinases (Stleger, Charnley and Cooper, 1987; Stleger, Cooper and Charnley, 1987). The fungus then grows into the haemocoel, then the rest of the body of the host insect, which is typically killed in four to six days by physical damage and secretion of fungal metabolites (Butt

and Goettel, 2000). Spores are then produced on the surface of the cadaver, which may then inoculate other insect hosts.

The most widely used entomopathogenic bacteria species for the control of insect pests is *Bacillus thuringiensis* (*Bt*) Berliner (Bacillales: Bacillaceae) (Lacey *et al.*, 2015). When it sporulates, Bt produces a bipyramidal protein crystal comprised of δ-endotoxins that are lethal to insects when ingested but are not toxic to vertebrates (Bond *et al.*, 1971; Siegel, 2001). In order for the toxin to be activated, the pH must be 9.0 to 10.5 (high pH), conditions found in insect guts, but not in the human gut, which has a lower pH (Broderick, Raffa and Handelsman, 2006). Once in the digestive system of the insect, the δ-endotoxins become soluble and bind to receptors located on midgut cells. This leads to the creation of pores in the cell membranes, which creates an osmotic imbalance and results in cell death. Insect death usually occurs 48h after ingestion, as a result of septicemia (Glare, Jurat-Fuentes and O'callaghan, 2017). Two *Bt* subspecies are known to kill coleopteran insects: *Bt* subsp*. tenebrionis* (Krieg *et al.*, 1983) was shown to be up to 100% effective against the larvae of the white-spotted rose beetle *Oxythyrea funesta* (Poda) (Coleoptera: Cetoniidae) (Robert, Chaufaux and Marchal, 1994); and subsp. *san diego* (Herrnstadt *et al.*, 1986), which was shown to be effective against larvae of the Colorado potato beetle *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) and is commercially available in the USA (Zehnder and Gelernter, 1989).

Physically acting products may be defined as having a non-specific mechanical or physical mode of action (IRAC, 2022). A widely used example is maltodextrin, which is made from starch, vegetable oil, and water, and causes death by blocking the spiracles thus suffocating the insect (EFSA, 2011). Fatty acids are another widely used example. The active substance of fatty acids products are unsaturated carboxylic acids (e.g., C14-C20, potassium salts (Bayer, 2021)). Fatty acids affect the insect by removing the waxy layer covering the cuticle, and then penetrate through the cuticle and disrupt cellular membranes, leading to cytolysis. Treated insects become dehydrated as a result of water loss, feeding is disrupted and death typically follows soon after (Convertini *et al.*, 2018; Suma *et al.*, 2019; Bayer, 2021).

In the present laboratory study with adult CSFB, we investigated the efficacy of a range of biopesticides: the botanical biopesticide azadirachtin; the entomopathogenic fungus *Beauveria bassiana* strain GHA (Balsamo) Vuillemin; three formulations of *Bt* subsp. *tenebrionis* (*Btt*); and two formulations of fatty acids. In the case of fatty acids, despite a long history of research (tested since the 1920s for their insecticidal potential (Siegler and Popenoe, 1925)) to our knowledge no previous published study has investigated the efficacy of these physically acting biopesticides against hard-bodied insects such as adult Coleoptera. We also looked at the efficacy of a conventional synthetic pyrethroid insecticide, lambda-cyhalothrin.

2. Material and Methods

2.1. Insects and plants

CSFB adults were collected in July 2019, 2020, and 2021 at harvest from farms in Shropshire, UK. The insects were kept in ventilated mesh cages (30x30x30 cm) in a growth room (Fitotron® SGR 122, Weiss Technik UK Limited, UK) at a constant 20°C temperature and 60% RH and fed by placing potted oilseed rape plants (variety Mirakel) grown under glasshouse conditions to growth stage 12 (BBCH system (Lancashire *et al.*, 1991)) into each cage. Potted oilseed rape plants were replaced every two weeks. Insect populations were kept under these conditions for up to nine months before being used in a bioassay. Beetles were taken straight from the cages for bioassays, and the sex of the tested individuals was not determined. Surviving CSFB were only returned to the cages to be used in future bioassays if they were part of the control group, for which only water was used. Surviving CSFB that were treated with biopesticides were not used again.

First and second true leaves were used as a food source for CSFB in the bioassays. The leaves were collected from young potted oilseed rape plants (variety Mirakel) grown under glasshouse conditions and that had reached a minimum growth stage of 12 (BBCH system (Lancashire *et al.*, 1991)). Within the same experiment, fully expanded leaves were collected from several plants of a similar growth stage.

Details of the products tested are shown in Table 1, including the trade names, manufacturers, the active ingredients, the rates tested, and the number of replicates. Biopesticide efficacy was compared to water, which was used here as a negative control.

Table 1. Product name, manufacturer, active ingredients, application concentrations, number of replicates of products used in the laboratory bioassays against adult cabbage stem flea beetle (*Psylliodes chrysocephala*). A water control was tested alongside each product, except for the bioassay with lambda-cyhalothrin for which the control was acetone.

Product Name	Manufacturer	Active ingredient	Concentrations tested	Replicates
Azatin®	Certis Belchim BV, Utrecht, The Netherland	217g/l azadirachtin	0.5ml/l, 1ml/l and 1.4ml/l (field dose)	3
INBS32	Andermatt Biocontrol UK Ltd, Henfield, UK	*Bacillus thuringiensis tenebrionis* undisclosed strain	10ml/l (field dose)	6
CEU-40770-I-WG	Certis Belchim BV	*Bacillus thuringiensis tenebrionis* strain SA-10	2.5g/l (field dose)	6
CEU-40780-I-WG	Certis Belchim BV	*Bacillus thuringiensis tenebrionis* undisclosed strain	1.25g/l (field dose)	6
Botanigard® WP	Certis Belchim BV	*Beauveria bassiana* strain GHA, 4.4 x 10^{10} spores/g	0.32g/l, 0.63g/l (field dose) and 1.26g/l	6
Fluka™ Lambda-cyhalothrin reference material	Honeywell	Lambda-cyhalothrin (pyrethroid)	0.16µg (4% of field dose), 0.78µg (20%) and 1.95µg (50%)	3
FLiPPER™	Bayer (Leverkusen, Germany)	Fatty acids C7-C20	8, 16 (field dose) and 32ml/l	3
Neudosan®Neu	Certis Belchim BV/Progema GmbH (Aerzen, Germany)	Fatty acids	10, 20 (field dose) and 40 ml/l	3

2.2. Azadirachtin product leaf disc bioassay

Solutions of the botanical biopesticide azadirachtin were prepared by diluting the product Azatin in tap water to produce three concentrations which were tested simultaneously (0.5ml/l, 1.0ml/l and 1.4ml/l). Bioassays were replicated three times with a separate solution prepared and used for each replicate. Tap water was used as the control and again a separate sample of water used for each replicate. Each concentration of Azatin or the tap water control was poured into a rectangular plastic tray (17x11x5cm) and an oilseed rape leaf was fully immersed for 5 seconds and then left to dry. Incubation chambers (cylindrical plastic containers, 12cm/7cm diameter top/bottom, 6 cm height) were prepared by placing four layers of damp paper towel on the base of the container. Six-centimetre diameter leaf discs (1 disc per leaf) were cut from the soaked leaves, and 1 disc was placed on the damp paper towel in the base of each incubation chamber. Fifteen adult CSFB (mixed sexes) were placed in each chamber, and the incubation chambers were then closed with a mesh lid (4cm diameter opening, mesh aperture 1mm x 1mm, with the open area of a mesh [A°] = 50% mesh holes) to provide ventilation.

The 12 incubation chambers were placed randomly inside a plant growth room (Fitotron® SGR 122, Weiss Technik UK Limited, Loughborough, UK) with a 16/8h day/night photoperiod, 20°C temperatures and 60% RH. Mortality was assessed every day for eight days by counting the number of dead CSFB in each chamber. The antifeedant activity of azadirachtin was assessed at the end of the assessment period by taking photographs of the leaf discs and analysing leaf area consumption using with the ImageJ software (version 1.53e). This bioassay was completed in February 2020.

2.3. Bacillus thuringiensis subsp. *tenebrionis* products leaf disc *bioassay*

This bioassay method was adapted from methods described in the literature (Zehnder and Gelernter, 1989). The efficacy of the three products INBS32, CEU-40770-I-WG and CEU-40780-I-WG, which are all based on *Bt* subsp. *tenebrionis*, were tested at the same time at 10ml/l, 2.5g/l and 1.25g/l respectively, i.e., the rates recommended by the manufacturers. The solutions were prepared by diluting each product in tap water to obtain the desired concentrations. Each concentration of a product was prepared six times so that a separate solution was used for each replicate. Incubation chambers were prepared as described in 2.2. Tap water was used as the control and again a separate sample of water used for each of the six replicates. Oilseed rape leaves were treated, and discs cut in the same way as in 2.2.

Ten CSFB adults (mixed sexes) were placed in each incubation chamber, which was then closed. The lid of each chamber was pierced with small holes to allow air exchange.

The 24 incubation chambers were placed randomly and kept in the same conditions as 2.2. Mortality was assessed every day for twelve days by counting the number of dead CSFB in each chamber. This bioassay was completed in December 2020.

2.4. *Beauveria bassiana* strain BHA product whole leaf bioassay

The efficacy of Botanigard WP (entomopathogenic fungus *Beauveria bassiana* strain GHA) was tested at three concentrations simultaneously, based on the recommended concentration indicated on the label (0.32, 0.63 (field rate) and 1.26g/l) and tap water was used as a control. Each concentration and control were replicated six times. Solutions of Botanigard WP were prepared by diluting the product in tap water. Each solution of Botanigard WP or the tap water was poured separately into a 200ml hand-held atomiser bottle. A separate preparation of Botanigard WP and water control was used for each of the six replicates.

Two hours before the bioassays were started, adult CSFB were collected from cages and placed in tubes (10 insects per tube, unsexed) and were refrigerated at 5°C to reduce insect activity. A fresh oilseed rape leaf was added on top of the paper towel in each incubation chamber (see 2.2) as a source of food. Ten CSFB adults were taken from the refrigerator and released from the tubes into each incubation chamber immediately before the test, then the test solution was sprayed into the chamber with three pumps of the atomizer, each pump applying 0.10ml of the test solution. In this way good coverage of the beetles and leaf inside each incubation chamber was achieved. Each incubation chamber was then closed with a similar lid as in 2.2.

The 24 incubation chambers were placed randomly inside a plant growth room (model MLR-351H, Sanyo, Osaka, Japan) with a 16/8h day/night photoperiod, a constant 20°C temperature and 85% RH. Mortality was assessed every two days for fourteen days by counting the number of dead CSFB in each chamber. This bioassay was completed in September 2021.

2.5. Physically acting products whole leaf bioassay

The fatty acid products FLiPPER and Neudosan were tested at the same time and each product was tested at three concentrations, based on the recommended concentrations indicated on the labels (8, 16 (field rate) and 32ml/l for FLiPPER and 10, 20 (field rate) and 40ml/l for Neudosan). Solutions of each product were prepared by diluting the product in tap water. Each combination of product and concentrations was replicated three times, and a tap water control was also replicated three times. Incubation chambers were prepared as described in 2.2, insects were prepared, and treatments applied as described in 2.4. The 21

incubation chambers were placed randomly and kept in the same conditions as 2.4. Mortality was assessed every day for four days. The bioassay was completed in April 2022.

To examine the effect of fatty acids on the beetle cuticle, five dead CSFB from the FLiPPER treatment and the control treatment were left to dry. Each specimen was then gold-coated with an Edwards S150 Sputter Coater and viewed at x2000 magnification using a scanning electron microscope (Cambridge Instruments Stereoscan 200, UK).

To improve the effectiveness of fatty acids, drop tests were performed on oilseed rape leaves with fatty acids FLiPPER and Neudosan mixed with one of two adjuvants: Silwet L-77 AG and Silwet STIK2 (Momentive, New-York, USA). Silwet L-77 AG is a super-spreader and Silwet STIK2 is a spreader-sticker that creates a film that protects contact biopesticides, keeping them wet for longer (Benjamin Langendorf, Momentive, personal communication). Details about the products used and the rates tested are gathered in Table 2. Three 10 µl drops of solution (water, fatty acid + adjuvant, fatty acid alone, or adjuvant alone) were deposited with a micropipette on oilseed rape leaves, and after 1 minute a picture was taken. The spread of the product (area in cm^2) was measured with the software ImageJ (version 1.53e). When depositing the drops, the time was recorded, and the leaves monitored until the leaves were dry when the time was then recorded again, to calculate the time it took for each solution to dry.

Collaborators at Momentive completed spreading tests and calculated dynamic surface tension (DST) of the various combinations of fatty acids and adjuvant, similar products as the present chapter, except that Momentive researchers only tested Silwet L-77, and the adjuvant was tested at the following concentration: 0.025%, 0.05%, 0.1% and 0.5% v/v. For the spreading test, they applied 10 µl of solutions to the bottom half of polystyrene Petri dishes (10cm diameter) and placed a hygrometer (Thermo-Hygro, Fisher, No. 11-661-13) next to the Petri dish and covered the Petri dish and hygrometer with a recrystallization dish to prevent evaporative air currents). After 30 seconds they removed the cover and marked the perimeter of the droplet with a marker (permanent ink, not water soluble) and measured the spread diameter (mm) of two perpendicular axes in triplicate (3 drops, each on different Petri dish bottoms) and calculated the average of the six diameters (2 axes x 3 drops). FLiPPER was diluted in water at pH = 10, while Neudosan was diluted in water at pH = 8.6. To calculate the DST over time, Momentive researchers used a KRÜSS Tensiometer (model BP2, Hamburg, Germany) using the bubble pressure method at 25˚C.

Table 2. Name, active ingredients, doses, and manufacturer of adjuvants used in the laboratory bioassays to improve the effectiveness of fatty acids.

Product Name	Manufacturer	Active ingredient	Doses tested
Silwet L-77 AG	Momentive (New York, USA)	Based on a trisiloxane ethoxylate	0.025%, 0.05%, 0.1%, 0.2%
Silwet STIK2	Momentive (New York, USA)	Siloxane Polyalkyleneoxide	0.15%, 0.25%
FLiPPER™	Bayer (Leverkusen, Germany), AlphaBio Control (Cambridge, UK)	Fatty acids C7-C20	1.6% v/v
Neudosan®Neu	Certis Belchim BV/Progema GmbH (Aerzen, Germany)	Fatty acids	2% v/v

2.6. Lambda-cyhalothrin (pyrethroid) glass vial bioassay

The lambda-cyhalothrin bioassay was done using the Insecticide Resistance Action Committee (IRAC) susceptibility test method 031 (https://irac-online.org/methods/weevils-and-flee-beetles/), using technical grade lambda-cyhalothrin (Fluka™ Honeywell). Glass vials (6cm high (h) and 1.25cm radius (r)) were selected and their surface area (SA, cm^2) calculated with the following formula:

$$SA = \pi \times r^2 + (2 \times \pi \times r) \times h$$
$$SA = \pi \times 1.25^2 + (2 \times \pi \times 1.25) \times 6$$
$$SA = 52\ cm^2$$

each lambda-cyhalothrin concentration was then calculated by multiplying SA by 0.0375µg/cm² (50% of field dose), 0.015µg/cm² (20% dose) and 0.003µg/cm² (4% dose) to give the following doses: 1.95µg, 0.78µg and 0.16µg respectively. The field doses were selected according to the IRAC susceptibility test method (cited above).

Solutions were prepared by diluting the lambda-cyhalothrin in acetone, then serial dilutions were made to reach the desired concentration. One millilitre of each concentration was separately pipetted into a vial (One millilitre of acetone was used as the control). Each lambda cyhalothrin concentration and the control were replicated three times. The 12 vials were then placed uncapped on a roller within a fume cupboard to let the acetone evaporate overnight. Ten adult CSFB were then placed in each vial, and lids were secured. Vials were kept in the controlled environment cabinet (as described in 2.2). Mortality was assessed after 24h. This bioassay was completed in January 2022.

2.7. Statistical analysis

Data were analysed using R (version 3.6.2) and RStudio (version 1.2.5033). CSFB mortality after treatment with azadirachtin and after treatment with entomopathogenic bacteria (*Bt*) were analysed after fitting the data to a Cox proportional hazards regression model following the modelling of Kaplan Meier survival curves using the packages survival, survminer, and dplyr. CSFB mortality data after treatment with entomopathogenic fungus (*Beauveria bassiana*) and after fatty acid treatments FLiPPER and Neudosan, however, were analysed using mixed effect models from the package *lme4* (Bates *et al.*, 2014) because no mortality was recorded in the control treatment for these two experiments, the hazard rates (coefficients) obtained during the statistical analysis were unrealistically high and it was not possible to generate satisfactory survival curves. CSFB feeding activity after treatment with azadirachtin and CSFB mortality data after treatment with lambda-cyhalothrin were analysed using a one-way ANOVA on a linear model of the data. Significance groups were computed using the *cld(lsmeans())* function included in the packages *multcomp* (Hothorn, Bretz and Westfall, 2015) and *lsmeans* (Lenth, 2016), or using the *HSD.test()* function included in the package *agricolae* (De Mendiburu and Simon, 2015). Degrees of freedom are referenced as 'df' and 95% confidence intervals are referred as '95% CI'. Box plot graphical illustrations were made with the boxplot function from the package graphics (Murrell, 2009) after the data was tidied with the mutate function from the package tidyverse (Wickham, Averick, *et al.*, 2019). The graphical illustrations provided by Momentive (New York, USA) were made using Microsoft® Excel® (version 2308).

The spread of fatty acids combined with adjuvants was analysed using a one-way ANOVA on a linear model of the data. The time to dry for fatty acids combined with adjuvants was analysed using generalised linear models (GLMs) fitted with Poisson probability distribution. The spread and time to dry of adjuvants alone were analysed using a one-way ANOVA on a linear model of the data after a square root transformation.

3. Results and Discussion

3.1. Azadirachtin product leaf disc bioassay

The CSFB survival curve after application of azadirachtin and water control treatments is illustrated in Figure 1. There were no significant differences in CSFB mortality between the water control and each azadirachtin application rate: 0.5ml azadirachtin/l (z = -0.373, HR = 0.752, 95% CI = 0.168 – 3.360, p = 0.709), 1ml azadirachtin/l (z = -0.795, HR = 0.502, 95% CI = 0.092 – 2.743, p = 0.427) and 1.4ml azadirachtin/l (z = 0.379, HR = 1.289, 95% CI = 0.346 – 4.801, p = 0.705). At the end of the experiment, no more than 40% of CSFB had died in any one treatment, and an overall mean of 20% mortality was recorded across all treatments tested and the water control. In terms of leaf consumption, less feeding damage (2.8% leaf area eaten) was recorded at the second highest dose (1ml/l) than the control (4.8% leaf area eaten), or when leaves were treated with the highest dose (1.4ml/l, 3.7% leaf area eaten)(F = 1.172, residuals df = 8, p = 0.379) with an overall mean of around 4% of leaf area eaten. Azadirachtin may be more effective when adults are feeding more actively, i.e., during maturation. Azadirachtin is usually used against smaller, soft-bodied insects such as whiteflies and aphids . In the case of flea beetles *Phyllotreta* spp., azadirachtin used in combination with entomopathogenic nematodes has been reported to decrease emergence of adult striped flea beetles *Phyllotreta striolata* (Fabricius) in a Chinese field study (Yan *et al.*, 2013a). Combined with fatty acids or petroleum spray oil, azadirachtin has also been reported to decrease leaf damage and increase yields in a US field study investigating control of the crucifer flea beetle *Phyllotreta cruciferae* (Goeze) (Reddy *et al.*, 2014b). It seems then that azadirachtin may be more effective against CSFB when used in combination with other products. However, more research is necessary to understand if this is indeed the case and, if so, how azadirachtin interacts with other products in these combinations and understanding which combination would be the most effective against CSFB in the field.

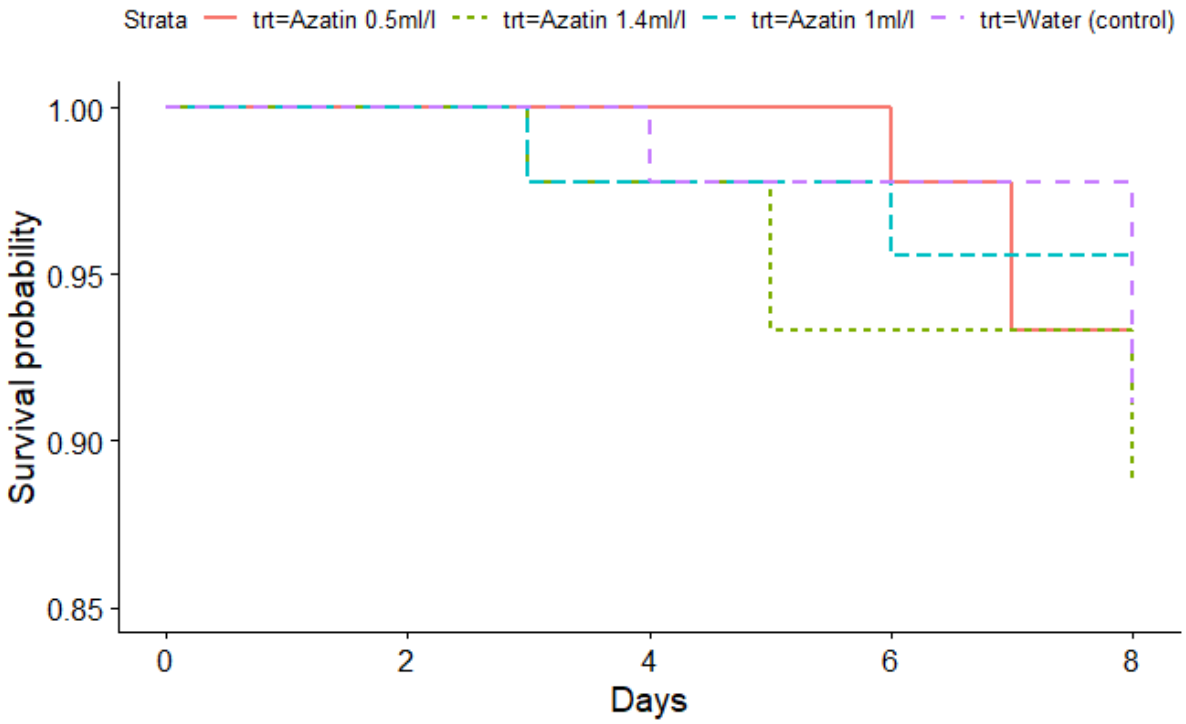


Figure 1. Survival curve of cabbage stem flea beetle (CSFB) (*Psylliodes chrysocephala*) after application of different rates of azadirachtin and water (control).

3.2. *Bacillus thuringiensis* subsp. *tenebrionis* products leaf disc bioassay

The CSFB survival curve after application of *Bacillus thuringiensis* subsp. *tenebrionis* and water control treatments is illustrated in Figure 2. There were no significant differences in CSFB mortality between the water control and each entomopathogenic bacteria treatment: INBS32 (z = -0.196, HR = 0.932, 95% CI = 0.461 – 1.885, p = 0.844), CEU-40770-I-WG (z = 1.369, HR = 1.568, 95% CI = 0.824 – 2.987, p = 0.171) and CEU-40780-I-WG (z = 1.438, HR = 1.591, 95% CI = 0.845 – 2.995, p = 0.150). At the end of the experiment, mortality remained low with 25% mortality for product INBS32, 36.7% mortality for product CEU-40770-I-WG, 40% mortality for product CEU-40780-I-WG and 26.7% mortality for the water control. The low mortality following treatment with the *Btt* based products could be explained by the fact that the individuals tested were adults and not larvae, as *Bt* is most typically used against the larval stages of insects (Bravo *et al.*, 2011). The only other study investigating the use of *Btt* against adult flea beetle is a patent in which reduced feeding activity of adult crucifer flea beetle (*Phyllotreta cruciferae*) was reported after they were exposed to treated leaves, but no mortality was reported (Payne *et al.*, 2000). The authors patented several *Btt* strains reported to be effective against coleopteran pests including the crucifer flea beetle. Despite this, no product has been registered and the results presented here do not indicate that *Btt* is likely to be effective against adult CSFB.

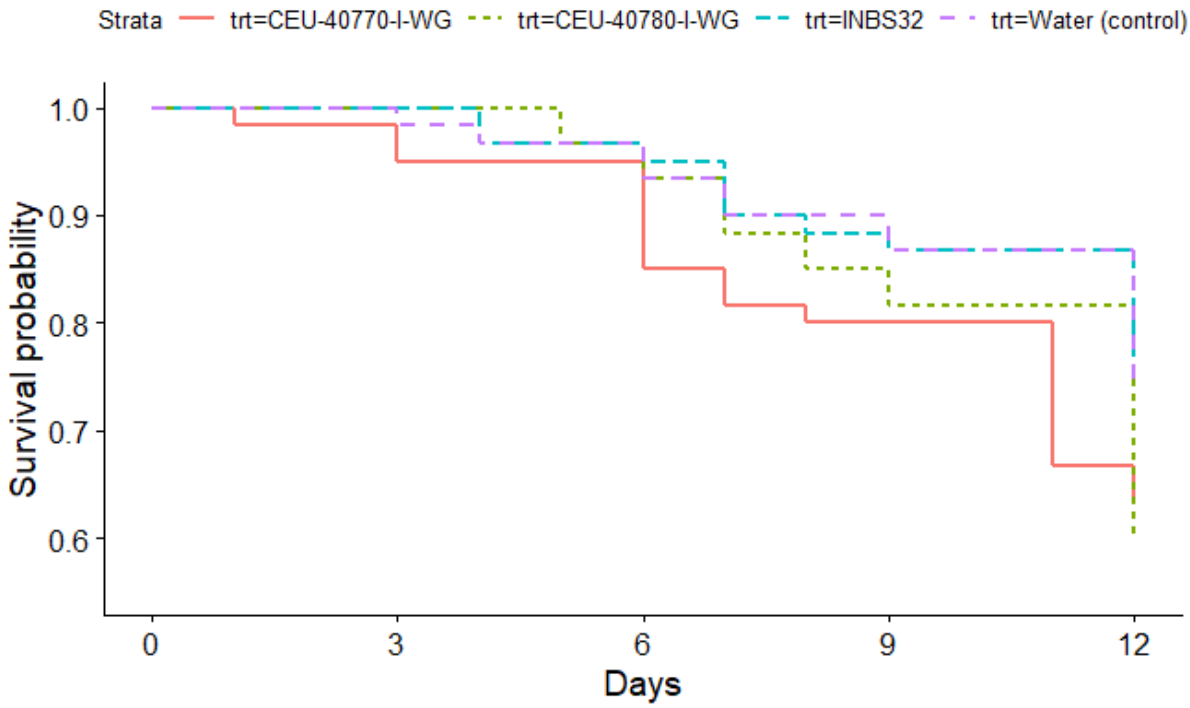


Figure 2. Survival curve of cabbage stem flea beetle (CSFB) (*Psylliodes chrysocephala*) after application of different strains of *Bacillus thuringiensis* sbsp. *tenebrionis* and water (control).

3.3. *Beauveria bassiana* strain GHA product whole leaf bioassay

Adult CSFB mortality increased significantly over time (t = 8,807, df = 143, p < 0.001) but only the application of double the field rate (1.26g/l, equivalent to 5.5 x 10^7 spores/ml) of *Beauveria bassiana* strain GHA significantly increased mortality compared to the control (t = 5.628, df = 20, p < 0.001), which is shown in Figure 3. Application of the field rate (0.63g/l, equivalent to 2.7 x 10^7 spores/ml) resulted in mortality similar to the control (t = 0.743, df = 20, p = 0.466), and application of half the field rate (0.32g/l, equivalent to 1.4 x 10^7 spores/ml) also resulted in mortality similar to the control (t = 0.601, df = 20, p = 0.555). Other laboratory studies have investigated the efficacy of various strains and isolates of *Beauveria bassiana* against adult flea beetles. For example, in one study, 15 isolates were tested using a concentration of 1 x 10^7 spores/ml against CSFB and a maximum mortality of 47% after 14 days was recorded when isolate V55 was used (Butt *et al.*, 1994). In another study, 14 isolates of *Beauveria bassiana* were tested at a concentration of 1 x 10^8 spores/ml against crucifer flea beetle, *P. cruciferae*, adults. Here mortality varied between 50 and 90%, 7 days after treatment (Miranpuri and Khachatourians, 1995). In the field, Menzler-Hokkanen *et al*. unpublished (cited in (Hokkanen, Menzler-Hokkanen and Butt, 2003a)) reported that a spray application and soil incorporation of *Metarhizium anisopliae* (strain/isolate unidentified) led to reductions in adult

Phyllotreta spp. emergence of 41% and 34%, respectively in turnip rape (*Brassica rapa*) fields in Finland. In the USA, a commercial formulation of *B. bassiana* (Botanigard ES) was tested under laboratory and field conditions against adult crucifer flea beetle. However, here only low mortality (<40%) was recorded in the laboratory, and high leaf damage was recorded in the field leading the authors to conclude that Botanigard ES was not effective against this species (Antwi, Olson and Carey, 2007; Antwi, Olson and Knodel, 2007). Despite this, the efficacy of combinations of *B. bassiana* GHA (Botanigard 22WP) and *M. anisopliae* F52 (Met52) has been tested against the crucifer flea beetle under field conditions in the USA (Reddy *et al.*, 2014b). Results from this study indicated reduced feeding damage and similar yields to canola crops where imidacloprid had been used when repeated applications of both Botanigard 22WP and Met52 were made (Reddy *et al.*, 2014b). This may be due to the insects receiving a higher total dose of fungal spores. Indeed, as environmental factors such as UV radiation, temperature and humidity are known to be detrimental to the survival of entomopathogens in general (Ignoffo and Garcia, 1992; Jaronski, 2010), entomopathogens are short lived in the field, and multiple applications allows for the replacement of the spores that did not survive following the first application.

Overall, the laboratory results presented here are similar to previously reported studies. As such, results from this study support the view that application rates of entomopathogens are an important factor in achieving effective control of a hard-bodied insect, such as adult CSFB. Frequency of application and use of combinations of entomopathogenic fungi may also help to counter the negative effects of abiotic factors. However, most studies so far completed on CSFB have been laboratory based (Butt *et al.*, 1992, 1994), so more research is needed, under both laboratory and field conditions, testing the efficacy of a wider range of combinations of fungal species and strains and isolates.

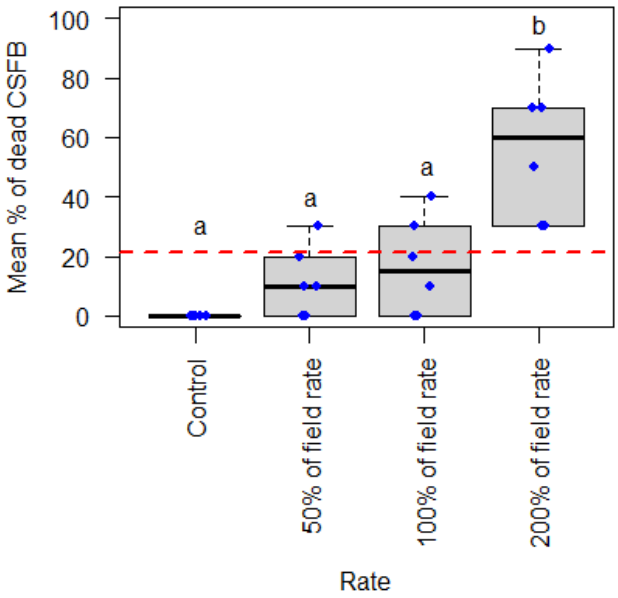


Figure 3. Percentage of dead cabbage stem flea beetle (CSFB) (*Psylliodes chrysocephala*) after 14 days of contact with entomopathogenic fungi *Beauveria bassiana* strain GHA and water (control). The dotted red line represents the overall mean of the data. The blue dots help visualise various data points. Different letters indicate significant differences (p-value < 0.05).

3.4. Physically acting products whole leaf bioassay

CSFB mortality results are illustrated in Figure 4a and 4b. All doses of FLiPPER led to higher CSFB mortality compared to the water control ($t = 4.409$, $df = 16$, $p < 0.001$) and all doses of Neudosan led to higher CSFB mortality compared to the water control ($t = 3.391$, $df = 16$, $p = 0.004$) after only 24h. Mortality did not increase further over time ($F = 2.4554$, $df = 62$, $p = 0.122$) and increasing the rates of fatty acids did not cause increased CSFB mortality ($F = 2.327$, $df = 16$, $p = 0.129$).

Both physically acting products were effective against CSFB adults under laboratory conditions reported here, which to our knowledge is the first demonstration of the potential of fatty acids against a flea beetle pest. Fatty acids have previously been reported to be effective against soft-bodied pest insects such as the larvae and the eggs of whiteflies *Trialeurodes vaporariorum* and *Bemisia tabaci* (Convertini *et al.*, 2018; Suma *et al.*, 2019), the aphid *Aphis gossypii* and the mealybug *Planococcus citri* (Suma *et al.*, 2019). Future work should focus on testing these physically acting products under field conditions.

Analysis of the CSFB elytra cuticle with the scanning electron microscope showed differences in the structure of CSFB elytra when treated with FLiPPER compared with the water control (Figure 5). The application of FLiPPER had the effect of disrupting the integrity

of the elytra by increasing the size of gaps between the scales that make up the cuticle on the elytra. This phenomenon has not been previously reported (Convertini *et al.*, 2018; Suma *et al.*, 2019) and further work is required to confirm whether disruption of the cuticle, as reported here, is directly linked to insect mortality and can be considered the mode of action of this biopesticide.

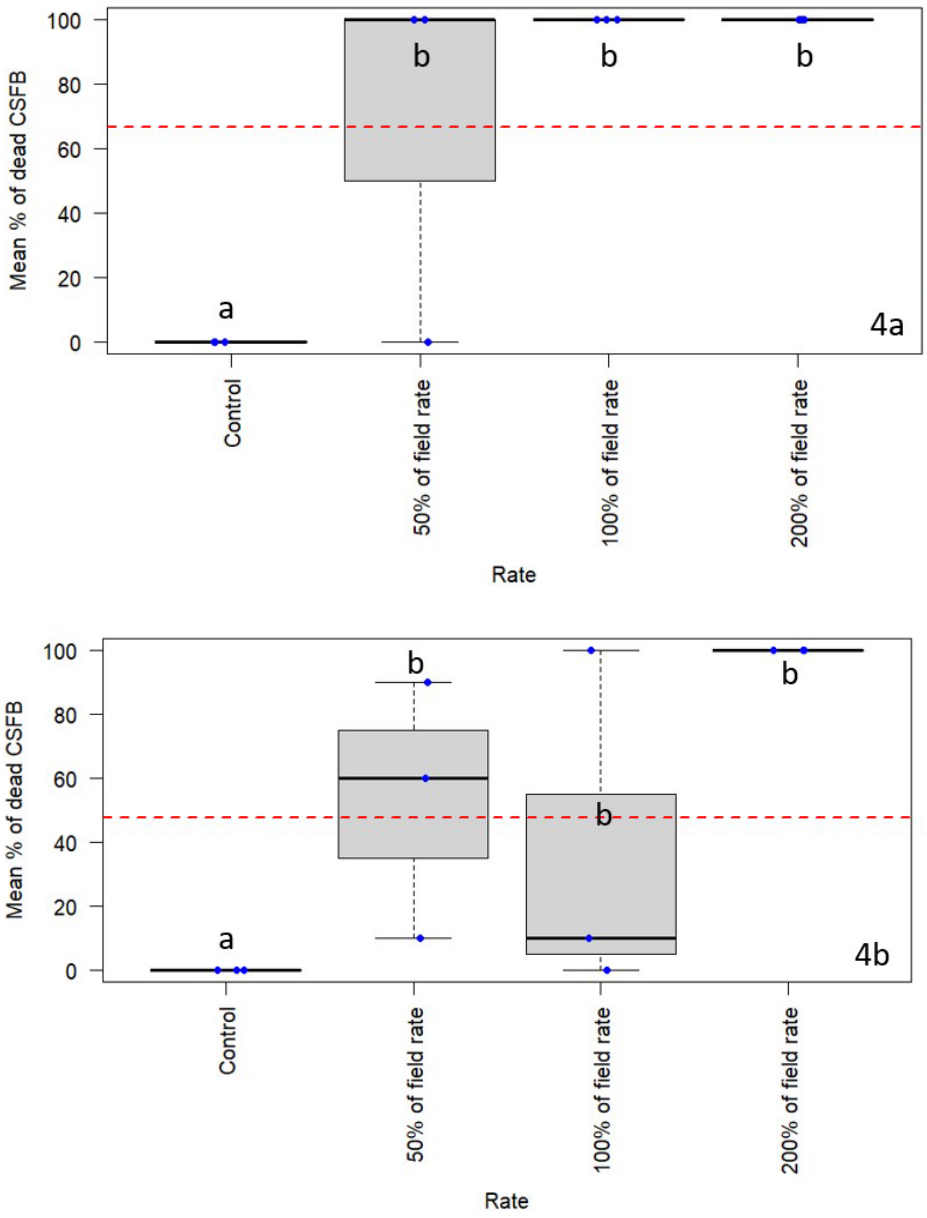


Figure 4. Percentage of dead cabbage stem flea beetle (CSFB) (*Psylliodes chrysocephala*) after 4 days of contact with fatty acid products FLiPPER (4a), Neudosan (4b). The dotted red line represents the overall mean of the data. The blue dots help visualise various data points. Different letters indicate significant differences (p-value < 0.05).

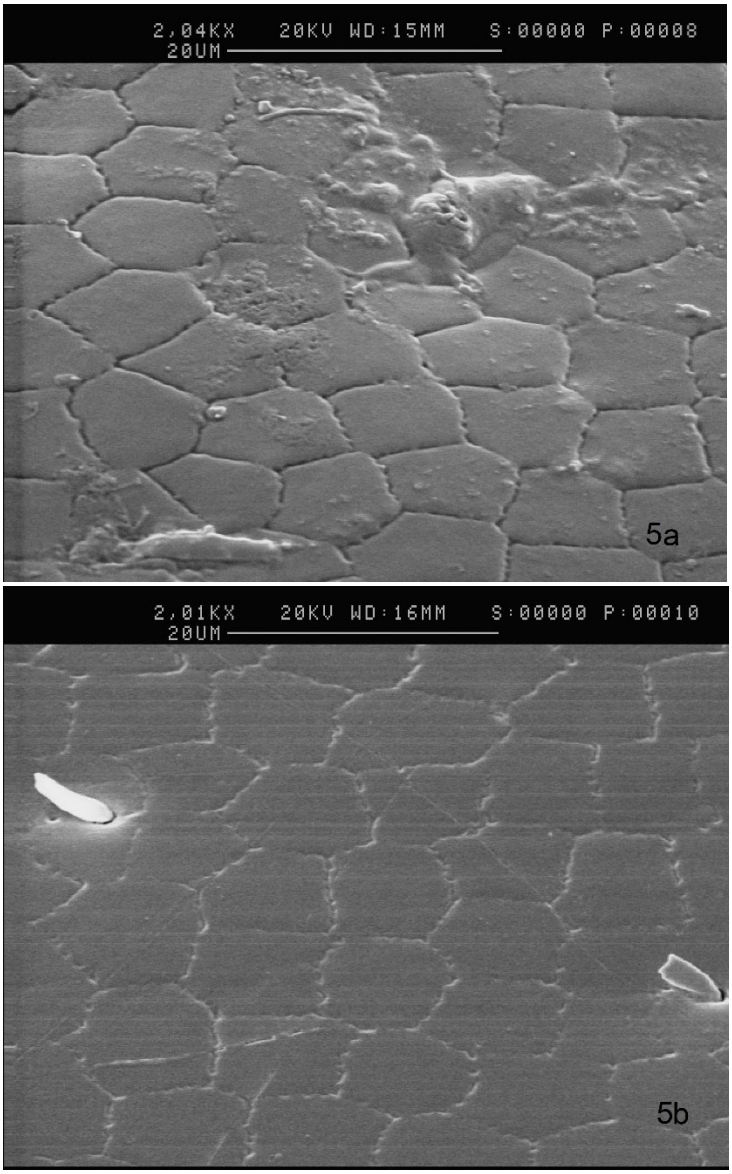


Figure 5. Cabbage stem flea beetle (*Psylliodes chrysocephala*) elytra cuticle observed through a scanning electron microscope, (x2000) after treatment with fatty acids (5a) and water (5b).

The results of the spreading tests of fatty acids when combined with adjuvants are illustrated in Figure 6. Figure 6a shows the significant increase in spreading with increased dose of adjuvants alone ($F = 23.14$, $df = 6$, $p < 0.05$), Figure 6b shows that when FLiPPER is combined with adjuvants there is no significant increase compared to FLiPPER alone ($F = 2.587$, $df = 6$, $p > 0.05$), and Figure 6c shows that when Neudosan is combined with adjuvants, there is a significant decrease of spreading compared with Neudosan alone ($F = 4.874$, $df = 6$, $p < 0.05$).

The results of the drying tests of fatty acids when combined with adjuvants are illustrated in Figure 7. Figure 7a shows the significant decrease of drying time with increased dose of adjuvants alone ($F = 74.61$, $df = 5$, $p < 0.05$), as they spread more as can be seen in Figure

6a. Figure 7b shows that when FLiPPER is combined with adjuvants there is a significant decrease of drying time with increased dose of adjuvants compared with FLiPPER alone ($F = 47.355$, $df = 14$, $p < 0.05$). Figure 7c shows that when Neudosan is combined with adjuvants there is a significant increase of drying time with increased dose of adjuvants compared with Neudosan alone ($F = 15.851$, $df = 14$, $p < 0.05$). Overall, these specific organosilicon-type adjuvants do not seem to efficiently increase the spread of fatty acids, nor increase their drying time.

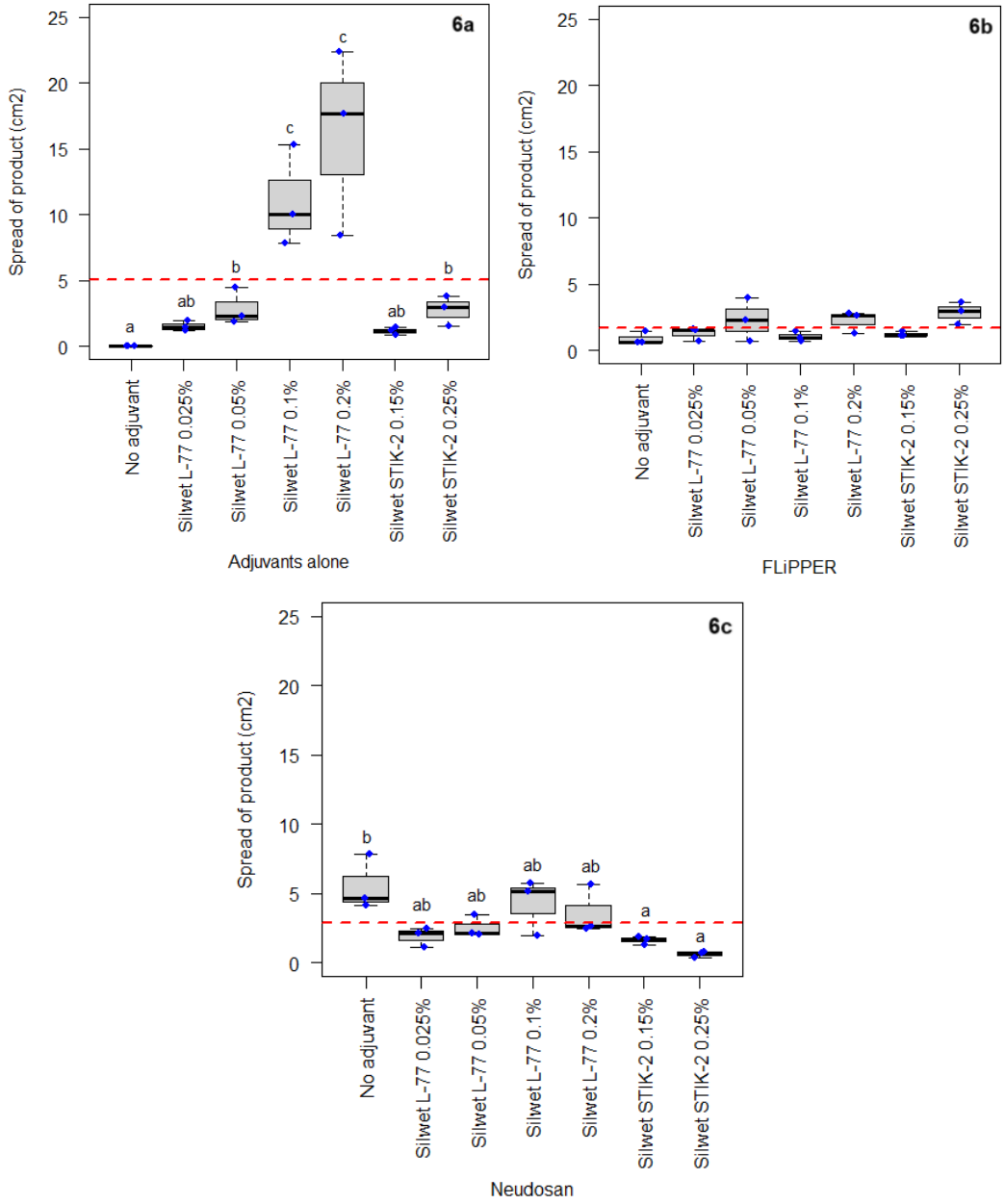


Figure 6. Spread of fatty acids combined with adjuvants: adjuvants alone (6a), FLiPPER (6b) and Neudosan (6c). The red line represents the overall mean of the data. The blue dots help visualise various data points. Different letters indicate significant differences (p-value < 0.05).

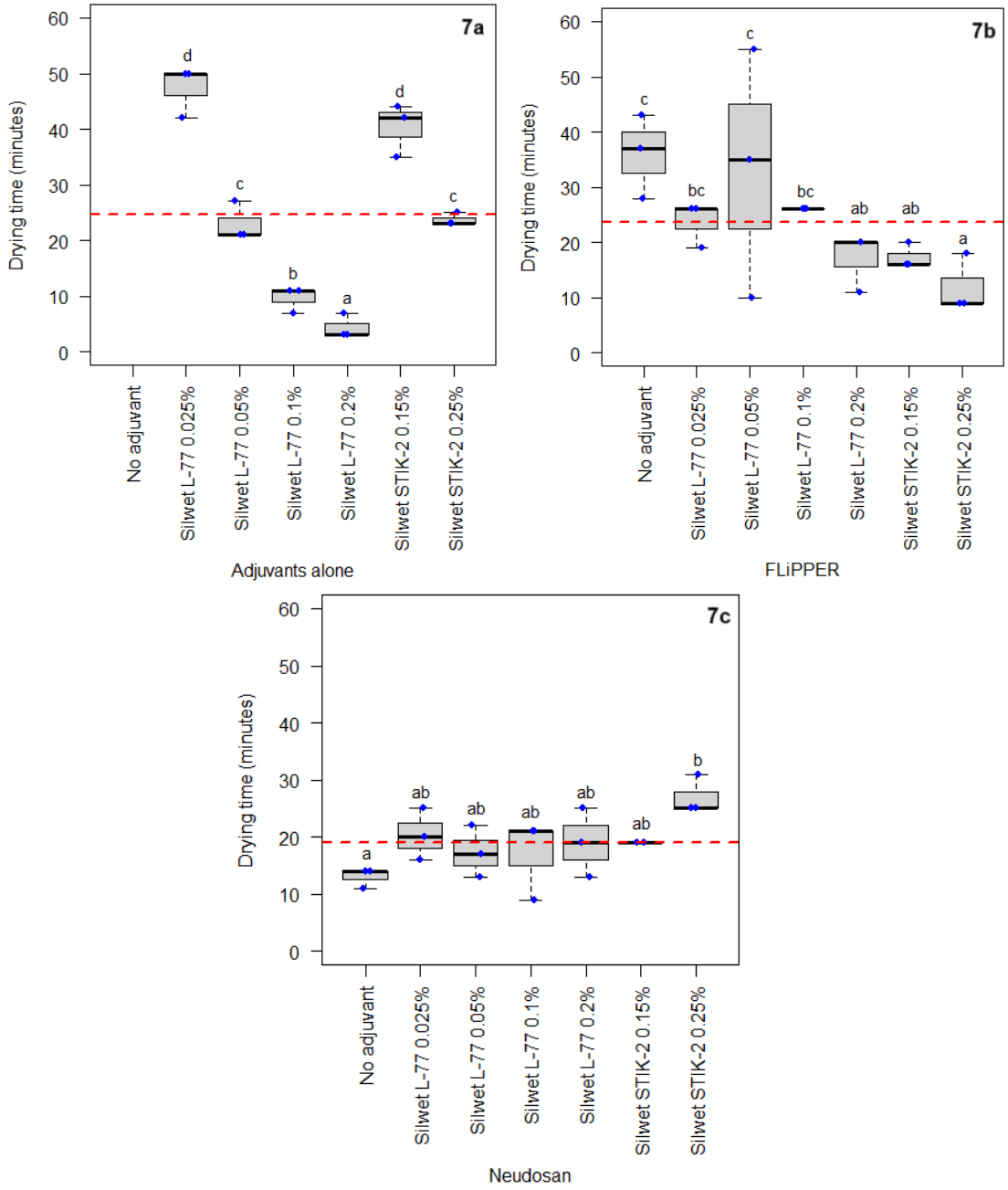


Figure 7. Drying time of fatty acids combined with adjuvants: adjuvants alone (7a), FLiPPER (7b) and Neudosan (7c). The red line represents the overall mean of the data. The blue dots help visualise various data points. Different letters indicate significant differences (p-value < 0.05).

Spreading tests results completed by Momentive researchers are presented in Figure 8. These results are similar to the results presented in Figure 6, with no significant effect of combining fatty acids with adjuvants compared to fatty acids applied on their own, and no antagonism identified.

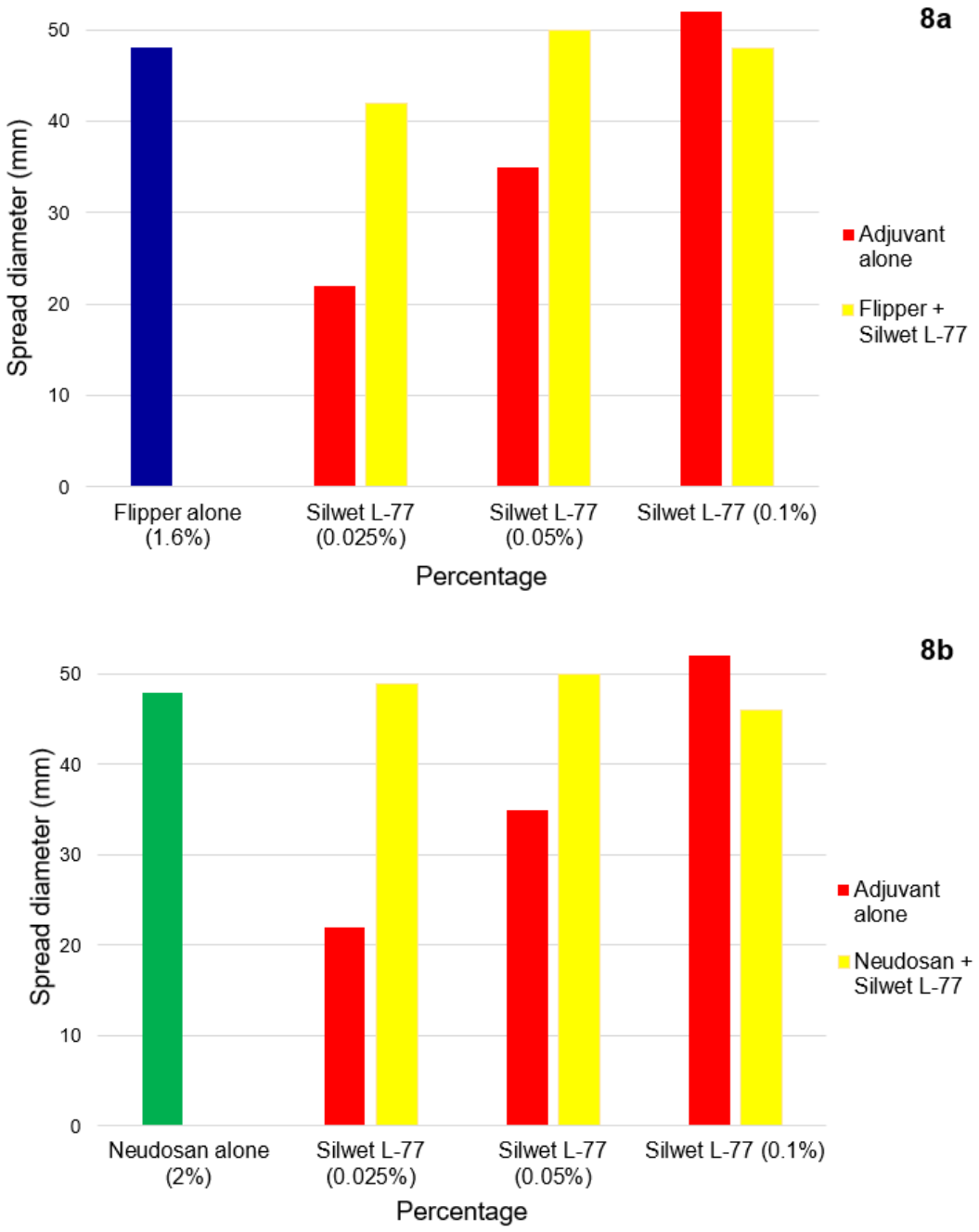


Figure 8. Spread of FLiPPER (8a) and Neudosan (8b) alone or combined with adjuvant Silwet L-77 at various concentrations (Source: Momentive).

Dynamic surface tension (DST) curves are presented in Figure 9. Adding Silwet L-77 to the fatty acids seem to reduce the surface tension when it is used at a concentration of 0.5%, which would lead to better deposition of the product and in turn is likely to lead to improved so better efficacy as it would stick more effectively to the leaves (Benjamin Langendorf, personal communication). As this test was completed using a bubble pressure tensiometer, the next step would be to test this on oilseed rape leaves to confirm these results.

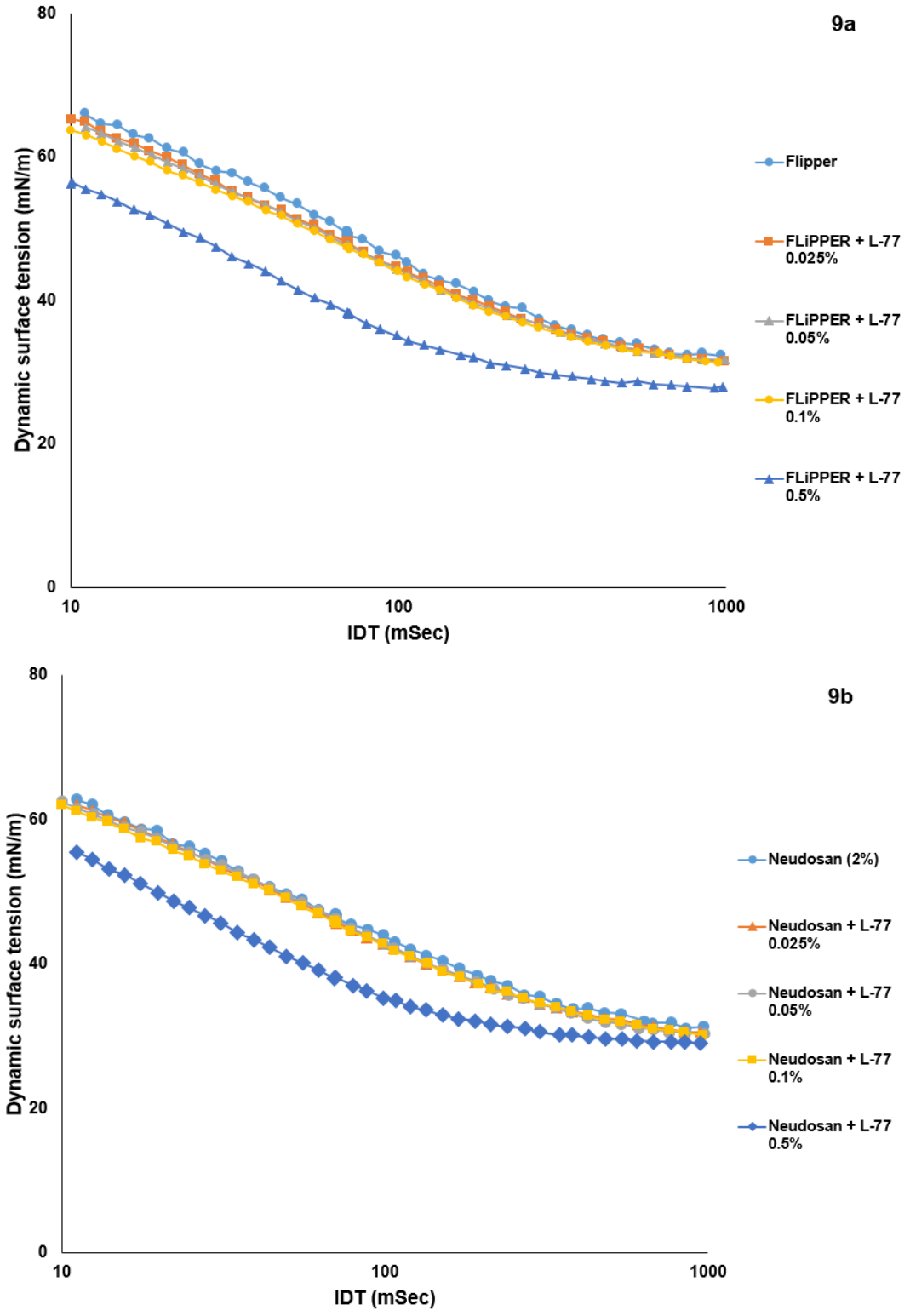


Figure 9. Evolution of the dynamic surface tension over time (IDT = Interface Development Time) of fatty acids alone or combined with adjuvant Silwet L-77 (FLiPPER (9a) and Neudosan (9b)) (Source: Momentive).

3.5. Lambda-cyhalothrin (pyrethroid) glass vial bioassay

CSFB mortality results are illustrated in Figure 10. The mortality of CSFB differed with lambda-cyhalothrin concentration, with the two highest concentrations causing higher mortality than the lowest concentration and the control (F-value = 40.07, df = 3, p-value < 0.001). According to the IRAC protocol (Insecticide Resistance Action Committee, no date) a mortality lower than 90% at 20% of the field rate indicate a suspected resistance to lambda-cyhalothrin. As our results fall into this category (76% mortality at 20% of the field rate), the tested population of CSFB was likely to be resistant to lambda-cyhalothrin. More generally, these results are to be expected given that a recent survey has reported that most CSFB populations in the UK, including samples taken from the same farm site used in this study in 2019 and 2020, are now highly resistant to pyrethroid insecticides (Willis *et al.*, 2020).

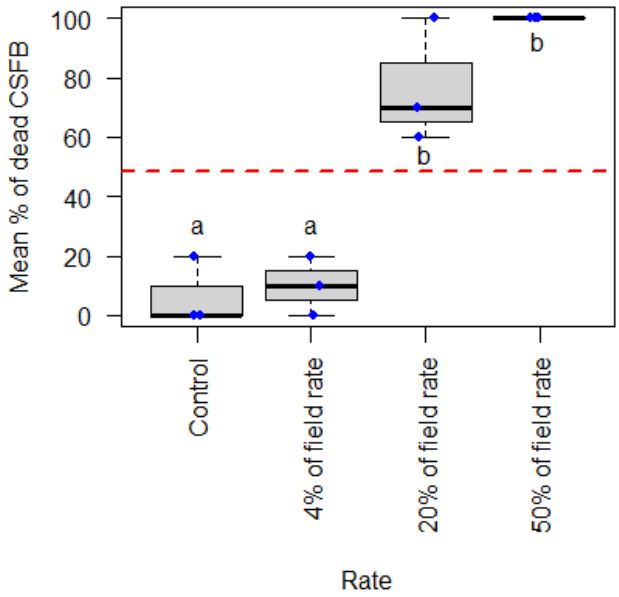


Figure 10. Percentage of dead cabbage stem flea beetle (CSFB) (*Psylliodes chrysocephala*) one day after treatment with lambda-cyhalothrin (pyrethroid) and control. The dotted red line represents the overall mean of the data. The blue dots help visualise various data points. Different letters indicate significant differences (p-value < 0.05).

4. Conclusion

The fatty acid-based products FLiPPER and Neudosan were effective against CSFB adults under laboratory conditions. As such, this study is the first to report on the potential of fatty acids against a flea beetle pest. In addition, the entomopathogenic fungus *Beauveria bassiana* strain GHA was also found to be effective against CSFB adults in this study. Azadirachtin was

not effective when applied on its own but available literature suggests that this botanical biopesticide may be effective when combined with other biopesticides.

Further work is required to investigate potential non-target effects of the products tested here, as biopesticides have a range of attractive properties that make them good components of IPM programmes (Chandler *et al.*, 2011) but, it is important to consider the potential negative impacts of these products on non-target organisms. There is for example uncertainty as to how safe azadirachtin is to non-target organisms with some studies concluding that it is safe (Charleston *et al.*, 2006; Biondi *et al.*, 2012), while others have questioned this conclusion (Qi, Gordon and Gimme, 2001; Medina *et al.*, 2004; Cordeiro *et al.*, 2010; Arnó and Gabarra, 2011; Efrom *et al.*, 2012; Tomé *et al.*, 2013; Barbosa *et al.*, 2015). Similarly, the entomopathogenic fungus *Metarhizium anisopliae* (Sorokin) is known to be pathogenic to natural enemies such as the lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and the plant bug *Dicyphus tamaninii* Wagner (Hemiptera: Miridae) (Thungrabeab and Tongma, 2007). These examples highlight the need to carefully investigate the impact of widespread applications of biopesticides.

In addition, there remain gaps in knowledge around the specific modes of action of each product tested, the importance of sublethal effects, and the extent to which improvements in product formulation and application techniques can improve efficacy and reliability of products under field conditions. Regarding product formulation, the use of adjuvants needs to be explored further. Even though according to my results, adding the tested adjuvants does not seem to improve spreading or drying time of fatty acids and it is yet not known if it would affect efficacy against CSFB, it did not show antagonistic interactions, and it would be interesting to evaluate whether organosilicon adjuvants such as Silwet L-77 and Silwet STIK-2 could improve the effectiveness of other biopesticides.

Each product shown to be effective in the laboratory must be tested under field conditions where it will be subject to a wider range of biotic and abiotic factors that may influence efficacy. An important aspect of field testing will be to consider the cost effectiveness of these biopesticides, which has been reported to be a barrier to widespread uptake due to the cost of the products themselves and the need for these products to be applied more frequently than conventional insecticides (Hoarau *et al.*, 2022b). The work presented here is an important first step in identifying potentially effective tools that may be included in future Integrated Pest Management (IPM) programmes. Biopesticides may then form one part of an IPM pyramid (Hoarau *et al.*, 2022a) that would also include other tools for management of CSFB such as crop rotation, stubble management, seed rate, companion cropping, organic amendments and resistant or tolerant varieties (White *et al.*, 2020; Ortega-Ramos *et al.*, 2021) alongside monitoring and the use of natural enemies, with which to manage CSFB in a sustainable way.

Chapter 4: Potential of entomopathogenic nematodes to control the cabbage stem flea beetle *Psylliodes chrysocephala*[2]

Abstract

Cabbage stem flea beetle (CSFB) is an important pest of oilseed rape that was controlled by neonicotinoid seed treatments until they were banned for this use in 2013. Since then, CSFB has been a difficult pest to control, partly due to widespread resistance to pyrethroid insecticides. Alternate solutions are necessary. Here, four entomopathogenic nematode (EPN) species were tested against CSFB adults under laboratory conditions. In addition, a bioassay was completed to test for EPN compatibility with a range of adjuvants (glycerin, xanthan gum and flame retardant) to protect EPNs from UV radiation and desiccation. Results show that EPNs have the potential to control CSFB adults under laboratory conditions. *Heterorhabditis bacteriophora* caused 75% CSFB mortality at a concentration of 4000 nematodes/mL after six days, *Steinernema feltiae* caused 80% CSFB mortality when applied at a concentration of 40,000 nematodes/mL after two days, *Steinernema carpocapsae* caused 85% mortality at a concentration of 10,000 nematodes/mL after six days, and *Steinernema kraussei* caused no more than 70% CSFB mortality overall compared to the water control, which led to 23% mortality. *Steinernema feltiae* and *H. bacteriophora* survival was 100% when exposed to adjuvants, except *S. feltiae* with glycerin and *H. bacteriophora* with flame retardant. Further research to evaluate the efficacy of EPN and adjuvants under field conditions is necessary.

1. Introduction

Oilseed rape (*Brassica napus*, Linnaeus) is the third most widely grown and the fourth most productive crop (in terms of tonnes/ha) grown in the United Kingdom (UK) (Defra, 2022). The production and value of oilseed rape in the UK has, however, decreased over recent years due largely to the increasing threat of pests, such as cabbage stem flea beetles (CSFB, *Psylliodes chrysocephala* Linnaeus) (Coleoptera: Chrysomelidae) (Scott and Bilsborrow, 2019). This has led to the area of oilseed rape grown to decrease from 756,000 hectares in 2012 to 307,000 hectares in 2021 (Defra, 2022).

[2] Partly published as: Price, C., Campbell, H., and Pope, T., **2023**. Potential of Entomopathogenic Nematodes to Control the Cabbage Stem Flea Beetle *Psylliodes chrysocephala*. *Insects*, 14, 665. https://doi.org/10.3390/insects14070665

Cabbage stem flea beetles invade oilseed rape at crop establishment, where they are often the most important pest species (Alford and Gould, 1976b; Winfield, 1992b; Ferguson *et al.*, 2003b; Nicholls, 2016b; Ortega-Ramos *et al.*, 2021). Damage by adults in the autumn can lead to seedling death (Leach *et al.*, 1994b), and damage by larvae can lead to stem wilting, delayed flowering, higher susceptibility to frost damage and pathogens (Schulz and Daebeler, 1984; Alford, Cooper and Williams, 1991; Broschewitz, Steinbach and Goltermann, 1993) or total plant collapse (Bonnemaison and Jourdheuil, 1954; Williams and Carden, 1961b; Graham and Alford, 1981b; C. Nilsson, 1990; Winfield, 1992b; Nilsson, 2002b; Ingrid H. Williams, 2010).

Until their ban by the European Union in 2013 (European Commission, 2013b), neonicotinoid insecticides applied to oilseed rape crops as a seed dressing were the primary means of protecting plants against CSFB (Bass and Field, 2018). Since 2013, pyrethroid insecticides applied as a foliar spray have been the only conventional synthetic insecticide option that oilseed rape growers have had for the control of CSFB. The overreliance on pyrethroid insecticides threatens non-target organisms such as pollinators and natural enemies (Ingrid H. Williams, 2010), and has led to development of widespread resistance to this type of pesticide in CSFB populations (Højland *et al.*, 2015b; Willis *et al.*, 2020).

One alternative to the current reliance on synthetic pesticide is the use of bioprotectants (IBMA, no date). The term bioprotectant includes crop protection tools such as semiochemicals (substances emitted by plants, animals, and other organisms), microbials including bacteria, fungi, protozoans, viruses, and invertebrate biocontrol agents/macrobials, which include entomopathogenic nematodes (EPNs). Along with entomopathogenic fungi, EPNs have been described as the organisms with the greatest potential to provide effective control of oilseed rape pests (Hokkanen, Menzler-Hokkanen and Butt, 2003b). EPNs, in particular, are attractive options with which to control a pest such as CSFB, as they actively search for their host once applied to the crop, present no harm to vertebrates and can kill an individual insect even when nematodes are applied at very low densities (Bednarek and Nowicki, 1986).

EPNs in the nematode genera *Steinernema* and *Heterorhabditis* are used to control many pest insects, such as the soil-dwelling larvae of leafminers, thrips, craneflies, garden chafers and various species of moths and weevils (Shapiro-Ilan, Hazir and Glazer, 2017). There are three juvenile stages of EPNs, but the only free-living stage is the third-stage juvenile, also known as the infective juvenile (IJ), that searches for and infects a host. The IJ enters the host through natural openings (mouth, anus and spiracles), or by piercing the cuticle in the case of *Heterorhabditis* spp. with the help of an anterior tooth (Bedding and Molyneux, 1982; Peters and Ehlers, 1994) and reaches the haemolymph (Akhurst and Boemare, 1990). Once within the haemolymph, the IJ releases bacteria that live in the gut of the nematodes and with which

they have a mutualistic, symbiotic relationship: *Photorhabdus* sp. for species of *Heterorhabditis*, and *Xenorhabdus* sp. for species of *Steinernema* (Boemare, Akhurst and Mourant, 1993; Gaugler, 2002). Once released into the haemolymph, the bacteria proliferate and kill the insect through septicemia and physical action within 24-72h. The digested tissues provide a food source for the IJs, which develop into adult nematodes and reproduce. Depending on the size of the host, two or more generations of nematodes can develop in the same cadaver (Shapiro-Ilan, Hazir and Glazer, 2017), but once nutrients are exhausted, the next generation of IJs will begin to search for a new host (Poinar, 1990).

Despite being highlighted as having the greatest potential to provide the effective control of oilseed rape pests (Hokkanen, Menzler-Hokkanen and Butt, 2003b), in a recent laboratory study, it was concluded that EPNs are not effective against CSFB adults (Godina *et al*. 2023). This conclusion was reached after testing the efficacy of Nemaplus® (*Steinernema feltiae* (Filipjev)), Nematop® (*Heterorhabditis bacteriophora* (Poinar)) and Nemastar® (*Steinernema carpocapsae* (Weiser)) against CSFB adults. Each EPN product was tested at a concentration of 2000 nematodes/mL by pipetting 1mL of the nematode suspensions into Petri dishes filled with sand (7% RH) before adding CSFB adults. Despite the current uncertainty around their effectiveness, testing EPNs under laboratory conditions is necessary as a first step in determining if the pest is susceptible to these control agents. Importantly, this can be performed where confounding variables such as biotic and/or abiotic factors can be excluded (Hassan, 2017). A clearer picture on the efficacy of EPNs against flea beetle pests of brassica crops is required as, to date, most studies have been performed under field conditions. This includes work on the striped flea beetle *(Phyllotreta striolata* (Fabricius)) (Li and Wang, 1990; Wei and Wang, 1993; Hou, Pang and Liang, 2001; Yan *et al.*, 2013b, 2018; Noosidum, Mangtab and Lewis, 2021), the crucifer flea beetle (*Phyllotreta cruciferae* (Goeze)) (Morris, 1987; Reddy *et al.*, 2014a) and an unnamed *Phyllotreta* species (Hokkanen, Menzler-Hokkanen and Butt, 2003b; Hokkanen *et al.*, 2006; Hokkanen, 2008). The results from these studies have been variable and not been based on results from preliminary laboratory work to evaluate the potential of the EPN species tested.

EPNs are sensitive to UV radiation and desiccation (Ignoffo and Garcia, 1992; Jaronski, 2010) and so their effective use under field conditions requires that these organisms are protected from these abiotic factors. The use of adjuvants in combination with EPNs against flea beetles has previously been tested in canola fields (Antwi and Reddy, 2016; Briar *et al.*, 2018) and is one way in which limitations on the use of EPNs can be overcome. Glycerin has been reported to be an antidesiccant and may help nematodes to persist on foliage for longer (Prabhuraj, Girish and Shivaleela, 2005). Xanthan gum has been reported to prevent nematode sedimentation and as such may improve application to crops (Beck *et al.*, 2013). Finally, flame retardants have been reported to protect nematodes against abiotic factors and

enhance their efficacy against flea beetles and other pests (Shapiro-Ilan *et al.*, 2010, 2016; Antwi and Reddy, 2016; Briar *et al.*, 2018).

In this chapter, I will present work investigating the efficacy of commercial formulations of the EPNs *S. feltiae* (Nemasys), *S. carpocapsae* (Nemasys C), *Steinernema kraussei* (Steiner) (Nemasys L) and *H. bacteriophora* (Nemasys H) against CSFB adults under laboratory conditions. These species of nematode were chosen for their ability to remain active at low temperatures (Grewal, Selvan and Gaugler, 1994; Long, Richardson and Fenlon, 2000), a useful quality in oilseed rape crops grown in the UK, as the pest establishes in the autumn or spring (AHDB, 2020). The objective of this study was to determine whether the selected EPN species are suitable candidates for further work under semi-field and field conditions.

2. Material and Methods

2.1. Insects and plants

CSFB adults were collected in July 2019, 2020, and 2022, at harvest from farms in Shropshire, UK. The insects were kept in ventilated mesh cages (30 × 30 × 30 cm) in a controlled environment room (Fitotron® SGR 122, Weiss Technik UK Limited, Loughborough, UK) at a constant 20°C temperature, 60% RH and 16/8h day/night cycle and fed by placing potted oilseed rape plants (variety Mirakel) grown under glasshouse conditions to growth stage 12 (BBCH system) into each cage. Potted oilseed rape plants were replaced every two weeks. Insect populations were kept under these conditions for up to three months before being used in a bioassay. The sex of the tested individuals was not determined before the bioassays and beetles were taken straight from the cages for bioassays. Fully expanded first and second true leaves were used as a food source for CSFB in the bioassays. The leaves were collected from young potted oilseed rape plants (variety Mirakel) grown under glasshouse conditions and that had reached a minimum growth stage of 12 (BBCH system). A water treatment was included alongside nematode treatments in each bioassay as a control. Tap water pH was 7 and hardness ($CaCO_3$) was 425 parts per million.

2.2. Entomopathogenic nematodes

I used commercial formulations of EPNs (see Table 1) supplied by BASF Agricultural Solutions UK (Littlehampton, UK). Packs of nematodes were kept refrigerated at 5°C until use and were used within the stated use by date.

Table 1. Entomopathogenic nematode (EPN) products used in the cabbage stem flea beetle (CSFB) mortality bioassays. Temperature range refers to the soil temperature indicated on the product label. All packs contained 5×10^7 individuals. The percentage values represent the proportion of nematodes in relation to the inert carrier contained in each pack.

Product Name	Manufacturer	Nematode species	Temperature range
Nemasys®	BASF Agricultural Solutions, Littlehampton, UK	*Steinernema feltiae* (90%),	10-30°C
Nemasys® C		*Steinernema carpocapsae* (87%)	12-30°C
Nemasys® L		*Steinernema kraussei* (88%)	5-30°C
Nemasys® H		*Heterorhabditis bacteriophora* (82%)	12-30°C

2.3. Mortality bioassay

2.3.1. Preliminary bioassay

Here, the four EPN species were each tested at three concentrations of live nematodes: 4000, 10,000, and 40,000 nematodes/mL. Each concentration was replicated three times. EPN suspensions were prepared by suspending a commercial formulation of EPNs in 1.5 L of tap water (stock suspension). EPNs were activated by vigorously stirring the stock suspension for five minutes, then a 1 mL sample was taken from the stock suspension using a micropipette. This 1 mL aliquot was then diluted by adding it to a conical flask containing 200 mL of tap water. From this dilution, 1 mL was pipetted into each of three 90 mm diameter Petri dishes and additional water added to each Petri dish to create a thin film of water over the base of the dishes. The number of active nematodes in each Petri dish was counted using a stereo microscope at magnification × 40 (Microtec Microscopes LTD, Somerset, UK). Using the mean number of live nematodes from the three Petri dishes, the number of live EPNs in the stock suspension was calculated and dilutions required to create the desired concentrations for the bioassays completed.

The following method was adapted from published literature (Svendsen and Steenberg, 2000; Trdan *et al.*, 2008b). Petri dishes (6 cm diameter) were prepared with two layers of filter paper (Whatman No. 1, Cytiva, Marlborough, MA, USA). One millilitre of each nematode suspension (or water for the control) was applied to the filter paper in each Petri dish with a micropipette. Then, ten CSFB adults were added per dish. A small piece of oilseed rape leaf was included in each dish as a source of food. Once prepared, the Petri dishes were placed in a fully randomised design inside a controlled environment cabinet set to a constant 25°C, 60% RH and 16/8h day/night cycle (SL2/RH, LEEC Ltd, Nottingham, UK).

CSFB mortality was assessed every two days for a period of eight days by counting the number of dead beetles in each dish. As CSFB feign death when threatened, death was confirmed when the individuals were not moving when 'prodded' repeatedly with a dissecting needle for more than ten seconds, and when the beetles' legs were in a splayed position. Moribund insects were considered dead.

2.3.2. Recommended concentrations bioassay

Steinernema feltiae and *H. bacteriophora* were used in this bioassay, at rates recommended by the manufacturer BASF Agricultural Solutions against CSFB in oilseed rape (125,000, 250,000 and 500,000 IJ/m^2, equivalent to 1,250, 2,500 and 5,000 nematodes/ml) and replicated six times. The bioassay procedure was similar to 2.3.1, except that the commercial formulations were dissolved in 10L of tap water and activated using an aquarium pump, the 10L bottle was regularly shaken upside down to prevent nematode sedimentation and keep the suspension homogenised, and the Petri dishes were placed in incubation chambers (round plastic boxes, 12cm/7cm diameter top/bottom, 6 cm height) lined with wet paper towel to maintain high humidity, closed with a lid perforated with 1mm holes to allow air transfers. Two data loggers (Tempo Disc, BlueMaestro, London, UK) were placed in similar conditions the day before the bioassay and confirmed a humidity of 100% in these conditions. Incubation chambers were placed in a fully randomised design in a plant growth cabinet (Panasonic MLR-352-PE, Osaka, Japan) at 20°C.

CSFB mortality was assessed every day for five days, by counting the number of dead beetles in each dish. As CSFB feign death when threatened, death was confirmed when the individuals were not moving when prodded repeatedly with a dissecting needle, and when the beetles' legs were in a spread-out position. Moribund insects were considered dead.

2.4. Compatibility between entomopathogenic nematodes and adjuvants

Nemasys (*S. feltiae*) and Nemasys H (*H. bacteriophora*) were used in this bioassay and each species was tested in combinations with each adjuvant. The concentrations of the used adjuvants are listed in Table 2. Each adjuvant was dissolved in tap water.

Table 2. Adjuvants tested alongside entomopathogenic nematodes (EPNs).

Product Name	Manufacturer	Active Ingredients	Concentrations	Replicates
Flametect Nitro D	Eco-Sol Ltd, Barry, UK	Nitrogen based solution (34% minimum), polymer binder system (30% minimum)	0.01, 0.1, 1 and 10%	3 per concentration
Xanthan gum	Sigma-Aldrich, St Louis, MO, USA	Xanthan gum from *Xanthomonas campestris*	0.001, 0.01, 0.1 and 1%	3 per concentration
Glycerol	Fisher Scientific, Loughborough, UK	Glycerol ≥ 99%	0.01, 0.1, 1 and 10%	3 per concentration

Commercial formulations of each EPN species were diluted separately in 5 L of tap water. IJs were activated by oxygenating using an aquarium pump for ten minutes prior the start of the bioassay and all the way until the end of the bioassay, and the 5 L bottle was regularly shaken to prevent nematode sedimentation and keep the suspension homogenised. Petri dishes (8.5 cm diameter) were lined with filter paper (Whatman No.1, Cytiva, Marlborough, MA, USA). In each Petri dish, approximately 5000 IJs were mixed with one of the adjuvants in 1 mL of water (only nematodes for the water control) and were added with a micropipette after thorough agitation of the suspensions. The Petri dishes were then sealed with paraffin film (Bemis™ Parafilm®, Neenah, Wisconsin, U.S.) to prevent EPNs from escaping, and wrapped in tin foil to protect nematodes from UV radiation emitted by the light bulbs. The dishes were then placed in incubation chambers (round plastic boxes, 12cm/7cm diameter top/bottom, 6 cm height, lined with four layers of wet paper towel) to maintain high humidity. All incubation chambers were placed in a growth room (Fitotron® SGR 122, Weiss Technik UK Ltd, Loughborough, UK) with a 16/8h day/night photoperiod and 20°C for seven days. Two data loggers (Tempo Disc, Blue Maestro, London, UK) were placed in similar conditions two days prior to the bioassay to confirm that these conditions resulted in 100% humidity.

Concentrations for xanthan gum were lower (see Table 2) than for other adjuvants due to the highest concentration of 10% not completely dissolving in water.

After seven days, the Petri dishes were opened, and each disc was rinsed into a second Petri dish using a squeeze bottle filled with tap water. Then, the first 30 nematodes counted within a consecutive series of fields of view were scored as either being dead or alive based on their response when repeatedly probed with a dissecting needle.

2.5. Statistical analysis

Data were analysed in R (version 4.2.2) and RStudio (version 2022.12.0). CSFB mortality was analysed using mixed effect models from the package *lme4* (Bates *et al.*, 2014). EPN survival when exposed to adjuvant was analysed using one-way ANOVA on a linear model of the data. Significance groups were computed using the *cld(lsmeans())* function included in the packages *multcomp* (Hothorn, Bretz and Westfall, 2015) and *lsmeans* (Lenth, 2016), or using the *HSD.test()* function included in the package *agricolae* (De Mendiburu and Simon, 2015). Graphical illustrations for CSFB mortality were made using the geom_col function from the package ggplot2 (Wickham, 2009) after the data were tidied with the mutate function from the package tidyverse (Wickham, Averick, *et al.*, 2019). Graphical illustrations for EPN survival with adjuvants were made with the boxplot function from the package graphics (Murrell, 2009) after the data were tidied with the mutate function from the package tidyverse.

3. Results

3.1. Mortality bioassay

3.1.1. Preliminary bioassay

CSFB mortality results are illustrated in Figure 1. All EPN treatments resulted in significantly higher mortality than was caused by the water control ($F = 7.14$, $df = 4$, $p < 0.001$). There was no difference in CSFB mortality between the two highest concentrations ($t = -1.18$, $df = 32$, $p = 0.247$) and both caused significantly higher mortality than the lowest concentration and the control ($t = -3.78$, $df = 32$, $p < 0.001$). Overall, CSFB mortality increased with time ($F = 38.56$, $df = 3$, $p < 0.001$).

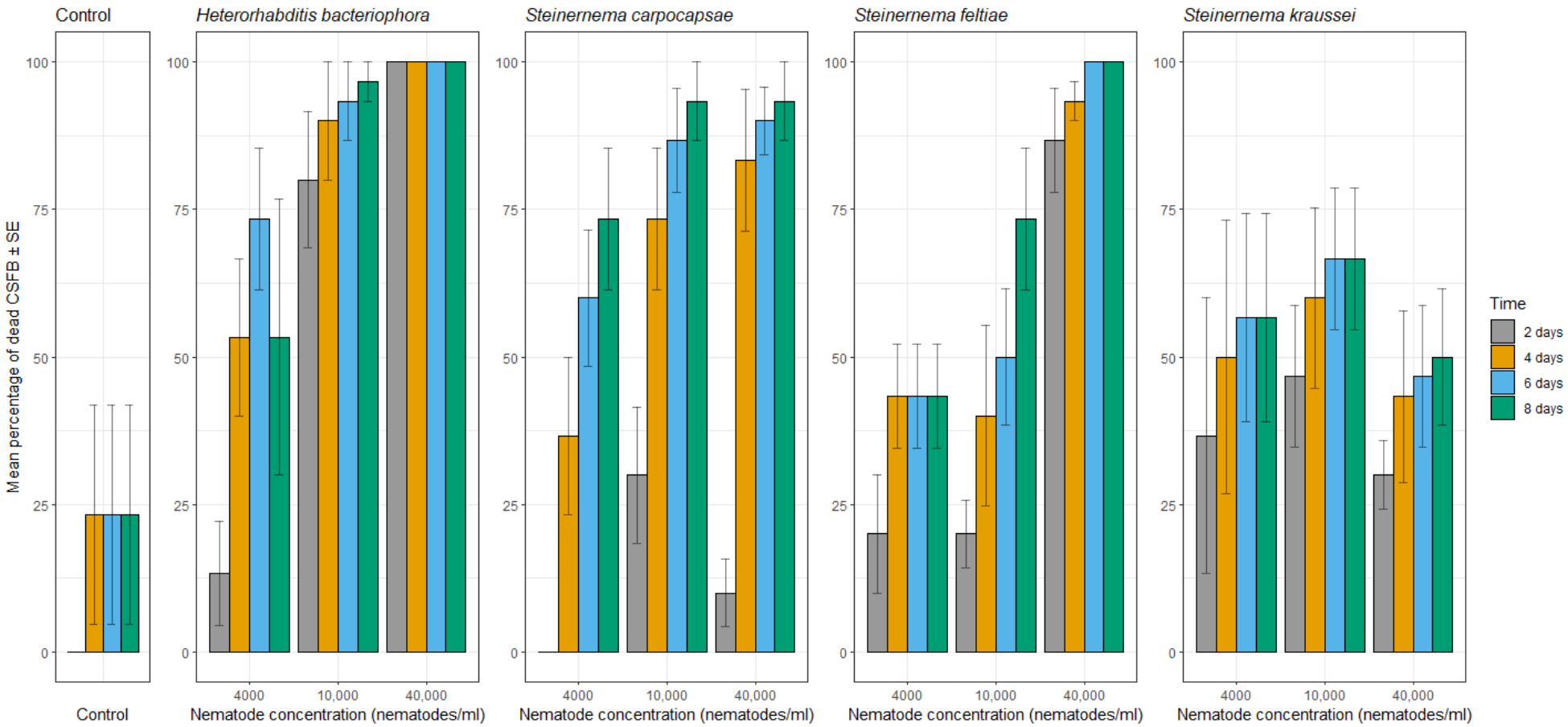


Figure 1. Mean percentage of dead cabbage stem flea beetle (CSFB) ± SE after two, four, six and eight days of contact with four different species of entomopathogenic nematodes (concentrations of nematodes/ml) or water (control). Values are cumulative over the days.

3.1.2. Recommended concentrations bioassay

CSFB mortality results are illustrated in Figure 2. Nematode treatments including *S. feltiae* caused significantly higher mortality than the water control (t = 5.93, df = 45, p < 0.001) but *H. bacteriophora* treatments resulted in CSFB mortality that was statistically lower than the water control (t = -2.30, df = 45, p = 0.026). There was no difference in mortality between concentrations (F = 0.16, df = 2, p = 0.851), but CSFB mortality increased with time (F = 42.90, df = 4, p-value < 0.001).

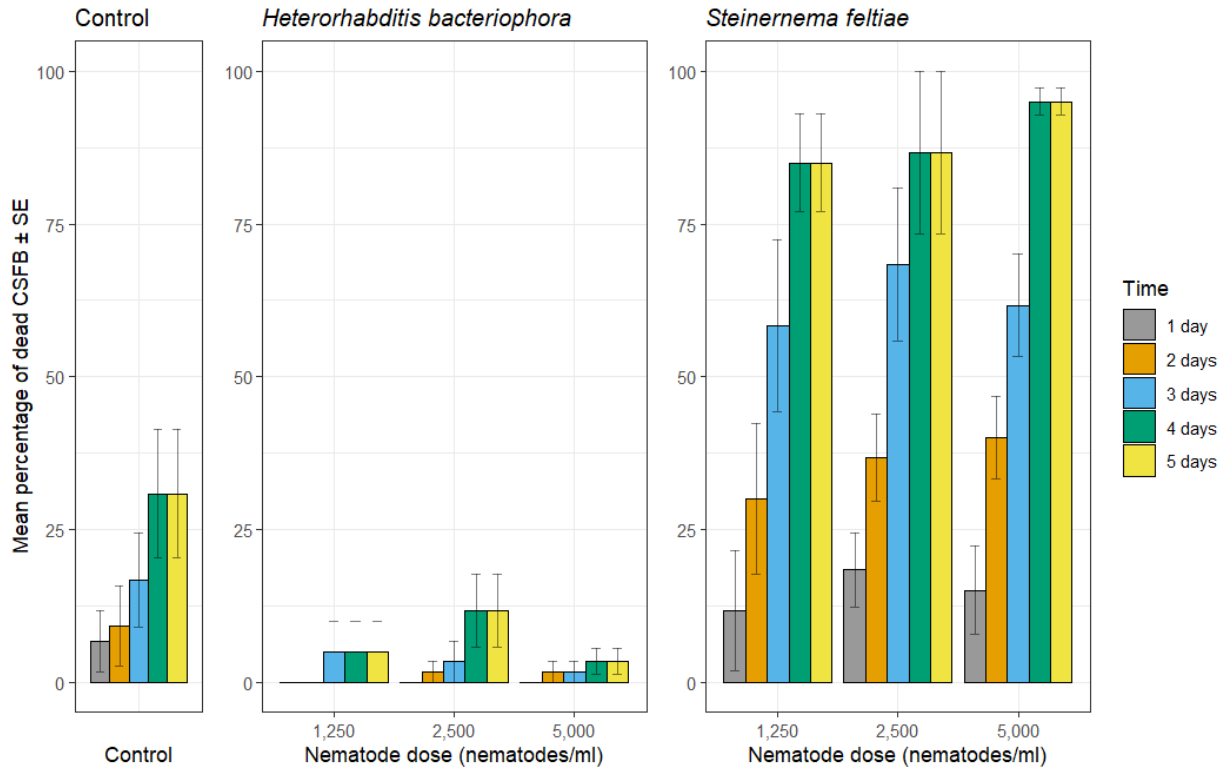


Figure 2. Mean percentage of dead cabbage stem flea beetle (CSFB) ± SE every day for five days, after contact with two different species of entomopathogenic nematodes (concentrations of nematodes/ml) or water (control). Values are cumulative over the days.

3.2. Compatibility between entomopathogenic nematodes and adjuvants

Steinernema feltiae survival was significantly lower when EPNs were exposed to glycerin compared with other adjuvants and the control (F = 6.308, df = 3, p = 0.001) whereas *H. bacteriophora* survival was significantly lower when EPNs were exposed to the flame retardant compared with other adjuvants and the control (F = 9.684, df = 3, p < 00.1).

Following these observations, the results of each adjuvant were analysed separately for each EPN species (Figure 3): there was no significant difference in *S. feltiae* survival when exposed to 0.01, 0.1, 1 or 10% of flame retardant (F = 2.702, df = 3, p = 0.116) and to 0.001, 0.01, 0.1 or 1% of xanthan gum (F = 2.703, df = 3, p = 0.116); however, when exposed to 0.01,

0.1, 1 or 10% of glycerin (Figure 3), survival was significantly lower at the highest concentration ($F = 20.53$, $df = 3$, $p < 0.001$). There was no significant difference in *H. bacteriophora* survival when exposed to the same concentrations of glycerin ($F = 3.609$, $df = 3$, $p = 0.065$) and to the same concentrations of xanthan gum ($F = 0.384$, $df = 3$, $p= 0.767$) but when exposed to a range of concentrations of flame retardant (Figure 4), survival decreased progressively with increasing concentration of this adjuvant; EPN survival was significantly different between each concentration except between 0.1% and 1% ($F = 5.11$, $df = 3$, $p = 0.029$).

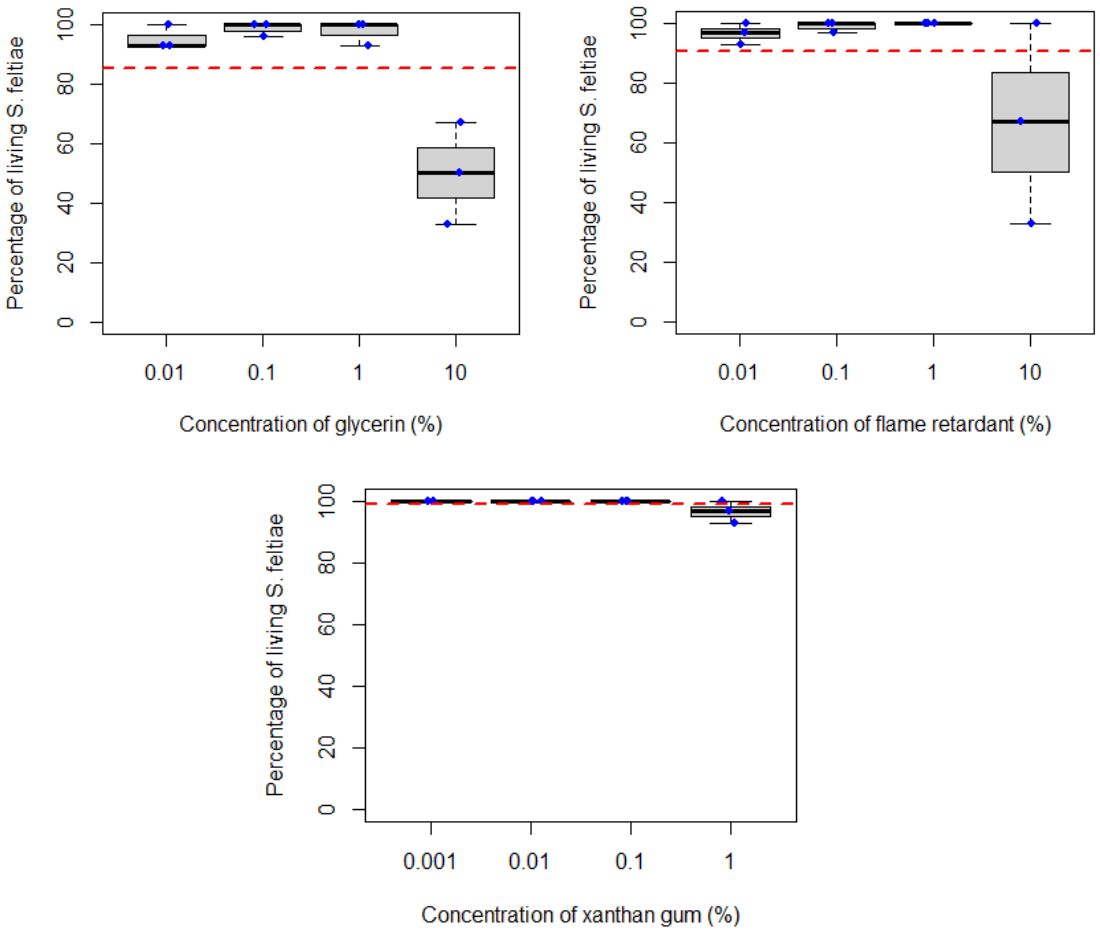


Figure 3. Percentage of living *Steinernema feltiae* after exposure to various concentrations of glycerin, flame retardant and xanthan gum. The red line represents the overall mean of the data. The blue dots help visualise various data points.

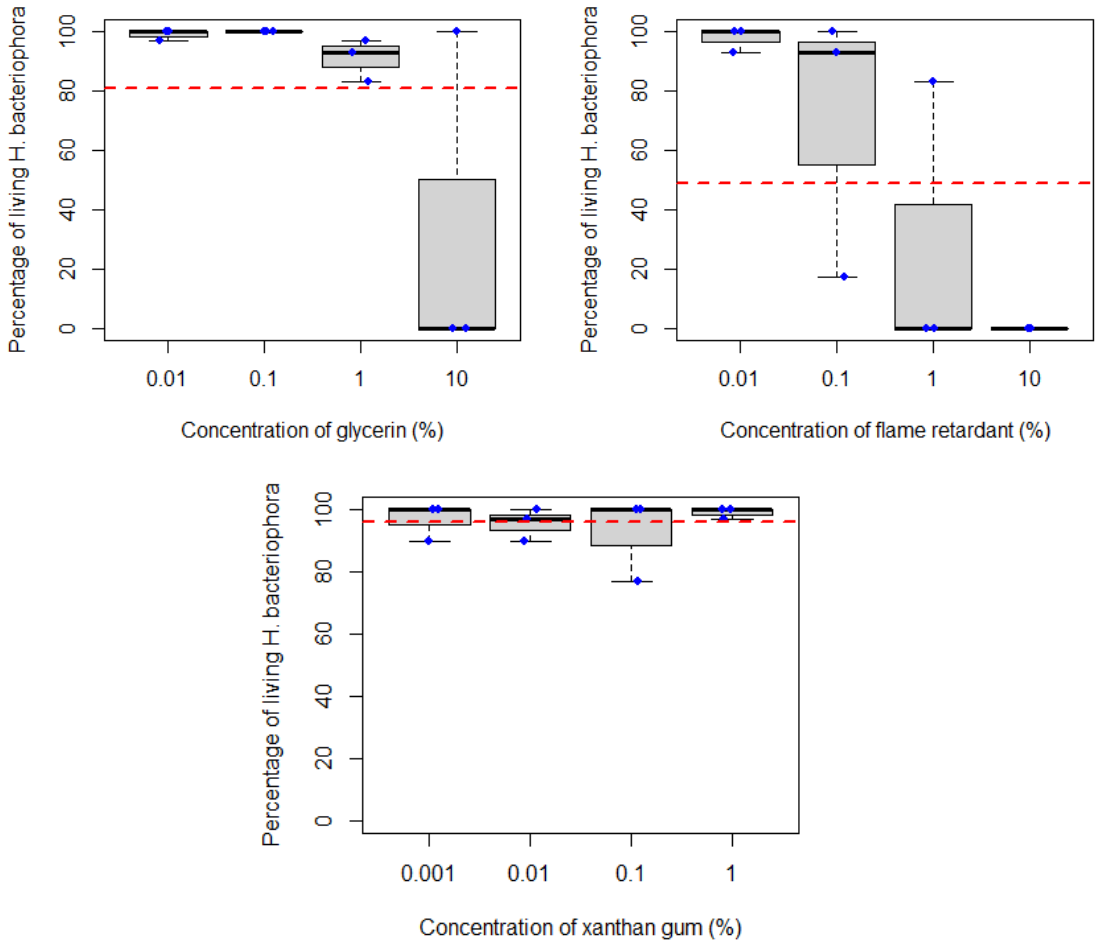


Figure 4. Percentage of living *Heterorhabditis bacteriophora* after exposure to various concentrations of glycerin, flame retardant and xanthan gum. The red line represents the overall mean of the data. The blue dots help visualise various data points.

4. Discussion

4.1. Mortality bioassays

Steinernema feltiae was the most effective species of nematode tested here. After four days, this species caused 80% CSFB mortality even when applied at the lowest concentration tested, 1,250 nematodes/mL. After two days, *Steinernema feltiae* caused 80% CSFB mortality when applied at the concentration of 40,000 nematodes/mL compared to 23% mortality in the water control treatment. *Heterorhabditis bacteriophora* efficacy varied between bioassays, causing approximately 75% CSFB mortality recorded after six days when a concentration of 4000 nematodes/mL was applied in the preliminary bioassay. However, CSFB mortality was less than 10% when 5,000 nematodes/ml was applied in the recommended concentrations bioassay, which might have been caused by various factors such as a poor batch of nematodes. *Steinernema carpocapsae* was effective in the preliminary bioassay at a concentration of 10,000 nematodes/mL when 85% mortality was recorded six days post

nematodes application. *Steinernema kraussei* was the least effective species tested here, with no more than 70% CSFB mortality recorded for any of the concentrations tested.

A previous laboratory study has investigated the efficacy of EPNs against CSFB adults. Godina *et al*. (2023) recorded 25% adult CSFB mortality with *S. carpocapsae*, 16% CSFB mortality with *S. feltiae* and 7% CSFB mortality with *H. bacteriophora*, and only *S. carpocapsae* was found in dead CSFB. The authors concluded that CSFB adults were not the most appropriate growth stage to be targeted using EPN and indeed results from this study against CSFB larvae were more encouraging. Differences in adult CSFB mortality between the results recorded in previous work and the results presented in the Results section are likely explained by differences in the methods used. While I used filter paper and incubated the dishes at 60-100% RH, Godina *et al*. (2023) used sand to cover the bottom of Petri dishes with 7% RH, which might have hindered EPN activity as they need moisture to survive and move around (Shapiro-Ilan, Hazir and Glazer, 2017). The authors also only tested one nematode concentration of 2000 nematodes/mL, while here I tested various concentrations of up to 40,000 nematodes/mL.

Previous work has also tested commercial formulations of *S. feltiae, S. carpocapsae, H. bacteriophora* and *H. megidis* (Poinar, Jackson and Klein) against *Phyllotreta* spp. flea beetle adults (Trdan *et al*., 2008). Here, the authors tested concentrations of 2000, 10,000 or 20,000 nematodes/mL and at three temperatures in Petri dishes on filter paper and found that for all EPN treatments, flea beetle mortality was higher than in the water control treatment. They observed that *S. feltiae* caused high mortality at every assessment date, while *H. bacteriophora* was the most effective when only the end of the experimental period was considered, after eight days. It was also reported that EPNs were in general more effective with increasing temperature. Indeed, temperature had a greater effect on flea beetle mortality than changing EPN concentration. Results presented here against CSFB are similar to those reported previously for *Phyllotreta* spp. by Trdan *et al*., however, the role of temperature should be included in future studies with CSFB, as well as testing whether incubating the Petri dishes in the dark would produce different results. The fact that these EPN species seem to be more effective at higher temperatures is an important finding, as winter oilseed rape crops grown in the UK and northern European countries are at their most vulnerable growth stage in the autumn when temperatures are declining.

Two recent studies have tested the efficacy of EPNs against the striped flea beetle (Xu *et al.*, 2010; Noosidum, Mangtab and Lewis, 2021). Both studies produced results broadly similar to the results reported in the present study. For example, all species of EPNs tested were found to successfully infect and kill the striped flea beetle (Noosidum, Mangtab and Lewis, 2021). Four days after treatment application, *S. carpocapsae* at a concentration of 50 nematodes/insect caused more than 50% adult mortality. Five days after treatment, 79-83%

adult mortality was recorded at 200 nematodes/insect with *S. carpocapsae* and *S. siamkayai*. These results appear to be broadly like the results reported in the present study.

Xu *et al.* (2010) reported that mortality of third instar striped flea beetle larvae increased from 30% to 100% as concentrations of *H. indica* increased from 4 to 36 nematodes/cm^2. In addition, at a concentration of 1,000 nematodes/ml, 30% first instar mortality was recorded when *H. indica* 212-2 was applied, more than 60% second instar mortality was recorded when *S. carpocapsae.* All and *H. indica* LN2 were applied, and more than 90% third instar and pupae mortality recorded when each isolate was applied, except *S. carpocapsae* All, which caused around 80% mortality. These results agree with the findings reported in the present study, with the same species of EPN (or closely related species) causing similar levels of control, though they did not test the EPN against flea beetle adults.

Testing the efficacy of EPN under field conditions is important to ensure that results from the laboratory can be translated successfully. While several field studies have reported encouraging results using EPNs against various flea beetle species such as CSFB (Hokkanen, Menzler-Hokkanen and Butt, 2003b; Godina *et al.*, 2023), unspecified *Phyllotreta* species (Li and Wang, 1990; Hokkanen *et al.*, 2006; Hokkanen, 2008) and crucifer flea beetle and striped flea beetle (Morris, 1987; Wei and Wang, 1993; Yan *et al.*, 2013b; Reddy *et al.*, 2014a; Noosidum, Mangtab and Lewis, 2021), these have not been based on initial laboratory testing results.

Future studies should test a wide range of temperatures relevant to crop conditions when evaluating EPN efficacy against CSFB. Godina *et al.* (2023) stated that larval stages of CSFB may be a better target for EPNs, but more work is required using potted plants infested with larvae under laboratory conditions to provide further information on the suitability of this growth stage of the target pest. There is also evidence that a specific strain of *H. bacteriophora*, SDT1-IL1, was more effective than the commercial *H. bacteriophora* Nematop® at killing CSFB larvae (60% mortality versus 30%), so using different strains of EPNs could also be a potential of future studies.

4.2. Compatibility between entomopathogenic nematodes and adjuvants

Overall, the adjuvants tested with *S. feltiae* and *H. bacteriophora* were compatible over a period of seven days of exposure, except for glycerin which affected *S. feltiae* negatively at the highest concentration tested, and the flame retardant, which negatively affected *H. bacteriophora* survival as concentrations increased. In the case of these two adjuvants, it appears that nematode survival is linked to concentration. Prabhuraj *et al.* (2005) working with *H. indica* Poinar, Karunakar and David, a nematode species closely related to *H. bacteriophora*, reported 81% survival of nematodes after two hours of exposure to 0.1%

glycerin, which was significantly higher than nematode survival when exposed to other adjuvants tested (Triton X-100, paraffin liquid, castor oil, palm oil and sunflower oil). However, no nematodes survived more than eight hours of contact with any of the adjuvants, while in the present study 100% of *H. bacteriophora* survived for at least seven days when exposed to the same concentration of glycerin.

As evidence of the potential importance of developing suitable EPN-adjuvant combinations, it is useful to consider other pests. For example, to control the lesser peachtree borer, *Synanthedon pictipes* Grote and Robinson (Lepidoptera: Sesiidae), (Shapiro-Ilan *et al.*, 2010, 2016) applied *S. carpocapsae* alongside Barricade® (Barricade International (firegel.com) (accessed on 11 April 2023)), a sprayable polymer gel typically used as a fire retardant and similar in composition to the fire retardant used in the present study, and compared it with chlorpyrifos. The authors concluded that applying *S. carpocapsae* and 2% Barricade® at the same time was more effective than applying them separately or not treating, and that the combination was at least as effective as chlorpyrifos. These results contrast with the results reported here where the flame retardant was not compatible with *H. bacteriophora*.

Based on these previous studies, Antwi and Reddy (2016) and Briar *et al.* (2018) have tested commercial formulations of *S. feltiae* and *S. carpocapsae* against crucifer flea beetle adults in canola fields combined with 1% Barricade®, or with an imidacloprid insecticidal product (Gaucho). *Steinernema feltiae* was only found to be effective in reducing leaf damage by adult beetles and improving yield when combined with the Barricade® or when combined with imidacloprid. One other advantage of polymer gels like Barricade® or the fire-retardant product used in the present study is that they are non-toxic; hence, they do not have negative environmental impacts.

The next steps for future studies would be to explore whether the adjuvants tested in the present study are effective in improving nematode persistence and application on oilseed rape plants under field conditions. These results give encouragement that the apparent compatibility of Flametect Nitro D with *S. feltiae*, as shown in this study, should be progressed to testing under more realistic environmental conditions.

Chapter 5: Field assessment of biopesticides to control the cabbage stem flea beetle *Psylliodes chrysocephala*

Abstract

Cabbage stem flea beetle (CSFB) is an economically important pest of oilseed rape in the UK. Until their ban by the European Union in 2013, CSFB was controlled with neonicotinoid seed treatments. Currently, the only authorized insecticides in the UK are synthetic pyrethroids, which are applied as foliar treatments, and to which there is now widespread resistance in CSFB populations. It is then necessary to find novel effective controls for this pest, such as the use of biopesticides as part of Integrated Pest Management (IPM) programmes.

Selected biopesticides screened under laboratory conditions in Chapter 3 were tested in a commercial oilseed rape crop. Biopesticides tested included physically acting products, entomopathogenic fungi, entomopathogenic nematodes and plant extracts. Each product was tested using the field rate indicated on the product label or rates recommended by the manufacturers. Efficacy of biopesticide products was compared with the synthetic pyrethroid insecticide lambda-cyhalothrin (Hallmark with Zeon technology) as a positive control, water as a negative control and untreated plots. Product efficacy was determined by recording foliar damage caused by CSFB adults and numbers of CSFB larvae inside the plants.

Only the physically acting product FLiPPER, when combined with Hallmark significantly reduced leaf damage compared to the water treatment and untreated plots. Larval density did not vary significantly among the treatments tested.

Several limitations of this study were identified, and changing the methods used may improve the quality of results. In particular, assessment methods could be improved, the timing of treatment application and the formulation of these biopesticides, particularly those based on entomopathogens, may be important in order to protect these organisms from negative abiotic factors.

1. Introduction

The cabbage stem flea beetle (CSFB; *Psylliodes chrysocephala*, Linnaeus, Coleoptera: Chrysomelidae) is the most important stem-mining pest of oilseed rape crops grown in Europe (Alford, Nilsson and Ulber, 2003; Nicholls, 2016a). After pupating in the soil for a few months, adults emerge in late spring-early summer, complete a summer diapause before migrating to oilseed rape crops in early September where the adults feed on young seedlings, mate and

lay eggs in the soil (Kaufmann, 1941; Ebbe-Nyman, 1952; Williams and Carden, 1961a; Alford, 1979; I. H. Williams, 2010). In late September the first larvae hatch, move through the soil and then tunnel into the petioles of the oilseed rape plants, feeding through the winter and spring, during which time the larvae move to the main stem of the plant to feed (Kaufmann, 1941; Ebbe-Nyman, 1952; White, 2015).

Adult CSFBs feed on leaves from early September, creating characteristic damage known as 'shot holing' which can kill seedlings if pest pressure is high enough (Leach *et al.*, 1994a; Alford, Nilsson and Ulber, 2003), while larvae migrating to the main stem in spring can also kill plants (Williams and Carden, 1961a; Graham and Alford, 1981a; Williams, 2004). Before being banned in December 2013 by the European Union (European Commission, 2013a), neonicotinoid insecticides were used to control CSFB (I. H. Williams, 2010). Oilseed rape growers in the UK seeking to control this pest through the use of conventional insecticides are now restricted to the use of pyrethroids, but this overreliance on a single insecticide mode of action has resulted in the development of widespread resistance to this group of insecticides, making them ineffective in most oilseed rape growing areas (Højland *et al.*, 2015a; Willis *et al.*, 2020), with some UK populations being 100% resistant to approved application rates of pyrethroid insecticides (Willis *et al.*, 2020). Pyrethroids have also been found to be detrimental to non-target organisms such as parasitoid wasp species that are natural enemies of CSFB (I. H. Williams, 2010).

Alternatives to the use of pyrethroid insecticides are urgently needed in order to reduce the economic impact of CSFB. The economic impact of this pest can be seen through changes in oilseed rape grown in the UK. In the period from 2012 to 2021, the area of oilseed rape grown decreased from 756,000 hectares to 307,000 hectares (Defra, 2022).

One potential solution to this pest problem is the use of biopesticides. Fatty acids are biopesticides based on unsaturated carboxylic acids as the active ingredient. Fatty acids have been known since the 1920s for their insecticidal potential (Siegler and Popenoe, 1925) and affect the target insect by penetrating the external cuticle and interacting with vital metabolic processes and feeding activity, resulting in the death of the insect (Convertini *et al.*, 2018; Suma *et al.*, 2019; Bayer, 2021). Despite this long history of research, to my knowledge no previous study has investigated the effect of fatty acids against hard-bodied insect such as adult Coleoptera.

Two species of entomopathogenic fungi, *Metarhizium anisopliae s.l.* (*brunneum*) (Metchnikov) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin, have been studied for their potential against CSFB, as well as *Phyllotreta spp.* flea beetles, flea beetle species that are closely related to CSFB. The insect is infected when spores adhere to its cuticle and germinate, penetrating the target using a combination of mechanical pressure and the secretion of enzymes such as proteases and chitinases (Stleger *et al.*, 1987; Stleger *et al.*, 1987). The

fungus then grows into the haemocoel, then the rest of the body of the host insect, which is killed within four to six days as a result of physical damage and secretion of fungal metabolites (Butt and Goettel, 2000). Spores are then produced on the surface of the cadaver, which may then inoculate other insect hosts, which makes it even more valuable as a biocontrol agent. In this field study, I tested the species *B. bassiana* strain GHA.

Another biopesticide tested in this study is azadirachtin, a tetranotriterpenoid produced by the neem tree (*Azadirachta indica* A. Juss., Meliaceae) (Schmutterer, 1990). Azadirachtin has both lethal (Karnavar, 1987; Mordue and Blackwell, 1993) and sublethal effects, including reduced growth, longevity, fertility, reproduction, oviposition and feeding (Mordue and Blackwell, 1993; Nisbet, 2000; Mancebo *et al.*, 2002). It is considered one of the most biologically active natural insecticide (Mordue and Blackwell, 1993; Morgan, 2009), the mode of action of azadirachtin is through disruption of vital hormones such as ecdysones. By targeting such a key hormone, this biopesticide has been reported to impact more than 400 insect species (Zehnder and Warthen, 1988; Schmutterer, 1990; Vietmeyer, 1992; Di Ilio *et al.*, 1999; Atawodi and Atawodi, 2009; Mordue, Morgan and Nisbet, 2010; Sahak, Pourmirza and Ghosta, 2010). Azadirachtin has been found in past studies to be effective when combined with other biopesticides (Yan *et al.*, 2013a; Reddy *et al.*, 2014b), which is why in this field study I combined it with the fungus *B. bassiana* in addition to testing it alone.

Entomopathogenic nematodes in the nematode genera *Steinernema* and *Heterorhabditis* are used against many pest insects, such as the soil-dwelling larvae of leafminers, thrips, craneflies, garden chafers and of various species of moths and weevils (Bélair, Wright and Curto, 2005; Cabanillas, Wright and Vyas, 2005; Cowles *et al.*, 2005; Grewal, Koppenhöfer and Choo, 2005; Tomalak, Piggott and Jagdale, 2005; Van Tol and Raupp, 2005; BASF, 2021). The free-living stage is the third-stage juvenile, also known as the infective juvenile (IJ), that searches for and infects a host. The IJ enters the host through natural openings (mouth, anus and spiracles), or by piercing the cuticle in the case of *Heterorhabditis* spp. with the help of an anterior tooth (Bedding and Molyneux, 1982; Peters and Ehlers, 1994) and reaches the haemolymph (Akhurst and Boemare, 1990). Once within the haemolymph, the IJ releases bacteria that live in the gut of the nematodes and with which they have a mutualistic, symbiotic relationship: *Photorhabdus* sp. for species of *Heterorhabditis* sp., and *Xenorhabdus* sp. for species of *Steinernema* sp. (Boemare, Akhurst and Mourant, 1993; Gaugler, 2002). Once released into the haemolymph, the bacteria proliferate and kill the insect through septicemia within 24-72h. The digested tissues provide a food source for the IJ, which will develop into an adult nematode and reproduce. The next generation of IJs will then exit the host and search for a new one (Poinar, 1990). In this field study, I tested entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora*.

Finally, these biopesticides were compared with a conventional pyrethroid insecticide lambda-cyhalothrin as a positive control. In addition, lambda-cyhalothrin was also combined with fatty acids to observe potential synergistic effects. Water was used in this study as a negative control and no treatment was used as an untreated control.

The objective of this study is to evaluate the efficacy of a range of biopesticide products, each used on its own or in selected combinations, against CSFB under field conditions. Product efficacy determined through recording adult CSFB feeding damage and larval density in oilseed rape plants. Comparisons of biopesticide efficacy with a conventional synthetic insecticide was made possible through the inclusion of a lambda-cyhalothrin treatment.

2. Material and Methods

2.1. Field location, experiment set up and clean up

The experiment was completed between September and November 2021 in a 10.5 Ha oilseed rape crop grown in Norton, Shropshire, UK (52.595863866039025, -2.41151437038343). The experiment covered 1300 m^2 of the field and its position, advised by the farmer as an area where historically CSFB damage had been high, is indicated in Figure 1. The products applied are listed in Table 1. For each product, the rate of application was the 'field rate' recommended by the manufacturer. The two treatments of Hallmark with Zeon Technology differ by the number of applications: Treatment 3 (T3) was applied three times while Treatment 9 (T9) was applied once. Applying a pyrethroid treatment more than once was for experimental purposes and is not recommended for common practice.

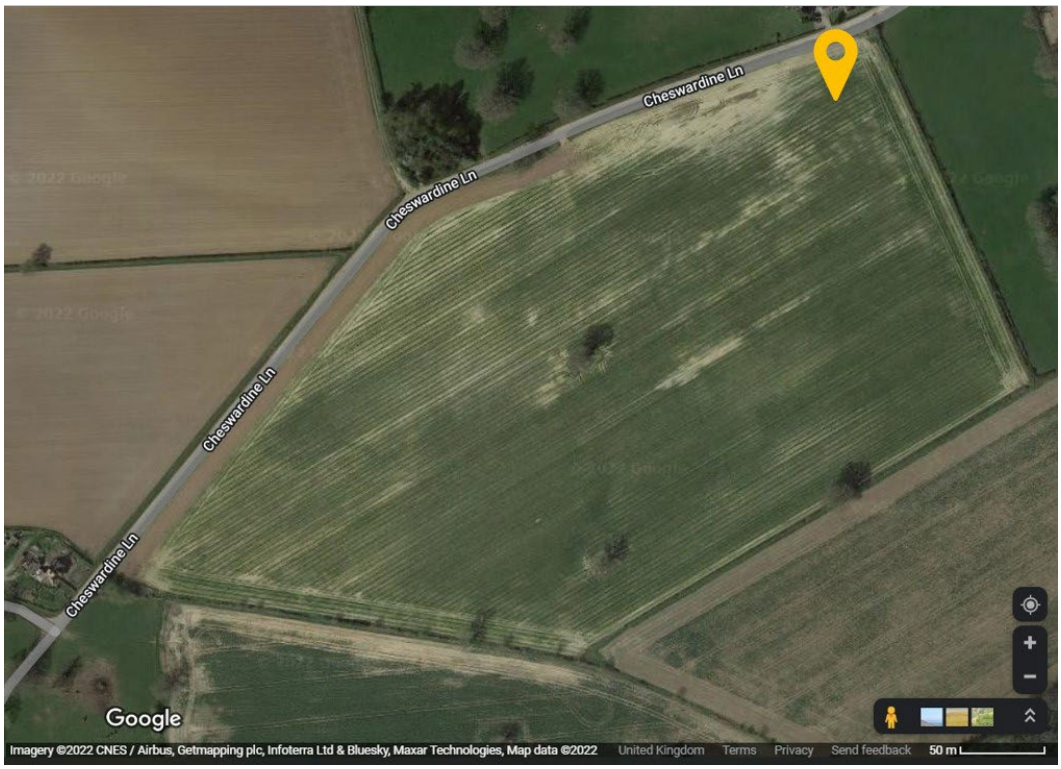


Figure 1. Oilseed rape field where the experiment was completed viewed on Google Maps (52.595863866039025, -2.41151437038343). The exact position of the experiment is indicated with a yellow pin.

The oilseed rape crop was drilled on 4th September 2021. The variety was Crossfit. The field was sub soiled prior to drilling with a Horsch Pronto min till drill and rolled straight after drilling. The seeds were treated with Integral Pro (bio-fungicide based on *Bacillus amyloquefaciens*) and Radiate (root-booster based on kinetin and auxin hormones). Until harvest in July 2022, the crop was treated with slug pellet Gusto®IRON (ferric phosphate); herbicides Clayton Satchmo (propaquizafop), Kerb®Flo 500 (propyzamide), Garryowen XL (glyphosate) to desiccate the crop before harvest; fungicides Toledo® (tebuconazole), Sparticus Xpro (bixafen, prothioconazole, tebuconazole); and fertilisers boron, potash, Sulphan 29 (sulphur), Brassitrel Pro (colemanite, manganese carbonate, pyridine-2-thiol 1-oxide), and OMEX Nitroflo (nitrogen). Total yield on the field reached 4.1t/ha (Adrian Joynt, personal communication).

The trial was separated into two experiments, one with treatments from T1 to T10, and the other with the entomopathogenic nematodes TA and TB (see Figure 2). The plots were delimited with yellow cross pegs at each corner (42 cm long, Norman Smith Equipment Ltd, Nottingham, UK) on 10th and 13th September. Each treatment was replicated six times, following a Latin rectangle design, with plots of 3x4 m^2 and 1m buffer zones in between each plot.

Table 1. Treatment number, product name, active ingredients, application rates, number of applications and manufacturer.

Treatment (T) number or letter & Product Name	Active ingredient	Rates tested	Applications	Manufacturer
T1: FLiPPER™	Fatty acids C7-C20	1.6L/100L, 300L/ha	3	Bayer (Leverkusen, Germany), AlphaBio Control (Cambridge, UK)
T2: CEU-40640-I-SL	Confidential	2L/100L, 300L/ha	3	Certis Belchim BV, Utrecht, The Netherlands
T3: Hallmark with Zeon technology	Lambda-cyhalothrin (pyrethroid)	0.05L/ha, 200L/ha	3	Syngenta, Basel, Switzerland
T4: Botanigard® WP	*Beauveria bassiana* strain GHA, 4.4 x 10^{10} spores/g	62.5g/100L, 300L/ha	3	Certis Belchim BV, Utrecht, The Netherlands
T5: Azatin®	217g/l azadirachtin	0.46L/100L, 300L/ha	3	Certis Belchim BV, Utrecht, The Netherlands
T6: Botanigard WP + Azatin	*Beauveria bassiana* strain GHA, 4.4 x 10^{10} spores/g + 217g/l azadirachtin	62.5g/100L + 0.46L/100L, 300L/ha	3	Certis Belchim BV, Utrecht, The Netherlands

T7: FLiPPER + Hallmark Zeon	Fatty acids C7-C20 + Lambda-cyhalothrin (pyrethroid)	1.6L/100L + 0.05L/ha, 200L/ha	3	Bayer, AlphaBio Control + Syngenta
T8: Water	N/A	300L/ha	3	N/A
T9: Hallmark with Zeon technology	Lambda-cyhalothrin (pyrethroid)	0.05L/ha, 200L/ha	1	Syngenta, Basel, Switzerland
T10: Untreated	N/A	N/A	N/A	N/A
TA: Nemasys	*Steinernema feltiae*	125,000, 250,000, 500,000 and 1,000,000 IJ/m^2, 1000L/ha	4	BASF Agricultural Solutions, Littlehampton, UK
TB: Nemasys H	*Heterorhabditis bacteriophora*	125,000, 250,000, 500,000 and 1,000,000 IJ/m^2, 1000L/ha	4	BASF Agricultural Solutions, Littlehampton, UK

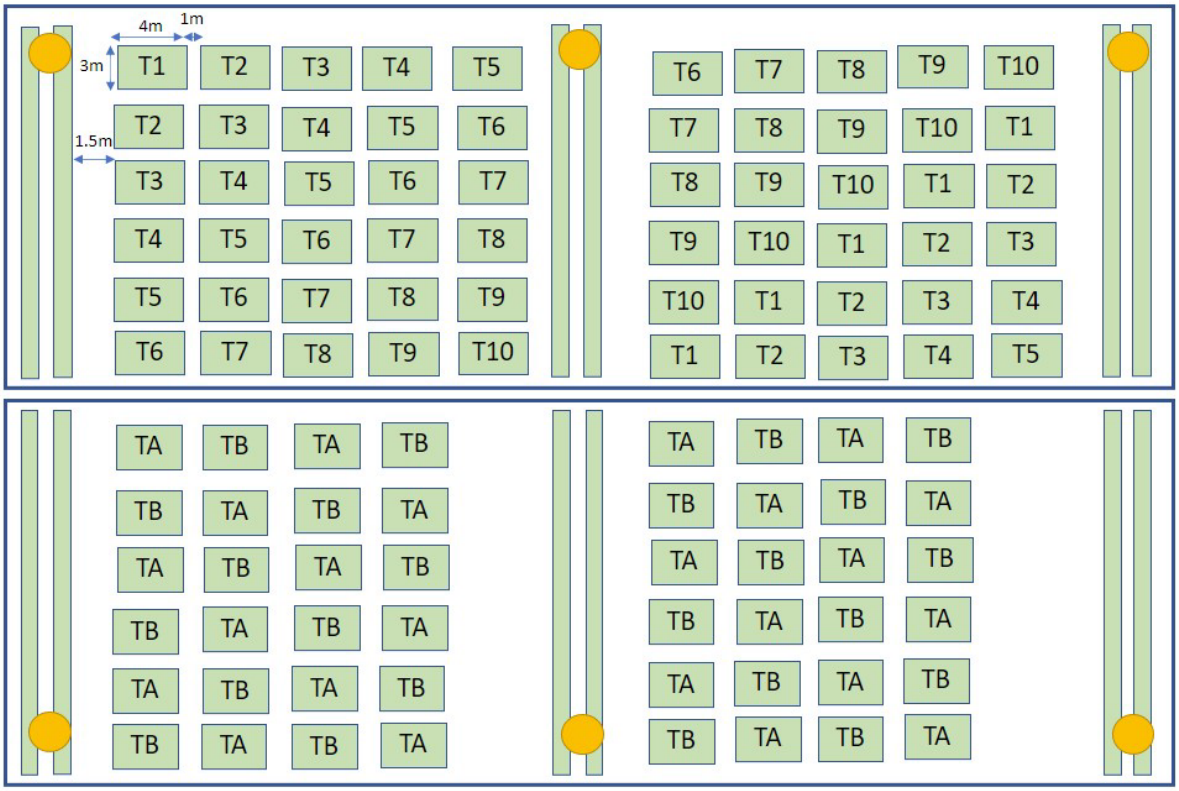


Figure 2. Experimental design of the two experiments. The yellow circles represent the yellow water traps used to monitor adult CSFB numbers (T1: FLiPPER; T2: CEU-40640-I-SL; T3: Hallmark with Zeon Technology applied three times; T4: Botanigard WP; T5: Azatin; T6: Botanigard WP + Azatin; T7: FLiPPER + Hallmark with Zeon Technology; T8: Water; T9: Hallmark with Zeon Technology applied once; T10: Untreated; TA: Nemasys; TB: Nemasys H).

Temperature (°C), windspeed (miles per hour, mph) and general weather conditions on the days of products application were recorded using current data from the website The Weather Channel (weather.com).

As required by the Administrative Trial Permits (202101498 and 202101499) obtained from Health and Safety Executive (HSE), the plants treated with entomopathogenic fungi, fatty acids, CEU-40640-I-SL and azadirachtin were destroyed by desiccation using the herbicide Rodeo (360 g/l glyphosate; Monsanto UK Ltd, Cambridge, UK) in March 2022, before plants had begun to flower.

2.2. Adult CSFB numbers recording

Six yellow water traps (26 cm diameter, 9.5 cm deep, Flora Insect Traps, Nickerson Brothers Ltd, Binbrook, UK) were set-up on 10th September on both edges and in the middle of the trial area in order to monitor adult CSFB numbers, adapted from Green (2008). Each water trap

was filled with water and approximately 1% detergent (Lipsol®, SciLabware Ltd) to break surface tension. Adult CSFB numbers were recorded before and during the experiment. Traps were first checked on the 22nd of September, and they were checked every two days until the 5th of November. Adult flea beetles were counted, then the traps were emptied and refilled each time.

2.3. Products application

Treatments T1-T9 were applied three times, applications were completed on 29th of September, 7th of October and 14th of October, except for the treatment with Hallmark Zeon applied once only (T9), and treatments TA and TB (nematodes) applied four times on the 30th of September, 7th of October, 13th of October and 21st of October. All products were applied using a hand-held knapsack boom sprayer (Lunchbox Sprayer, Trials Equipment UK Ltd, Braintree, UK) with four flat fan nozzles operating at a pressure of 2 bars. The knapsack sprayer was calibrated by calculating the flow rate using the following formula, which was then used to choose the correct nozzles:

Flow rate (L/min) = Volume (L/ha) * walking speed (km/h) * spray width (m) / 600

The total spray width was 2m and the walking speed was 6 km/h. As the label recommendation for lambda-cyhalothrin is that the product should be applied using a water volume of 200L/ha, treatments containing this product were applied using F110-015 nozzles with a combined flow rate of 2L/min. Nematodes are recommended to be applied at a water volume of 1000L/ha so the nematodes treatments were applied with F110-15 nozzles for a total flow rate of 20L/min. The other products were applied with a water volume of 300L/ha, so the relevant treatments were applied with F110-04 nozzles for a combined flow rate of 6L/min (Hypro Flat Fan VP 110° Nozzles, Hypro, Pentair, Minneapolis, USA). Water pH was 7 and water hardness was 425 ppm.

2.4. Leaf damage and larval density assessments

Leaf damage was evaluated by taking pictures of the plants in the field (no collection) and then completing visual assessments of leaf damage by making comparisons with the EPPO guide to percentage leaf area eaten by CSFB (Figure 3). Leaf damage pre-treatment was assessed on 22nd, 24th and 29th September, with 25 plants selected haphazardly across the field each time. Leaf damage after treatment application had begun was assessed on 30th September, 6th October, 12th October, and 19th October, with five plants per plot selected

haphazardly (300 plants in the T1-T10 experiment and 240 in the TA & TB experiment for each assessment session).

Five plants per plot (300 plants in the T1-T10 experiment and 240 in the TA & TB experiment) were collected haphazardly in the field on the 3rd of November and kept in a cold room at 3°C on the University campus. Plants were dissected by hand in the laboratory and the number of larvae per plant recorded. Larvae were discarded and plants destroyed following the permits directions, which specified that 'harvested portions of treated crops must be destroyed by either burning, burying or disposal in a facility licensed for this purpose.'

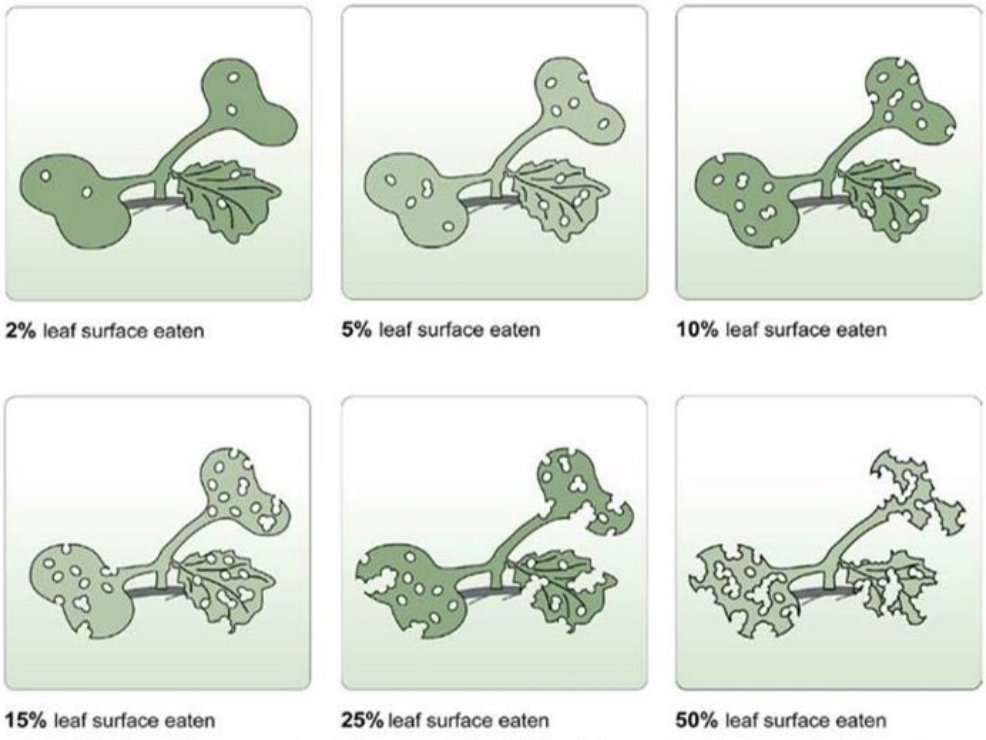


Figure 3. Guide to evaluate the percentage of oilseed rape leaf surface eaten by adult cabbage stem flea beetle (CSFB) (EPPO, 2020).

2.5. Statistical analysis

Datasets were analysed using R (version 4.2.2) and RStudio (version 2022.12.0). Data analysis methods were chosen based on the distribution of the data. Significance groups were computed using the *cld(lsmeans())* function included in the packages *multcomp* (Hothorn, Bretz and Westfall, 2015) and *lsmeans* (Lenth, 2016).

Statistical differences are illustrated in figures with letters (a, b, c…). Graphical illustrations were made with the *boxplot* function from the package *graphics* (Murrell, 2009) after the data was tidied with the *mutate* function from the package *tidyverse* (Wickham, Averick, *et al.*, 2019), except for Figure 4 which was made using Microsoft Excel 2019 (version 2208).

Leaf damage:

The pre-treatment leaf damage assessment was analysed using Kruskal-Wallis rank sum tests followed by a Wilcoxon test for post hoc pairwise comparisons with Bonferroni adjustment. The leaf damage recorded for plants treated with T1-T10 was analysed using a one-way ANOVA on a linear model of the data. The leaf damage for plants treated with TA and TB (nematodes) was analysed using a one-way ANOVA on a linear model of the data after a log transformation was performed to normalise the distribution of the data.

Larval numbers:

The numbers of larvae in nematodes-treated plots (TA and TB) were analysed using a one-way ANOVA on a linear model of the data after a log transformation was performed to normalise the distribution of the data. The numbers of larvae recorded in plants treated with T1-T10 were analysed a one-way ANOVA on a linear model of the data.

3. Results

Over all the treatment application dates, the mean temperature was 14.5°C, the mean windspeed was 10.5 mph, and the weather conditions were mostly cloudy or cloudy for five out of the six treatment application sessions, and mostly sunny for one session.

Table 2. Date, mean temperature, wind speed and weather recorded on spray days.

Date	Mean temperature (°C)	Wind speed (mph)	Weather
29/09/2021	14	9	Partly cloudy
30/09/2021	12	17	Cloudy
07/10/2021	18.5	8.5	Cloudy
13/10/2021	*16*	6	Mostly cloudy
14/10/2021	15.5	10.5	Cloudy
21/10/2021	11	11	Mostly cloudy

3.1. Adult CSFB numbers recording

Mean numbers of adult CSFB caught in yellow water traps between 22nd September and 5th November is shown in Figure 4. Numbers did not exceed a mean of 14 beetles per trap on a single assessment date, which is lower than the advised threshold for treatment of 100 beetles per trap checked weekly (AHDB, no date).

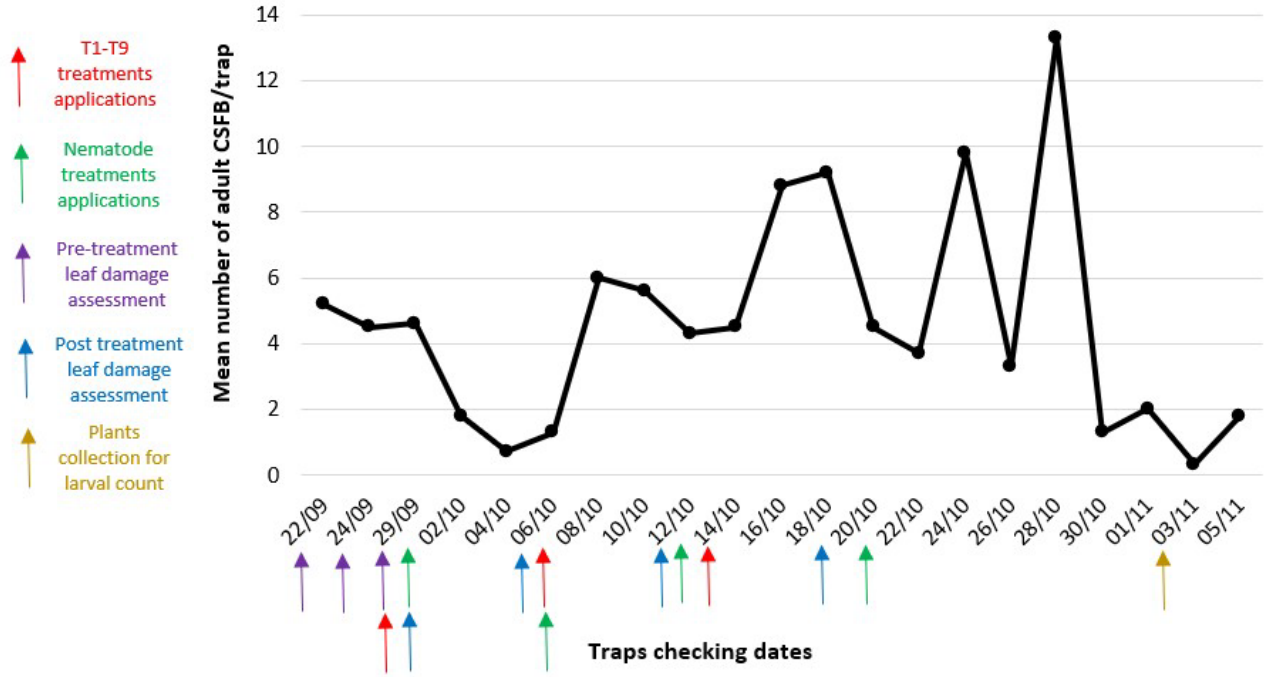


Figure 4. Mean number of adult cabbage stem flea beetle (CSFB) per trap between 22nd September and 5th November.

3.2. Leaf damage assessment

Leaf damage pre-treatment increased significantly between the first and the third assessment (Kruskal-Wallis chi-squared = 14.043, df = 2, p = 8.92 x 10^{-4}). The mean damage increased from 8.16% on 22nd September to 15.6% on 29th September (Figure 5). Due to the way pre-treatment assessments were done, it wasn't possible to make comparisons between treatments.

In plots treated with T1-T10, there was significantly less leaf damage on the last assessment date with a mean damage of 12% (19th October) compared to the three earlier assessment dates (30th September, 6th October and 12th October) (F = 15.663, df = 3, p = 3.45 x 10^{-9}). Leaf damage was not significantly different across treatments on 30th September (F = 0.683, df = 9, p = 0.72) and on 6th October (F = 0.672, df = 9, p = 0.73). On 12th October, plots treated with lambda cyhalothrin applied four times (T3) and applied once (T9) had lower leaf damage compared to other treatments. Plots treated with azadirachtin (T5 had the highest lead damage (F = 2.802, df = 9, p = 0.00962) (Figure 6). On 19th October, plots treated with fatty acids + lambda cyhalothrin (T7) or lambda-cyhalothrin applied once (T9) had lower leaf damage compared to other treatments. Plots treated with the entomopathogenic fungus *Beauveria bassiana* strain GHA (T4) had the highest leaf damage (F = 3.566, df = 9, p = 0.002) though it was not significantly different from other treatments apart from T7 and T9 (Figure 7).

In plots treated with TA and TB, the leaf damage between dates was significantly lower on the last assessment date (19th September) compared to earlier assessment dates (F = 48.522,

df = 2, p = 3.61 x 10^{-16}). There were however no significant differences between the different rates (F = 0.514. df = 3, p = 0.6731) and nematodes species applied (F = 0.571, df = 1, p = 0.451).

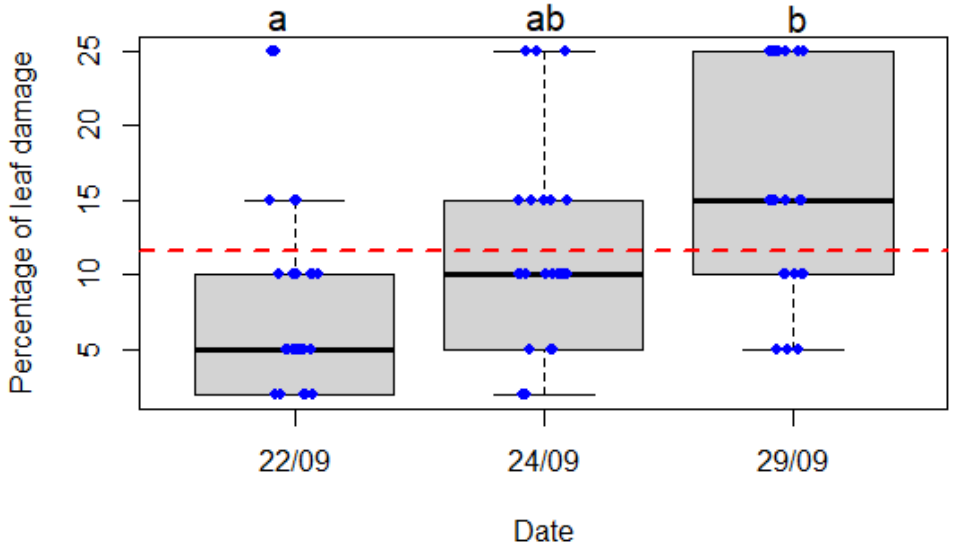


Figure 5. Percentage of leaf damage pre-treatment. The red line represents the overall mean percentage of leaf damage. The blue dots help visualise various data points. Different letters indicate significant differences (p-value < 0.05).

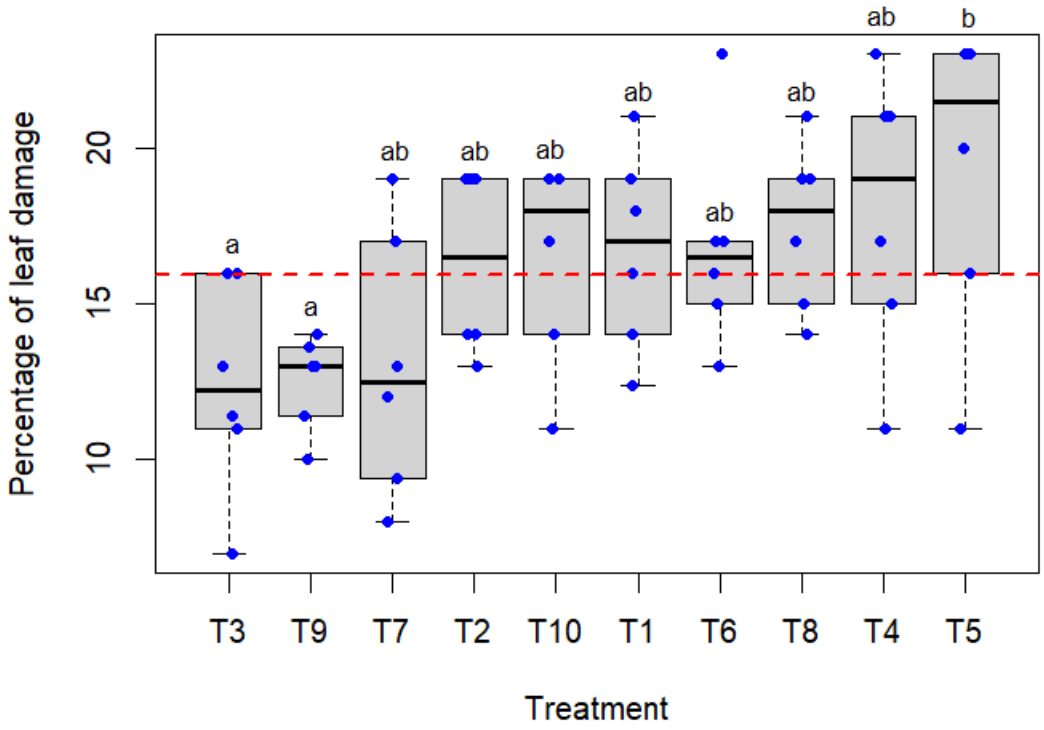


Figure 6. Percentage of leaf damage on the 12th of October. The red line represents the overall mean percentage of leaf damage. The blue dots help visualise various data points. Different letters indicate significant differences (p-value < 0.05).

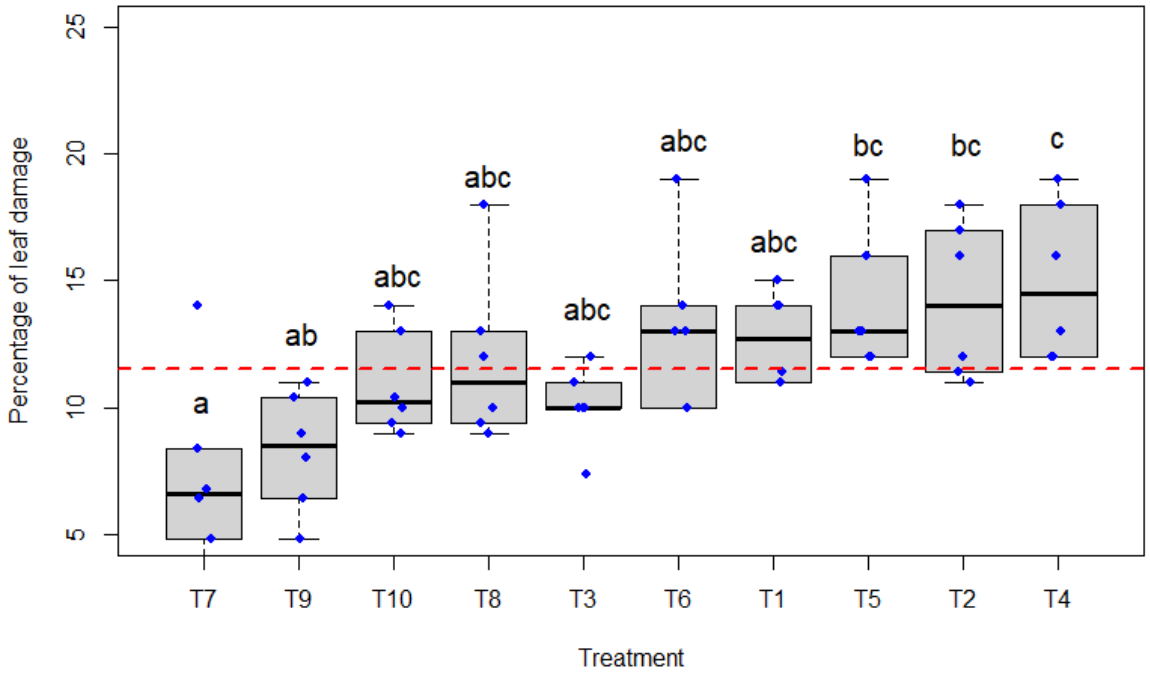


Figure 7. Percentage of leaf damage on the 19th of October (last of four assessment dates). The red line represents the overall mean percentage of leaf damage. The blue dots help visualise various data points. Different letters indicate significant differences (p-value < 0.05).

3.3. Larval density assessment

Larval density was not significantly different between nematode treatments (F = 0.270, df = 1, p =0.606) or between doses (F = 1.961, df = 3, p = 0.135) (Figure 8) with a mean number of 7 larvae/plant, a maximum number of 15.4 larvae per plant and a minimum number of 2.4 larvae/plant.

It was also not significantly different between T1-T10 treatments (F = 1.248, df = 9, p = 0.288, Figure 9) with a mean number of 7.1 larvae/plant, a maximum number of 15.6 larvae per plant and a minimum number of 1.8 larvae/plant.

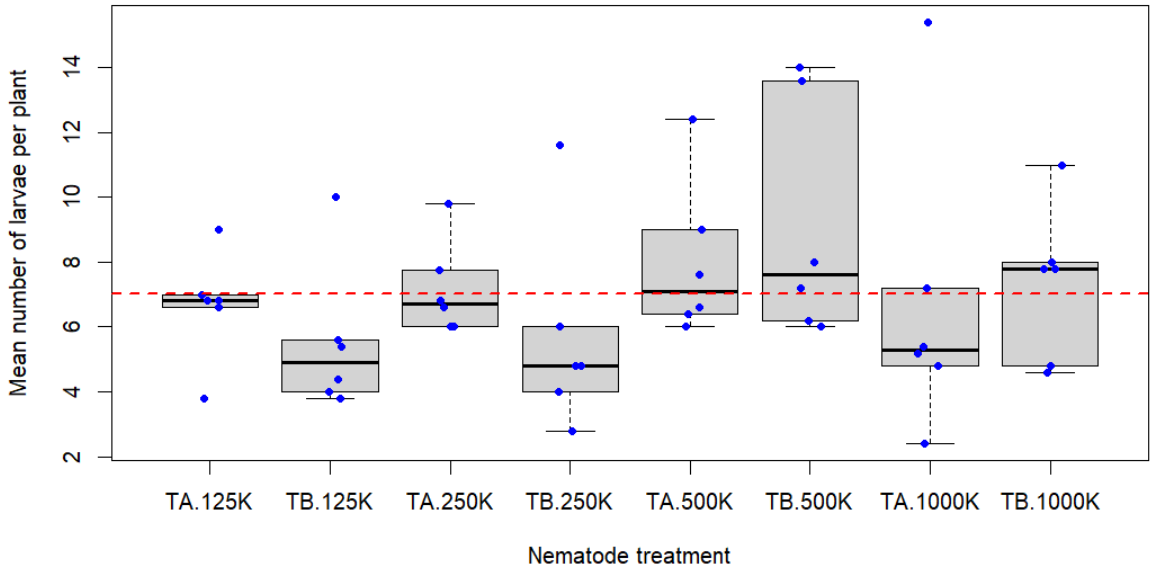


Figure 8. Mean number of larvae per plant treated with entomopathogenic nematodes (TA = *Steinernema feltiae*, Nemasys; TB = *Heterorhabditis bacteriophora*, Nemasys H; K = thousands of nematodes/m^2). The red line represents the overall mean percentage of leaf damage. The blue dots help visualise various data points.

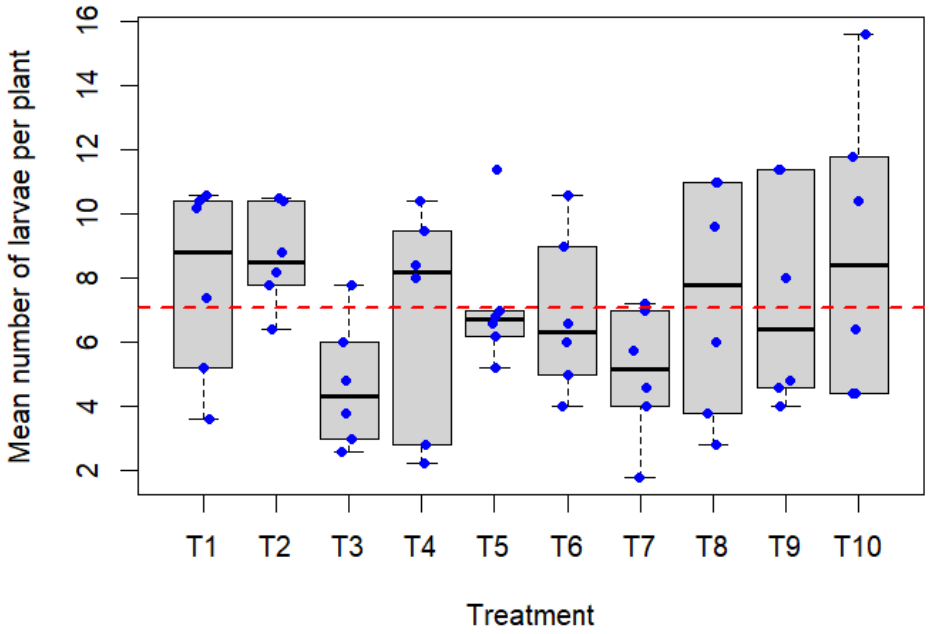


Figure 9. Mean number of larvae per plant. The red line represents the overall mean percentage of leaf damage. The blue dots help visualise various data points.

4. Discussion

4.1. Adult CSFB numbers recording

As mentioned in the Results section, the number of adult CSFB recorded in the traps were much lower than the advised threshold for treatment. Anecdotally, the numbers of adult beetles

that emerged in the summer of 2022 and collected at harvest in July was lower than the previous year. This decrease of flea beetle numbers found in oilseed rape grain stores at harvest is probably not limited to this field, as a trend of decreased CSFB damage was observed across the country (AHDB, 2022).

4.2. Leaf damage assessment

My results suggest that conventional pyrethroids are more effective at controlling adult CSFB damage compared to the biopesticides tested here, which were in most cases not more effective than water or no treatment in reducing damage. The azadirachtin products Azatin and the entomopathogenic fungus *B. bassiana* strain GHA were the least effective products found in this study. Other formulations of *B. bassiana* strain GHA than Botanigard WP used in this study have been tested in field studies in the US, such as Botanigard ES or Botanigard 22WP, tested by Antwi, Olson and Knodel (2007) and Reddy *et al.* (2014) respectively. Treatment with Botanigard ES led to high levels of leaf damage being recorded and the authors concluded that this product was not effective. Results presented in this study appear to be similar. Botanigard 22WP led to reduced leaf damage when applied repeatedly and combined with another fungal biopesticide, Met52 (*M. anisopliae* strain F52), which suggests that Botanigard WP could be effective if combined with another fungal product (Reddy *et al.*, 2014b). The fact that entomopathogens such as fungi and nematodes are susceptible to environmental factors such as UV radiations, temperature and humidity (Ignoffo and Garcia, 1992; Jaronski, 2010) may have contributed to the results presented here, but the low pest pressure in the studied field makes it difficult to draw firm conclusions. Azadirachtin was not effective at reducing leaf damage by adult CSFB when applied on its own or in combination with Botanigard WP, potentially for reasons mentioned in the previous paragraph. Other combinations using azadirachtin could be tested, such as fatty acids and azadirachtin, which Reddy *et al.* (2014) tested and recorded decreased canola leaf damage by the adult crucifer flea beetle in the US, or entomopathogenic nematodes and azadirachtin, which lead to decreased emergence of adult striped flea beetles in a Chinese field study (Yan *et al.*, 2013a).

Fatty acids by themselves were not effective, being statistically similar to the control, but when combined with the pyrethroid they led to significantly lower leaf damage compared to pyrethroid when used alone. The application of fatty acids and pyrethroid resulted in the lowest leaf damage of all treatments tested and was significantly better than T5 (azadirachtin), T2 (CEU-40640-I-SL) and T4 (*B. bassiana* strain GHA). This field experiment is the first, to my knowledge, to investigate the efficacy of fatty acids against adult flea beetles. As such, there are no other studies with which to compare results to, however, FLiPPER has been widely reported to be effective against soft-bodied insects such as some species of whiteflies, aphids

and mealybugs (e.g. Convertini *et al.*, 2018; Suma *et al.*, 2019). In future studies, it would be interesting to investigate the efficacy of fatty acids against CSFB when used in combination with conventional insecticides, such as lambda-cyhalothrin. The combination of difference modes of action may help overcome insect pest resistance to pyrethroid insecticides. However, as pyrethroids are known to be detrimental to non-target organisms such as parasitoid wasps that are natural enemies of CSFB (I. H. Williams, 2010), it is debatable whether it would be a good solution to try to prolong the life of old insecticides instead of focusing on developing new and safe solutions.

Entomopathogenic nematodes in my study did not lead to significantly lower leaf damage regardless of species or rates applied, which contradicts what other studies have found. Indeed, there are several field studies in the literature investigating the potential of entomopathogenic nematodes to reduce flea beetle feeding damage, albeit none on CSFB specifically. *Steinernema feltiae* in particular was found to be effective at reducing adult *Phyllotreta* spp. flea beetle emergence (Hokkanen *et al.*, 2006; Hokkanen, 2008) and leaf damage when combined with a polymer gel as UV protectant (Antwi and Reddy, 2016; Briar *et al.*, 2018). A relative species, *S. carpocapsae*, was found to be effective at reducing the number of *Phyllotreta* spp. flea beetle larvae in the soil, decreasing leaf damage and increasing yields (Yan *et al.*, 2013a; Reddy *et al.*, 2014b), though one study found that true only when a polymer gel was applied alongside the nematode (Antwi and Reddy, 2016), and at reducing damage on radish roots by larvae, increasing their weight and diameter (Noosidum, Mangtab and Lewis, 2021). No field study has previously investigated the potential of *H. bacteriophora* for control of feeding damage by flea beetles, but closely related species of nematodes have been studied. *Heterorhabditis indica* was found to be effective at reducing the number of *Phyllotreta* spp. flea beetle larvae in the soil, decreasing leaf damage and increasing yields by one study (Yan *et al.*, 2013a), however, in a second study the same species of nematode was found to be ineffective at reducing adult numbers (Yan *et al.*, 2018). The same species of nematode has also been reported to reduce damage on radish roots by flea beetle larvae and increased their weight (Noosidum, Mangtab and Lewis, 2021).

I identified several limitations in the methods used in this study. For example, as seen in Figure 3, the guide I used to assess leaf damage was appropriate for plants at cotyledon stage. However, as weeks went by, the plants were significantly bigger (3-4 leaf stage on the last assessment date), so I suspect the assessment method will need to be adapted to the growth of the plants or complete a more intensive sampling at the early growth stages of cotyledon and 1-2 true leaves. Spraying the plants in the evening could potentially help entomopathogenic nematodes and fungi survive longer on the plants, as entomopathogens are known to be sensitive to UV radiation and desiccation (Ignoffo and Garcia, 1992; Jaronski, 2010). Improving the formulation by using adjuvants could also possibly increase the spread

of the products over the leaves, decrease their drying time as these products need to be wet when encountering CSFB, and/or act as a protectant against UV radiations and low humidity during the day. Another limitation was the quality of the water used to prepare the biopesticides, as a water hardness of around 425 ppm is considered close to very hard. However, some biopesticide products such as the fatty acid FLiPPER are not recommended to be used with water which hardness exceed 300 ppm as it can lead to the occurrence of flocculation which could reduce efficacy (Bayer, 2021). Repeating this work using softer water could potentially lead to different results. Pest pressure was too low to draw firm conclusions but there is some evidence that fatty acids used with lambda-cyhalothrin and lambda-cyhalothrin used on its own may have had some positive effects. More work is required to explore this further.

4.3. Larval density assessment

There were no significant differences between larval numbers recorded in plots treated with any of the biopesticides tested, water control, the conventional synthetic insecticide, and left untreated. A recent study, (Godina *et al.*, 2023) also investigated the use of entomopathogenic nematodes to control CSFB larvae in potted plants and in the field and recorded encouraging results. They used commercial formulations of *Steinernema feltiae* (Nemaplus®), *Heterorhabditis bacteriophora* (Nematop®) and *H. downesi* (Nemamax®), as well as the strain SDT1-IL1 of *H. bacteriophora*. On potted plants, they sprayed each treatment at a concentration of 150,000 nematodes/plant and after five days incubation at 15°C they counted significantly less alive larvae in the plants treated by nematodes compared to the water treatment, with *S. feltiae* being the most virulent species. The incubation temperature used in this study was similar to the average temperature I recorded in the field for my own study (14.5°C) so the difference might lie in the application method, as my plants might have received less nematodes than the plants in (Godina *et al.*, 2023). Repeating the potted plants experiment in the UK could lead to more encouraging results. In the field, the authors completed four different experiments with nematodes applied at concentration of 500,000 nematodes/m^2 (similar to the third highest concentration in my study). In the first experiment they tested *H. bacteriophora* and *S. feltiae* applied together, *H. bacteriophora* and *S. feltiae* applied together at half the concentration, *H. bacteriophora*, *S. feltiae* and SDT1-IL1 compared to a water control. In October, they did not find significant differences between nematode treatments and the control. In November, they found significantly less larvae in the treatment with both *H. bacteriophora* and *S. feltiae* applied together compared to *S. feltiae* applied alone. In the second and third experiments they tested *H. bacteriophora* and *S. feltiae* applied together, *S. feltiae* and the pyrethroid insecticide Karate (lambda-cyhalothrin) compared to a

water control and found in December that Karate was significantly more effective than the control and the nematode treatments at reducing larval number in plants. In the fourth experiment, they tested *H. bacteriophora* and *S. feltiae* applied together, the conventional seed treatment Lumiposa and *H. bacteriophora* and *S. feltiae* applied together with Lumiposa, compared to a water control. In December they found significantly lower larvae in plants treated with *H. bacteriophora* and *S. feltiae* applied together compared to the Lumiposa treatments and the water control. The authors concluded that the performance of entomopathogenic nematodes in the field was variable and lower than under laboratory conditions.

The time taken to dissect plants could have been reduced by having several people complete the task or to use a different method other than dissection. An example of a different method is the Funnel method described by Conrad *et al.* (2016), in which the authors left the plants to dry in funnels with a collecting vessel placed under them, collecting the larvae that escape the drying plant tissue. Using this method would mean decreasing the amount of work necessary to complete the task and processing all the plants at the same time, but several downsides include the low speed of this method (in Conrad *et* al. (2016) the authors kept the plants in these conditions for 21 days), the space required to store all the plants in individual pots (540 plants in total were collected in my study) and the uncertainty about whether all larvae exited the plants before the plants were disposed of and larvae in collecting vessels counted.

5. Conclusion

This study did not show that the biopesticides tested were more or as effective as the conventional synthetic insecticide, lambda-cyhalothrin, in reducing damage due to CSFB in a commercial oilseed rape crop. However, as adult CSFB density recorded with yellow water traps was much lower than spray threshold, testing these products where pest pressure was higher may have yielded different results. My results were also counterbalanced by other limitations, which may have reduced the quality of results reported, such as spray timing, formulations used and assessment methods. The choice of using the entomopathogenic fungus product Botanigard WP (*Beauveria bassiana* strain GHA) would also need to be re-evaluated, as applying the product in the evening to avoid UV could mean exposing the fungus to temperatures that are likely to be below 15°C, which would be too cold for it to be effective (Etienne Hinh, Certis Belchim BV, personal communication). Some of the studies cited in this chapter also show that there are other variables that can be studied besides adult leaf damage and larval, such as adult emergence the following year.

Biopesticides have the reputation of not being effective in outdoor crops compared to synthetic insecticides and compared to laboratory results, as sub-optimal environmental

conditions in the field reduce many biopesticides efficacy (Ignoffo and Garcia, 1992; Jaronski, 2010). Therefore, before biopesticides can be included in IPM programmes for CSFB, more work is required to ensure their efficacy and their economic viability for oilseed rape growers. Currently most of these products are commercialised for horticultural crops and used against soft-bodied pest insects. Furthermore, these products are more expensive than conventional insecticide: based on current prices, and as such it would, for example, cost an oilseed rape grower 20 times more to apply a fatty acid product compared to a pyrethroid insecticide. It is mostly because biopesticides do not have the luxury to work at very low doses, unlike synthetic insecticides: for example, FLiPPER (fatty acids) is recommended to be sprayed at a rate of 4.8L/ha while Hallmark with Zeon technology (pyrethroid), is recommended to be sprayed at a rate of 0.05L/ha. More work is then needed to make these products more affordable and ensure their efficacy in the field.

Chapter 6: Sentiment analysis of the farming press about cabbage stem flea beetle management before and after the neonicotinoid seed treatments ban

Abstract

The cabbage stem flea beetle used to be controlled in the UK and the rest of Europe through the use of neonicotinoid seed treatments, which were placed under a moratorium for use in oilseed rape crops in 2013 by the European Commission. This was due to scientific evidence indicating a potential threat to bees. In this study a sentiment analysis of a sample of the UK farming press between 2010 and 2022 was completed in order to record changes over time in the opinion of agricultural professionals to the ban of neonicotinoid insecticides and its impact on cabbage stem flea beetle control. Results show that there was an increased interest in topics related to oilseed rape and cabbage stem flea beetle control after the initial moratorium of neonicotinoids in 2013, with an increasing number of articles published on this topic between 2013 and 2019, when the permanent ban of neonicotinoids came into effect. Neonicotinoids were frequently mentioned when the ban was first introduced, as well as pyrethroid insecticides, as farmers were concerned with the future of cabbage stem flea beetle control without neonicotinoid seed treatments. An important impact of the neonicotinoid ban has been that many farmers have increased their reliance on pyrethroid foliar sprays. The sentiment analysis results with the automated analysis completed using R (version 4.3.1) showed positive sentiment increasing in the articles over the whole study period. However, the manual analysis completed by reading 10% of the articles did not show a clear trend over time. Words associated with negative emotions such as anger, disgust and fear increased at the time of the neonicotinoid ban. However, future work is necessary to improve the methods and confirm this study's findings.

1. Introduction

The cabbage stem flea beetle (*Psylliodes chrysocephala*, Linnaeus) is an important pest of oilseed rape in the UK and the rest of Europe. It was successfully controlled through the use of neonicotinoid seed treatments before the three active ingredients used for this purpose (clothianidin, thiamethoxam and imidacloprid) were placed under a two-year moratorium starting December 2013, which prohibited the use of these products on bee-attractive crops, which include oilseed rape (European Commission, 2013a).

The moratorium on the use of neonicotinoid seed treatments followed the publication of new scientific evidence in spring 2012 that these insecticides may be harmful to non-target organisms such as bees. The European Commission asked the European Food Safety Authority (EFSA) to review this risk, and they published a report in January 2013 that identified 'high acute risks for bees' caused by use of insecticide products based on these three active ingredients (European Commission, 2013a). In particular, it was advised that 'the uses as seed treatment and soil treatment of plant protection products containing clothianidin, thiamethoxam or imidacloprid should be prohibited for crops attractive to bees and for cereals except for uses in greenhouses and for winter cereals. In addition, foliar treatments with plant protection products containing clothianidin, thiamethoxam or imidacloprid should be prohibited for crops attractive to bees and for cereals with the exception of uses in greenhouses and uses after flowering.' (European Commission, 2013a).

Following additional analysis of the scientific evidence, the European Commission recommended a complete ban of these three neonicotinoid active ingredients for all outdoor use, which was voted for by European Member States in May 2018 to be effective from December 2018 in the UK (Blake, 2018).

In this study, I conducted a sentiment analysis of magazine articles published in the UK farming press to analyse the opinion of agricultural professionals, and in particular oilseed rape growers, regarding the ban of neonicotinoid insecticides and its impact on oilseed rape crop production and cabbage stem flea beetle control.

Sentiment analysis can be defined as 'the field of study that analyses people's opinions, sentiments, evaluations, appraisals, attitudes, and emotions towards entities such as products, services, organizations, individuals, issues, events, topics, and their attributes' (Liu, 2012). It is used interchangeably with the terms 'opinion mining' and is usually used by businesses and organisations seeking to know more about consumers' opinions about the products or services they offer (Liu, 2012). It has also been used by researchers to analyse the opinion of members of the public or specific cohorts of people, such as agricultural professional. For example, Hooda and Hooda (2018) analysed the opinion of members of the public in India through Twitter® (now X®) posts mentioning agriculture, and Bermeo-Almeida *et al*. (2019) analysed Twitter® posts and Facebook® comments to evaluate people's opinion on insect pest control in crops. Rust *et al.* (2021) combined interviews of agricultural professionals and the analysis of the UK farming press to understand how sustainable farming practices are presented and whether their prominence changed over time.

In this study, I have reviewed the UK farming press, to analyse the opinion of agricultural professionals on the topic of cabbage stem flea beetle control in oilseed rape crops, particularly in the context of neonicotinoid insecticides being banned for use in oilseed rape crops, and the introduction of Integrated Pest Management (IPM) practices. I selected Farmers Weekly and

Farmers Guardian, the two most frequently read weekly farming magazines in the UK, with a weekly circulation of 35,484 for Farmers Weekly (ABC, no date b) and 24,818 for Farmers Guardian (ABC, no date a) from January to December 2022.

2. Material and Methods

2.1. Article harvesting

Article harvesting was completed following the method presented in Rust *et al*. (2021). The Nexis Uni™ online database was searched for articles published in Farmers Weekly and Farmers Guardian using the following Boolean queries:

'Oilseed rape' AND 'flea beetle' AND 'control' OR 'pressure' OR 'damage' OR 'pest' OR 'insecticide' OR 'losses' OR 'lost' OR 'attacks' OR 'neonicotinoid'.

This allowed the sourcing of all relevant articles available online on the database for these two publications from 1st January 2010 (approximately two years before the initial moratorium on the use of neonicotinoids) to 31st of December 2022. A total of 594 articles were selected and downloaded.

2.2. Article numbers and word usage

In R (version 4.3.1) and RStudio (version 2023.06.2+561), the *LexisNexisTools* package (Gruber, 2023) was used to upload the LexisNexis articles in a .doc format onto RStudio. Using the function *lnt_similarity* from the same package, duplicate articles were identified. Next, I removed duplicates where this was confirmed through manual checking. A total of 23 articles were removed in this way.

To evaluate the interest of the selected topic in the UK farming press over the years, the number of articles published per year was plotted for each year and sorted into four different categories:

- Before the initial moratorium (2010-2011), subsequently referred in this chapter as 'before the ban';
- When the European Commission asked the EFSA to review the risk of neonicotinoids on bees (2012), subsequently referred in this chapter as 'threat of ban';
- When the two-year moratorium was effective (2013-2018), subsequently referred in this chapter as 'initial moratorium period';
- When the permanent neonicotinoid was effective (2018-2022), subsequently referred in this chapter as 'permanent ban in effect'.

The following single words were filtered out based on Pickering *et al.* (2020) recommended IPM strategy and Ortega-Ramos *et al*. (2021):

"variety", "varieties", "varietal", "insecticide", "insecticides", "biopesticide", "biopesticides", "defoliation", "pyrethroid", "rotation", "pyrethroid", "pyrethroids", "neonics", "neonicotinoids", "neonicotinoid", “cultivation”, “cultivations”, “drilling”, “drill”, “sowing”, “companion”, “parasitoids”.

Words of the same root (e.g., "variety", "varieties" and "varietal") or with similar meaning (e.g., “drilling” and “sowing”) were pooled under the same name (e.g., “variety”). The usage of each word was then plotted for each year (from 2010 to 2022) in relation to the number of articles that was published each year and sorted out into the same four periods as above.

Word usage over the years was also manually evaluated by reading a proportion of the articles (10%) and recording the mention of the words listed above in each article.

2.3. Sentiment analysis

For the sentiment analysis, the first step was to remove words using a custom list, with the *anti_join* function from the *dplyr* package (Wickham, François, *et al.*, 2019), which are common words used that do not reflect the writer’s opinion and need to be removed from the analysis. The following words were removed as R wrongly associated them with specific sentiments, or in the case of "â", were corrupted symbols from the original texts:

"says", "will", "also", "can", "rape", "bee", "money", "treat", "rating", "pound", "cash", "stone", "tariff", "words", "powerful", "belt", "fleece", "honest", "nurture", "remains", "confidence", "bug", "â", "crop", "crops", "oilseed", "present", "late", "august", "immediately", "management", "cool", "tree", "usual", "gross", "forward", "backward", "manure", "major", "wild", "legal", "breakfast".

Sentiment words were selected and associated to specific sentiments based on the NRC lexicon using the *get_sentiments* function from the *tidytext* package (Silge and Robinson, 2016).

From this table of association, words linked to more complex emotions (anger, joy, fear, disgust, anticipation, sadness, surprise, and trust) were plotted as percentage of emotion words compared to the total number of words used.

To get a better understanding of the overall sentiment of the articles over the years instead of the sentiment carried by single words, single sentences were analysed using the *sentiment*

function from the *100entiment* package (Rinker, 2017), then a mean average score per year was calculated and plotted. A score ≥ 0 indicated a positive sentiment, while a score ≤ 0 indicated a negative sentiment. The year 2010 only had one article associated to it, so it was considered an outlier and was removed before plotting.

Line graphs and histograms were made using the *ggplot2* package (Wickham, 2009). Overall, sentiment of the articles were manually evaluated by reading and scoring the sentiment of 10% of articles, randomly chosen but weighed in based on number of articles published each year.

3. Results and Discussion

3.1. Article numbers and word usage

Figure 1 shows that the number of articles published in Farmers Weekly and Farmers Guardian containing the selected Boolean queries increased when the initial moratorium on the use of neonicotinoid treatments was announced in 2013. The number of articles then decreased between 2015 and 2018 and increased again in 2018 when the permanent ban was passed, showing that the interest of the press in this subject seemed to follow the various steps in the neonicotinoids ban process. In 2021 and 2022 the number of articles published had returned to similar numbers to those seen before the ban. However, it is unclear if this is due to reduced interest in cabbage stem flea beetle control in oilseed rape or because of the decrease in oilseed rape area grown in the country (Defra, 2023) meaning that there was less interest in this crop.

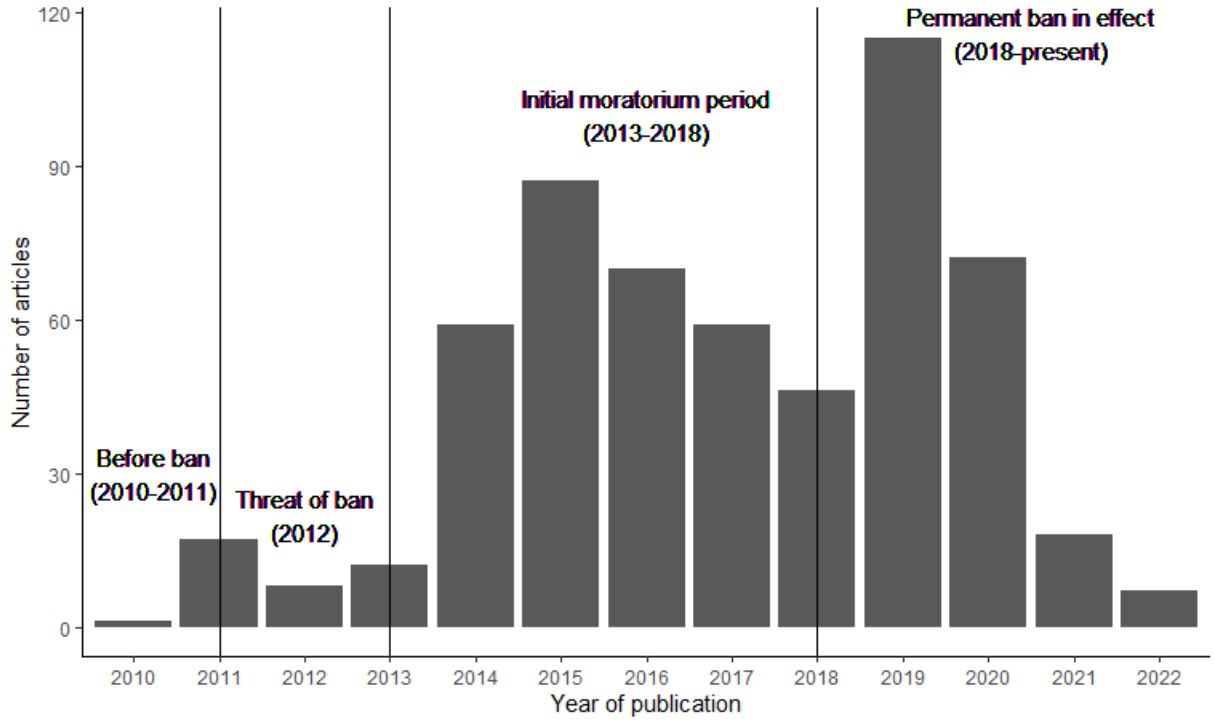


Figure 1. Number of articles used in this study published over the years, between the 1st of April 2010 and the 31st of December 2022.

Figure 2 follows a similar general pattern but shows the usage of words associated with conventional control and IPM related practices over this period of time. The words neonicotinoids, drilling/sowing and variety were the words that were used most frequently compared to all the other words.

Of these words, the use of the word neonicotinoids increased sharply at the time when the use of these insecticides was being reviewed by the European Commission and the EFSA in 2012. The use of the word variety followed the same pattern as the word neonicotinoids but increased a year later from 2013, with peaks of usage in 2016 and 2020, potentially illustrating how oilseed rape growers adapted to the loss of neonicotinoid by using oilseed rape varieties more tolerant of cabbage stem flea beetle damage (Farmers Guardian, 2020). It was also shown in a recent study that changing the use of varieties to improve crop production was a topic discussed positively by farmers in the UK (Rust *et al.*, 2021).

The word insecticide can be seen decreasing over time, matching the decrease in the use of insecticides in oilseed rape crops. Neonicotinoid insecticides were not used from 2013 and in addition the use of pyrethroids decreased (from around 1,73 million ha treated with a pyrethroid insecticide in 2012 (Garthwaite *et al.*, 2013) to around 1,28 million ha treated with a pyrethroid insecticide in 2016 (Garthwaite *et al.*, 2016) to 790,000 ha area treated with a pyrethroid insecticide in 2020 (Garthwaite *et al.*, 2021)). Scott and Bilsborrow (2019)

suggested that this decrease in pyrethroid use may reflect a decrease in oilseed rape crop area grown in the UK, which went from approximately 712,000 ha in 2012 to approximately 342,000 ha in 2023 (Defra, 2023). The word pyrethroid shows a slight increase in usage at the same time the word neonicotinoids did in 2012, potentially illustrating the need of the growers to identify their alternative control solutions of cabbage stem flea beetle when faced with the possibility of losing neonicotinoids, before decreasing with time. Indeed Scott and Bilsborrow (2019) reported that 82% of oilseed growers they had interviewed used insecticide sprays to fight CSFB during the 2014/15 growing season, with pyrethroids representing 87% of these sprays, which was an increase compared to previous years and was identified as a way to compensate for the neonicotinoid seed treatments ban.

The words biopesticides and parasitoids were only mentioned between 2015 and 2022 but their usage remained very low and stable over this period, and it shows that these two means of controlling cabbage stem flea beetle are not the main solutions being considered or used by oilseed rape growers at the time. The words rotation and cultivations were used throughout all of the periods studied here but their usage remained stable, though the use of companion, in relation to companion crops, seems to decrease very slightly. The word defoliation is only mentioned from 2019 and in a similar way to biopesticides and parasitoids, its use remained low and so was probably not one of the main methods considered by farmers at the time.

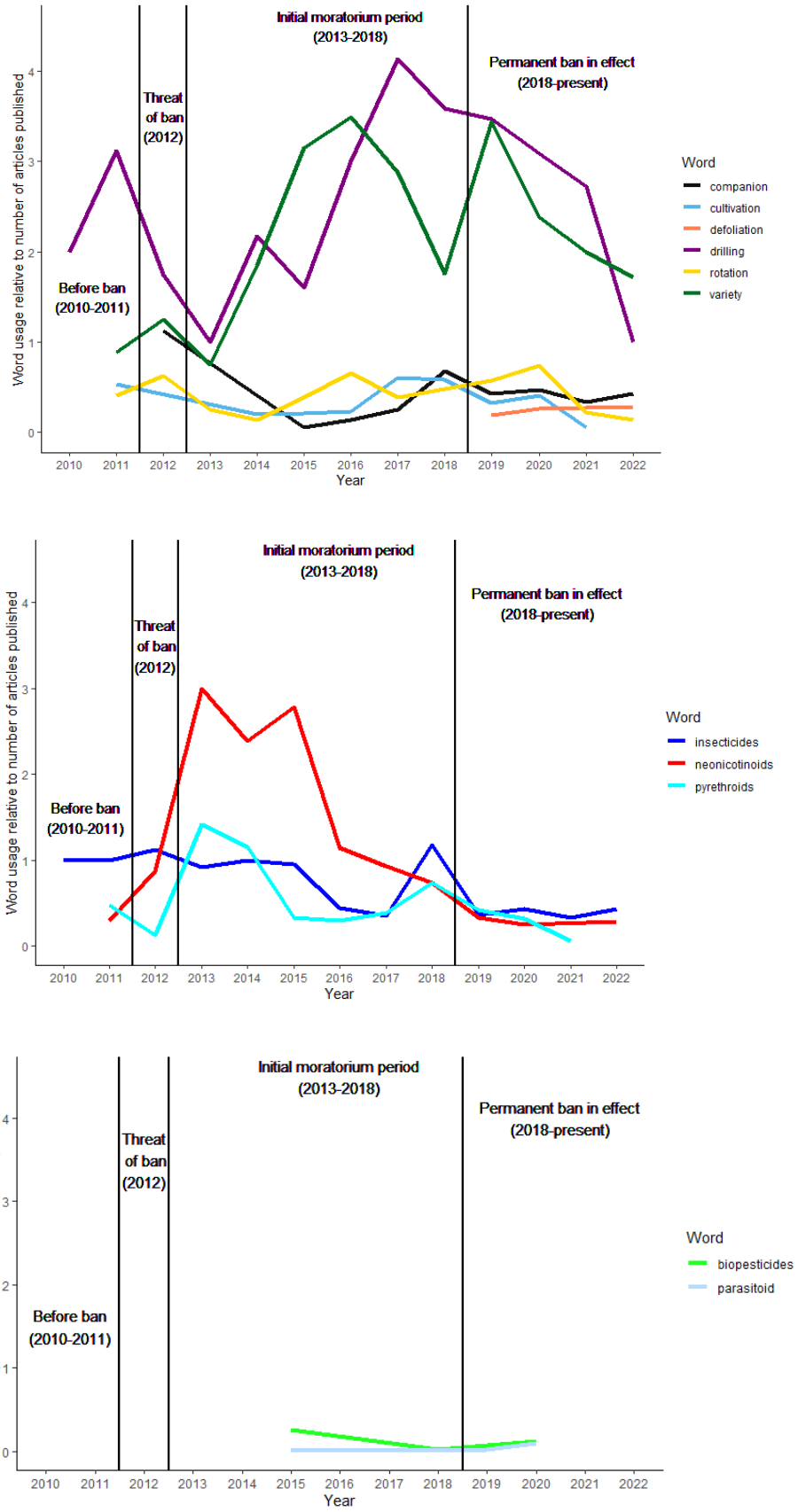


Figure 2. IPM methods word usage over the years, between the 1st of April 2010 and the 31st of December 2022.

The proportions of IPM-related words used in 10% of the articles are illustrated in Table 1. Drilling/sowing was used at least once in the majority of the articles read (between 60 and 80%), regardless of the period, which coincides with the results obtained by the R analysis in Figure 2.

Most of the results obtained by manually checking 105 of the articles coincides with the results obtained by the R analysis in Figure 2. Insecticide was used in the majority of articles before the permanent ban was put into place at the end of 2018, and then its usage decreased in frequency. Rotation was used in a minority of the articles read and the frequency only slightly decreased after the permanent ban. Variety was used in 40% of the articles read during the period of the initial moratorium before decreasing slightly. The use of the word neonicotinoids increased in the articles read from the period before the ban to the period of the initial moratorium before decreasing slightly. Pyrethroid was used increasingly when the period of the initial moratorium started before decreasing.

There are discrepancies between the R analysis and the manual analysis for only two of these words. Defoliation was used in 10% of the articles read but only after the initial moratorium started onwards. Companion was most frequently used in articles published after the permanent ban was put into place, whereas the R analysis shows that the usage of companion decreased over time instead.

Table 1. Usage of IPM-related methods in 10% of the articles depending on the period. Numbers represent the proportion of usage in the articles of the period. The word parasitoid was only used in one of the selected 10% articles so did not have any weight in the percentage calculations.

	Drilling	Insecticide	Rotation	Cultivation	Companion	Varieties	Neonicotinoids	Pyrethroids	Defoliation	Parasitoid
Before ban (2010-2013)	0.8	0.6	0.2	0.2	0.2	0	0	0	0	n/a
Initial moratorium (2014-2018)	0.8	0.4	0.2	0.1	0.1	0.4	0.4	0.2	0.1	n/a
Permanent ban (2019-present	0.6	0.3	0.1	0	0.5	0.2	0.3	0.1	0.1	n/a

3.2. Sentiment analysis

The percentage of sentiment words used in the articles are illustrated in Figure 3. Between the time before the neonicotinoid ban was being discussed (2010-2011) and the time when the European Commission was officially reviewing the risks that neonicotinoid insecticides pose to bees (2012), there were more words linked to negative emotions being used, such as “anger”, “disgust” and “fear”, while words linked to positive emotions such as “joy” and “trust” decreased, which would indicate an increase of negative feelings in the farming press when a potential ban of neonicotinoid insecticides was announced (Case, 2012). Proportions decreased evenly among all the emotions during the initial moratorium period, and remained the same during the permanent ban period, with the only notable increase being words linked with the positive emotion “trust”, which could reflect the hope that oilseed rape growers had from the novel control methods being introduced such as companion crops, early or late sowing dates, varieties more tolerant of CSFB (Dyer, 2021; Farmers Guardian, 2021).

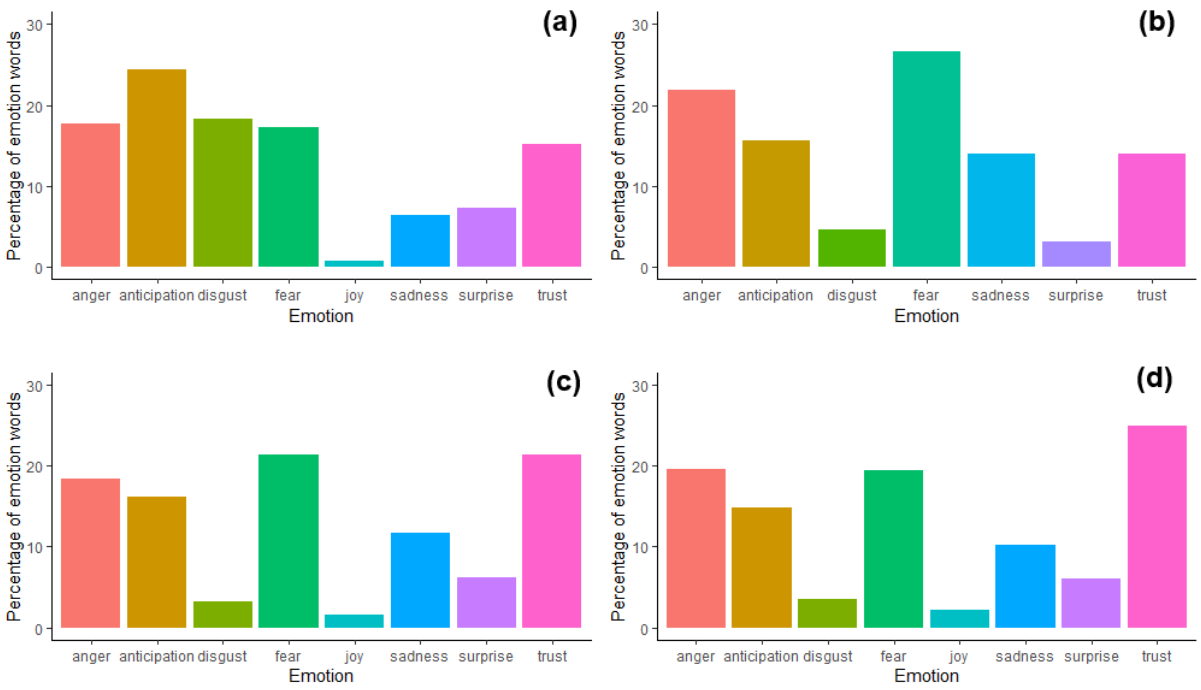


Figure 3. Percentage of sentiment words used associated to selected sentiments (nrc lexicon, positive and negative removed) before the neonicotinoids ban (a; 2010-2011), when there was a threat of ban (b; 2012), during the initial moratorium (c; 2013-2018) and after the permanent ban (d; 2018-2022).

The sentiment score of articles each year are illustrated in Figure 4. Sentiment was overall positive across the years included in this study, with a notable increase in positive sentiment between 2011 and 2012, before decreasing again between 2012 and 2013, and then slowly

and consistently increasing between 2013 and 2022. The increase in positive sentiment scores between 2011 and 2012 contrasts with the increase in negative sentiment words seen in Figure 3 for the same periods, potentially because words alone can convey emotions that would be interpreted differently when considered in the context of whole sentences.

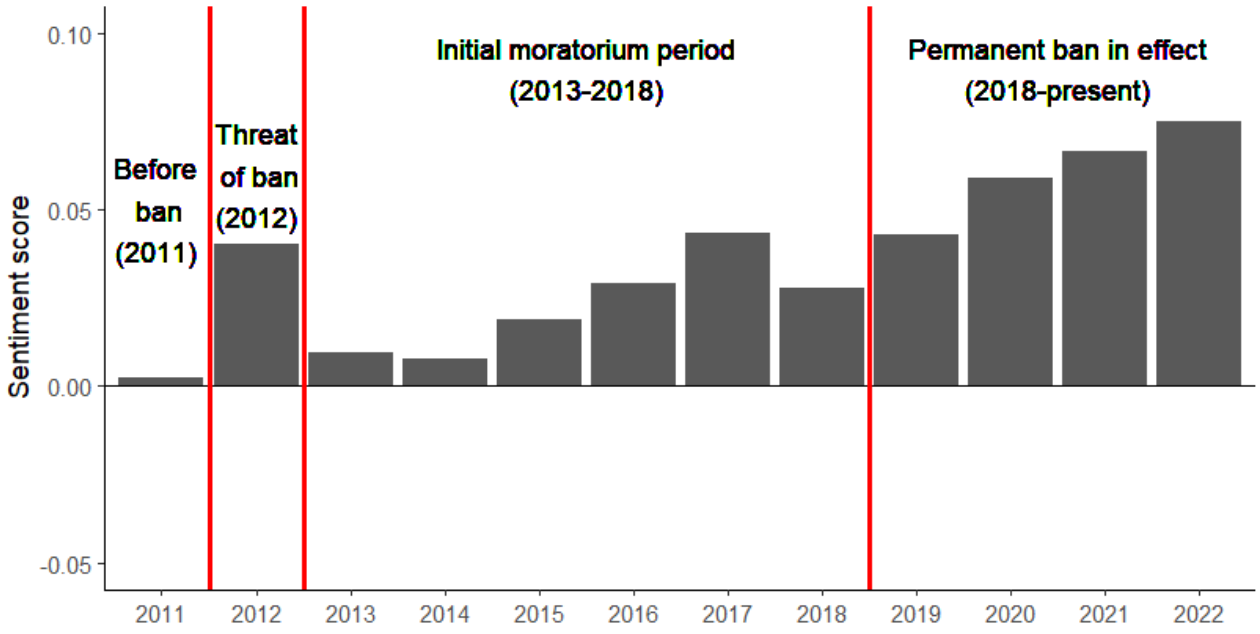


Figure 4. Sentiment score of articles, shown per year of publication (positive sentiment when y > 0, negative sentiment when y < 0).

The sentiment score results of the sample of articles checked manually are presented in Table 2. The mean average scores determined manually for 10% of articles differ from automated sentiment analysis performed on all articles. Instead of a steady increase in positive sentiment over time, negative sentiment scores are seen in 2016 and 2017, with no overall trend in sentiment score. This may be due to the smaller sample size used here, however there is no consensus on the proportion of articles to check manually (Caswell, 2014; Hill, 2016; Jones, 2020).

Table 2. Mean average sentiment score of articles performed manually on a sample of articles (10% of total number of articles). A score < 0 means a negative sentiment, a score > 0 means a positive sentiment, a score = 0 means a neutral sentiment.

Year	Average score
2010	0
2011	0
2012	1
2013	1
2014	0
2015	0.111
2016	-0.287
2017	-0.143
2018	0.6
2019	0
2020	0.5
2021	1
2022	1

4. Conclusion

This study shows that the topic of oilseed rape and cabbage stem flea beetle control increased in prominence in the weekly UK farming press over the period between 2010 and 2022. During that period, neonicotinoid seed treatments were first put under a two-year moratorium in 2013 and permanently banned in 2018. It also shows that although words linked to negative emotions were increasingly used when the ban was announced, over time sentiment improved, perhaps reflecting development of new control methods that benefited oilseed rape growers.

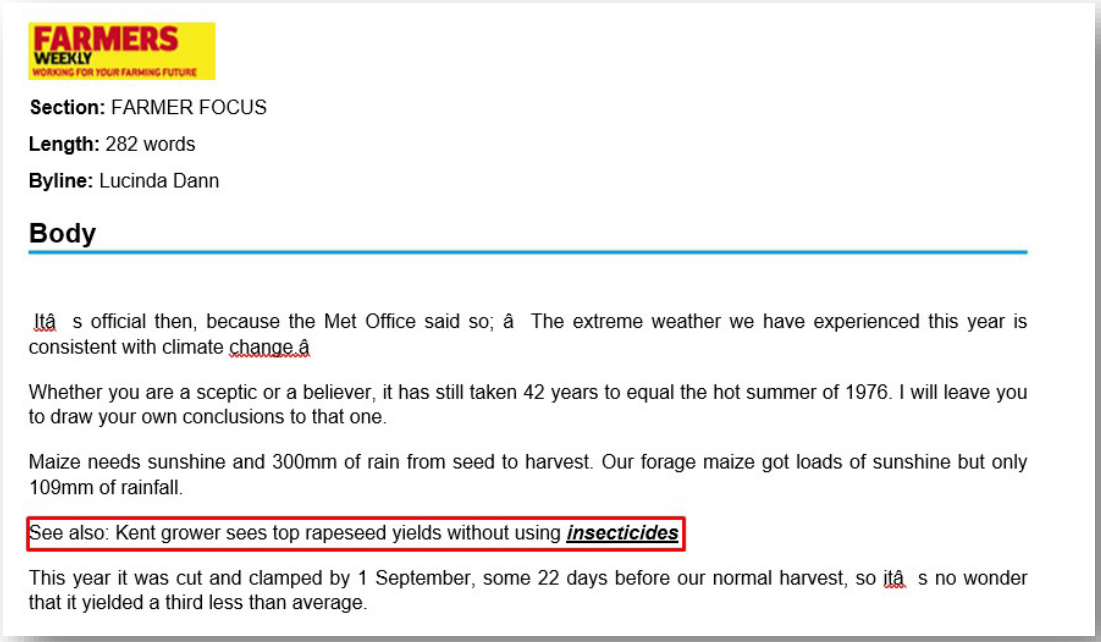
However, several limitations were identified regarding the presented methods when manually checking 10% of the articles. One is that 10% of the articles were manually checked by me only for sentiment analysis of whole articles. A more robust method would have been to have several people analysing the same sample of articles to compare sentiment scores and draw a mean average, instead of relying on the scores of just one person. Other limitations identified have the potential to affect the accuracy of word counts and are gathered in Table 3.

Future work needs to improve the methods of extracting the articles and find a way to fix the issues identified above to avoid under- and overestimation of word usage. Using a shorter type of media, such as social media posts or comments, would also limit the risk of analysing a text that only briefly mentions the topic of interest. Checking the articles manually and

removing portions of texts that are not relevant would help but is highly time consuming when faced with a large volume of articles (the current study analysed 571 articles).

Table 3. Limitations identified regarding the articles used in the present study and the effect they have on the analysis.

Error/issue	Details	Effect on word count
Focus of the article is irrelevant	Most of the articles do not exclusively talk about oilseed rape and cabbage stem flea beetle, only mentioning this topic in passing	Overestimation of usage as some words of interest are used a lot but not necessarily to discuss the topic of flea beetle control in oilseed rape
Articles downloaded contain bugs and formatting issues	A space missing between the end of a sentence and the beginning of the next one means that words are transformed into fake words	Underestimation of usage as key words are hidden and not picked up by the analysis when trying to filter for them
Presence of links to other articles	Titles of other articles are included in the body of the analysed articles that contain words of interest that are not in the article itself (see Figure 5 for an example)	Overestimation as some words are picked up even though they are technically not part of the article
Insertion of random characters	Replacement of characters such as quotation marks and apostrophes with “â” (see Figure 5 for an example)	Underestimation as which this error can influence how words are perceived by the analysis when “â” is attached to them
Spelling mistakes	See Figure 6 for an example	Underestimation as words are not picked up by the analysis when trying to filter for them



FARMERS WEEKLY
WORKING FOR YOUR FARMING FUTURE

Section: FARMER FOCUS

Length: 282 words

Byline: Lucinda Dann

Body

Itâ s official then, because the Met Office said so; â The extreme weather we have experienced this year is consistent with climate change.â

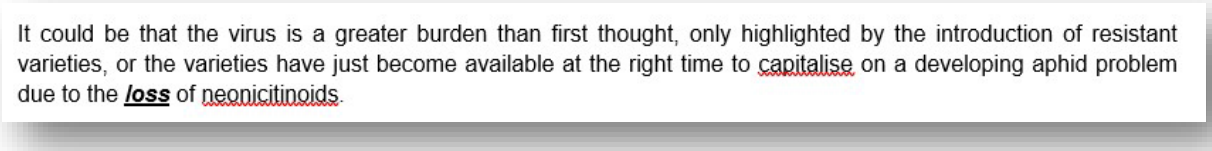
Whether you are a sceptic or a believer, it has still taken 42 years to equal the hot summer of 1976. I will leave you to draw your own conclusions to that one.

Maize needs sunshine and 300mm of rain from seed to harvest. Our forage maize got loads of sunshine but only 109mm of rainfall.

See also: Kent grower sees top rapeseed yields without using ***insecticides***

This year it was cut and clamped by 1 September, some 22 days before our normal harvest, so itâ s no wonder that it yielded a third less than average.

Figure 5. Article showing the inclusion of the title of another article in the article body, which contains the word “insecticides”, which is a word that was filtered for as part of the study. This is the only instance this word was used in the article.



It could be that the virus is a greater burden than first thought, only highlighted by the introduction of resistant varieties, or the varieties have just become available at the right time to capitalise on a developing aphid problem due to the ***loss*** of neonicitinoids.

Figure 6. The word “neonicotinoids” is misspelt in this sentence, so this instance will not be picked up by the analysis when trying to filter out this word.

Chapter 7: General Discussion

1. Efficacy of biopesticides against cabbage stem flea beetle, current knowledge, and future requirements

1.1. Current knowledge

The present study is the first to report on the potential of fatty acids against a flea beetle pest both under laboratory and field conditions. It showed that fatty acid-based products can be effective against CSFB adults under laboratory conditions, and their efficacy could potentially be further improved through the use of adjuvants to increase the spread of these products on the leaves of crop plants or increase the time it takes for them to dry. Although by themselves fatty acid-based products under field conditions did not lead to a reduction in CSFB adult feeding damage or lower larval numbers, combining them with a conventional pyrethroid insecticide led to the lowest feeding damage of all treatments tested. More work with fatty acids is necessary to confirm the results presented in previous chapters. The encouraging results presented under laboratory conditions in Chapter 3 were not matched under field conditions in Chapter 5. However, several ways to improve fatty acids efficacy have been identified, such as applying the fatty acids at night as colder temperatures could result in these products staying wet longer, which in-turn is likely to improve efficacy; combining fatty acids with other biopesticides or conventional insecticides, though compatibility would need to be proven, and as conventional insecticides such as pyrethroids are detrimental to natural enemies of CFSB (I. H. Williams, 2010), this last suggestion might not be compatible with sustainable crop protection practices.

Under laboratory conditions, all entomopathogenic nematode species tested were effective against CSFB adults, with *Steinernema feltiae* being the most effective species, followed by *Heterorhabditis bacteriophora*, *Steinernema carpocapsae* and *Steinernema kraussei*. Preliminary tests with adjuvants to potentially increase nematodes survival on oilseed rape foliage showed that fire retardant and xanthan gum were compatible with *Steinernema feltiae*, while glycerin and xanthan gum were compatible with *Heterorhabditis bacteriophora* over a period of seven days. However, applications of entomopathogenic nematodes did not succeed in lowering leaf damage or larval numbers under field conditions. This study is one of only two that I am aware of investigating the potential of entomopathogenic nematodes against flea beetles under laboratory conditions, the other one being that of Godina *et al.* (2023). Instead, most studies have investigated the potential of entomopathogenic nematodes under field conditions and focused on other species of flea beetles such as the crucifer flea beetle and the striped flea beetle. More work is then necessary both under laboratory and under field

conditions against CSFB to confirm the results presented here with improved methods with for example using adjuvants to protect nematodes from detrimental abiotic factors, testing a range of temperatures that more closely reflect crop conditions, targeting different CSFB life stages such as larvae, testing a wider range of nematode species and strains. Other potential work related to entomopathogenic nematodes would be the use of the bacteria living in their gut, or the metabolites that they produce (Shapiro-Ilan, Hazir and Glazer, 2017).

The work presented in this study showed that azadirachtin, tested on its own under laboratory conditions, and both on its own and in combination with the entomopathogenic fungus *Beauveria bassiana* strain GHA under field conditions, was not effective at controlling cabbage stem flea beetle (CSFB) in terms of adult mortality and feeding, and larval mortality. Similarly, the selected products based on *Bacillus thuringiensis* sbsp *tenebrionis* were not effective against CSFB under laboratory conditions, with mortality remaining low.

More encouraging results showed that the entomopathogenic fungus *B. bassiana* strain GHA can be effective against CSFB adults under laboratory conditions when double the recommended field rate for soft-bodied insects is applied. However, this entomopathogenic fungus was not effective, when tested under field conditions, at reducing leaf damage by CSFB adults when applied on its own or in combination with azadirachtin. Similarly, *B. bassiana* strain GHA was not found effective at reducing CSFB larval numbers. Future work using entomopathogenic fungi against CSFB will need to find a way to counteract the effect of UV radiation, temperature, and humidity. One solution to mitigate the negative effects of UV radiation would be to spray in the evening when the sun is down. However, field conditions of oilseed rape crops grown in the UK in the evening are such that this would mean applying the fungus when temperatures are too cold for *B. bassiana* strain GHA to be effective (below 15°C; Etienne Hinh, Certis Belchim BV, personal communication). It would also be necessary to evaluate the effect of entomopathogenic fungi against non-target organisms, as for example *Metarhizium anisopliae* (Sorokin) is pathogenic to the lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and the plant bug *Dicyphus tamaninii* Wagner (Hemiptera: Miridae) (Thungrabeab and Tongma, 2007), which are both natural enemies of insect pests. Testing entomopathogenic fungi against non-target organisms present in oilseed rape crops would then be an important part of future work to ensure that entomopathogens are safe to use. The present study focused on the application of entomopathogenic fungi as inundative sprays, but another solution could be the used of endophytic species, that could be applied as a seed coating or inoculated directly in the soil. Endophytic fungi would then grow inside the tissues of the plants without harming them (Stone, Polishook and White, 2004) while still having an insecticidal effect, which has been shown against other insect pest species (Mejía *et al.*, 2008; Brum *et al.*, 2012; Zhang *et al.*, 2014; Mantzoukas and Eliopoulos, 2020).

Field results presented in Chapter 5 suggest that conventional pyrethroids are more effective at controlling CSFB adults damage compared to the biopesticides tested here, which were in most cases not more effective than water or no treatment in reducing damage. These results highlight some of the challenges that are faced regarding the development and future availability for use on farms of biopesticides. However, as the intention would be to use these biopesticides as components of Integrated Pest Management (IPM) programmes, it is not necessary for these products to be as effective as conventional insecticides as stand-alone treatments.

1.2. Future requirements

If biopesticides, such as those tested in this study, are to be used as part of an IPM programme, it is crucial to understand the way they interact with each other and with other control methods in order to use them effectively. This is because there can be antagonistic behaviours (e.g., using fungicides and nematicides can be lethal to entomopathogenic fungi and nematodes (Chandler, 2017; Shapiro-Ilan, Hazir and Glazer, 2017). A way to overcome the sensitivity of entomopathogens to abiotic factors mentioned above would be the selection of new species and strains that are more tolerant to UK arable crops conditions, while the issue with some entomopathogens being detrimental to non-target organisms could be reduced by the selection of species and strains that are specific to CSFB. Another area of future research would be to determine what would be the lethal dose of these biopesticides to kill CSFB specifically, as in the present study, concentrations were chosen based on label recommendations, even though these products were often commercialised against soft-bodied insects such as aphids and whiteflies. To be able to effectively penetrate CSFB's tough cuticle a biopesticide might then need to be applied at higher concentrations than those recommended by manufacturers for control of other crop pests.

Future work also needs to focus on applying selected biopesticides to oilseed rape under field conditions. The results presented in Chapter 5 suggest that the biopesticides tested are not effective under field conditions in their current forms, but several ideas for improvements were identified and gathered in Table 1. While the original plan was to complete two field experiments as part of the present study, only one field experiment was completed due to lack of time as a result of the COVID-19 multiple lockdowns in 2020 and 2021. Completing field experiments would have allowed me to address the limitations listed in Table 1, potentially obtaining stronger datasets and confirming whether biopesticides are effective against CSFB under field conditions.

Table 1. Problems/challenges encountered during the field experiment and potential solutions for future work.

Problem/challenge	Potential solution
Plants being assessed as they grow using a scoring system designed for only early growth stages makes assessment inaccurate	Adjusting damage scoring system to reflect plant growth stage
UV radiation, temperatures and humidity variations are detrimental to entomopathogens survival	Spraying in the evening, adding adjuvants to biopesticides
Biopesticides not reaching the pest efficiently	Adding adjuvants to increase the spread of products on leaves and keep them wet longer
Efficacy of biopesticides reduced by poor water quality	Ensure that water hardness does not exceed label recommendations.
Low CSFB pressure in field, with the number of adults present in yellow water traps much lower than the spray threshold	Test biopesticides where pest pressure is high enough to increase robustness of results
No effect of biopesticides observed on CSFB adults feeding damage levels and number of CSFB larvae	Study other variables in addition, such as adult emergence in the spring
Plant dissection to assess larval number is time consuming, and lots of larvae may have exited the plants or died, which can make them difficult to spot as their body becomes soft	Use different larval number assessment methods such as the funnel method (Conrad, Brandes and Heimbach, 2016)

2. The impact of using biopesticides as a component of future IPM programmes

Before biopesticides can be widely used by oilseed rape growers to control CSFB, several aspects will need to be considered, such as their cost effectiveness. This is because biopesticides tend to be applied more frequently than conventional insecticides to be effective, and are also applied at higher doses (Hoarau *et al.*, 2022b). Indeed, it would be 20 times more expensive for an oilseed rape grower to apply a fatty acids product (e.g., FLiPPER applied at 4.8L/ha) instead of a pyrethroid insecticide (e.g., lambda cyhalothrin product Hallmark with Zeon technology applied at 0.05L/ha) at current prices. At the time of writing, no biopesticides have been approved for use in oilseed rape crops against CSFB (Health and Safety Executive, 2023). Other reasons why biopesticides are slow to be integrated into farmers' toolbox to control CSFB could be a lack of trust and knowledge on how to best apply them, the ease of applying conventional insecticides, and the absence of incentive for growers to use biopesticides (Chandler, 2017; Shapiro-Ilan, Hazir and Glazer, 2017). However, the lack of effective conventional insecticide solutions might with time lead oilseed rape growers to find other products to spray on their crop.

As observed in the previous paragraph, biopesticides tested under field conditions are not as effective as under laboratory conditions or greenhouse conditions, mainly because biopesticides based on living organisms (entomopathogenic nematodes, fungi and bacteria) are sensitive to abiotic factors such as temperatures, UV radiation and humidity levels (Ignoffo and Garcia, 1992; Jaronski, 2010). These abiotic factors cannot be controlled under field conditions. Biopesticides are currently commercialized for use in horticultural crops grown under protected cropping systems (e.g., glasshouse conditions) and used against soft-bodied insects. However, it would be unhelpful to make simple comparisons between biopesticides and conventional insecticides regarding their efficacy and cost, as the present study does not have the objective of purely replacing conventional insecticides with biopesticides. On the contrary, this study can be seen as a vital first step in identifying biopesticides as potentially effective alternatives to conventional insecticides as part of a wider IPM programme. The idea would be to use them alongside other tools such as cultural control methods including crop rotation, seed rate, seed drilling date, companion cropping, organic amendments, varieties more tolerant to CSFB damage, stubble management, good soil moisture, etc. (Pickering *et al.*, 2020; White *et al.*, 2020; Ortega-Ramos *et al.*, 2021), natural enemies such as parasitoid wasps (Jordan *et al.*, 2020; Pickering *et al.*, 2020) and monitoring and prediction methods (Ortega-Ramos *et al.*, 2023; Tixeront *et al.*, 2023). An example of an IPM pyramid can be seen in Chapter 1 of the present study. The base of the IPM pyramid consists of preventative measures such as cultural control methods, followed by monitoring practices, followed by using

biopesticides and natural enemies, and finally the use of conventional insecticides as a last resort, either on their own or combined with biopesticides to potentially reduce application rates. Another pyramid can be seen in Pickering *et al.* (2020), drawn in a different format with the different steps not based on the type of control, but based on the growth stage of the oilseed rape crop. The authors suggest that biopesticides could be used from pre-sowing of the crop in late summer all the way until spring, but that their efficacy needs to be proven under field conditions and more research is therefore necessary.

3. Recent research on CSFB IPM tools other than biopesticides

As CSFB has become such an important pest of oilseed rape crops grown in the UK and the rest of Europe since the ban of neonicotinoid insecticides by the European Union in 2013 (European Commission, 2013a), there is an extensive international research effort to find effective controls other than biopesticides, for use against CSFB. Most of these studies have been extensively reviewed in several publications (Pickering *et al.*, 2020; White *et al.*, 2020; Blake *et al.*, 2021; Ortega-Ramos *et al.*, 2021; Hoarau *et al.*, 2022a, 2022b), so the section below focuses on research that was published since then.

The study that investigated a method that is the most similar to biopesticides is Cedden *et al.*, (2023), who evaluated the lethal and sublethal effect of RNA interference (RNAi) on CSFB adults through feeding bioassays. They observed that when targeting the gene Sec23, mortality of CSFB reached up to 76%, and sublethal effects, such as decreased feeding activity and reduced mobility were also reported. They concluded that RNAi has potential to control CSFB but that more genes need to be identified and effects on non-target organisms need to be assessed.

Other studies published recently have focused on improving monitoring methods and to help assess CSFB populations levels to better inform farmers who need to decide how to manage their crop. In a recent study by Hausmann *et al.* (2023), the abundance of CSFB adults in UK oilseed rape crops was reported to depend on the distance of the crop from previous year's oilseed rape crops. They concluded that CSFB adults number in yellow water traps decreased with increasing distance, which could indicate that coordinating crop rotation at a regional level can potentially influence CSFB adults numbers, which was also suggested by Ortega-Ramos *et al.* (2021).

Tixeront *et al.* (2023) investigated the colonisation process of oilseed rape crops in France by CSFB adults in order to improve monitoring and forecasting methods. The authors placed yellow sticky traps on each side of the fields and in the centre and facing either inwards or outwards. They observed that more CSFB adults entered the crop than left it, caught more adults on traps placed nearest to the crop and caught more adults during the daytime. Combining these results with meteorological data, they also observed that CSFB numbers

increased with increasing temperatures but decreased with increasing humidity and wind speed. They recommend placing traps at the border of the fields and facing outwards to catch more CSFB adults and to adjust trap height as the plants grow in future studies.

Ortega-Ramos *et al.* (2023) studied the spatio-temporal distribution of CSFB larvae in UK oilseed rape crops from 2003 to 2017 to cover periods pre- and post-neonicotinoid insecticides moratorium. The objective of the study was to help with decision support systems to predict CSFB population changes. The authors observed that after the moratorium on neonicotinoids was implemented in 2013, the number of CSFB larvae in oilseed rape crops increased 10-fold, and that larval numbers varied depending on whether the crop was sown early or late, the size of the field, temperatures, and humidity levels. The authors of this study noted that hot and dry conditions were linked to high number of larvae after the 2013 moratorium, which could be due to higher temperatures leading to increased flight activity and crop invasion by CSFB adults. A key requirement for improvement management of CSFB is the ability to reliably monitor populations of this pest within crops.

Seimandi-Corda *et al.* (2022) compared different methods of estimating CSFB larval numbers in oilseed rape determined using extraction by plant dissection, extraction by desiccating the plants and calculating the percentage of leaves bearing scars left by larvae entering the plants petioles. The first method was noted as being time consuming and so the purpose of this study was to determine if the two other methods were good alternatives. The authors found that desiccating the plants led to 76% of the larvae being extracted after seven days, increasing to 82% after 14 days. They found a strong correlation between the number of scars and the number of larvae in the plants, but this method is not as accurate despite its advantage of being quick and non-destructive. They concluded that desiccating the plant was an effective alternative to plant dissection as it is less labour intensive, however they estimated that seven days might be too long a period in case spraying decision by farmers need to be taken quickly. One last study investigated a cultural control method. Seimandi-Corda *et al.* (2023) evaluated the potential of using companion plants and straw mulch to reduce CSFB damage in oilseed rape in UK and Germany. They observed that combining oilseed rape with either cereal companion plants or straw mulch significantly reduced feeding damage by CSFB adults, with also some positive effects from legume companion plants but did not observe any trend regarding larval infestation. This study however confirms that companion plants can potentially help reduce damage by CSFB adults.

4. Conclusion

CSFB is a pest that has become difficult to control following the ban of neonicotinoid seed treatments in 2013 and the development of resistance to pyrethroid foliar sprays, leaving oilseed rape growers with no effective synthetic insecticide controls. However, this study

shows that biopesticides have the potential to effectively control this pest, although more work is needed to ensure their efficacy on the farm under field conditions. Thankfully, several ideas were suggested in this study to allow future studies to improve the results presented here. It was also highlighted that as biopesticides are no silver bullets and will need to be implemented into an IPM programme, there are many other studies being continuously published focusing on other control methods that could potentially be implemented along with biopesticides in the future. There is then hope that CSFB will be effectively controlled by biopesticides as part of a wider strategy.

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