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Biological control agents against the cabbage stem flea beetle in oilseed rape crops

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Abstract

The cabbage stem flea beetle (CSFB[1]) *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[2]) such as *Metarhizium anisopliae* and *Beauveria bassiana*, entomopathogenic nematodes (EPN[3]) 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[4]) systems for the sustainable management of this pest.

Keywords: cabbage stem flea beetle; oilseed rape; entomopathogenic fungi; entomopathogenic nematodes; biopesticides.

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,

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[1] CSFB: cabbage stem flea beetle

[2] EPF: entomopathogenic fungi

[3] EPN: entomopathogenic nematode

[4] IPM: Integrated Pest Management

2022). In Europe, almost 9 million hectares were grown in 2020, which represented 25% of the global area grown (FAOSTAT, 2022). 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 (Nicholls, 2016; Williams, 2010). 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, 2019).

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 et al., 2003; Alford and Gould, 1976; Bromand, 1990; Ferguson et al., 2003; Garbe et al., 2000; Nicholls, 2016; Winfield, 1992). It can also damage other overwintering brassica crops (Newton, 1929; Roebuck, 1936) including turnip, mustard and cabbage (Ahuja et al., 2011). It is native to Europe, North Asia and North Africa (Bonnemaison, 1965; Cox, 1998; Gruev and Döberl, 2006; 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) (Bracken and Bucher, 1986; Lamb, 1984; Palaniswamy and Lamb, 1992; Weiss et al., 1991) 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., 2013).

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, 2016). 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 756,000 hectares in 2012 (Defra, 2019), 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, 2016)), to 307,000 ha in 2021, with yields on average of 3.2 tons/ha. 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, we 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, we 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 (Graham and Alford, 1981; Williams and Carden, 1961). 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 et al., 2016; Nicholls, 2016).

CSFB is a univoltine species in the UK and other northern temperate countries (Williams and Carden, 1961). 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, 2010; Williams and Carden, 1961). As soon as they emerge, adults feed on mature leaves, stems and pods of oilseed rape and other brassicaceous species for about a month (Alford, 1979; Kaufmann, 1941; Såringer, 1984; 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, 2010; Williams and Carden, 1961). 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 (Ebbe-Nyman, 1952; Kaufmann, 1941).

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).

Hatching of CSFB eggs starts in late September (Alford, 1979; 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; 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 (Ebbe-Nyman, 1952; Kaufmann, 1941). 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).

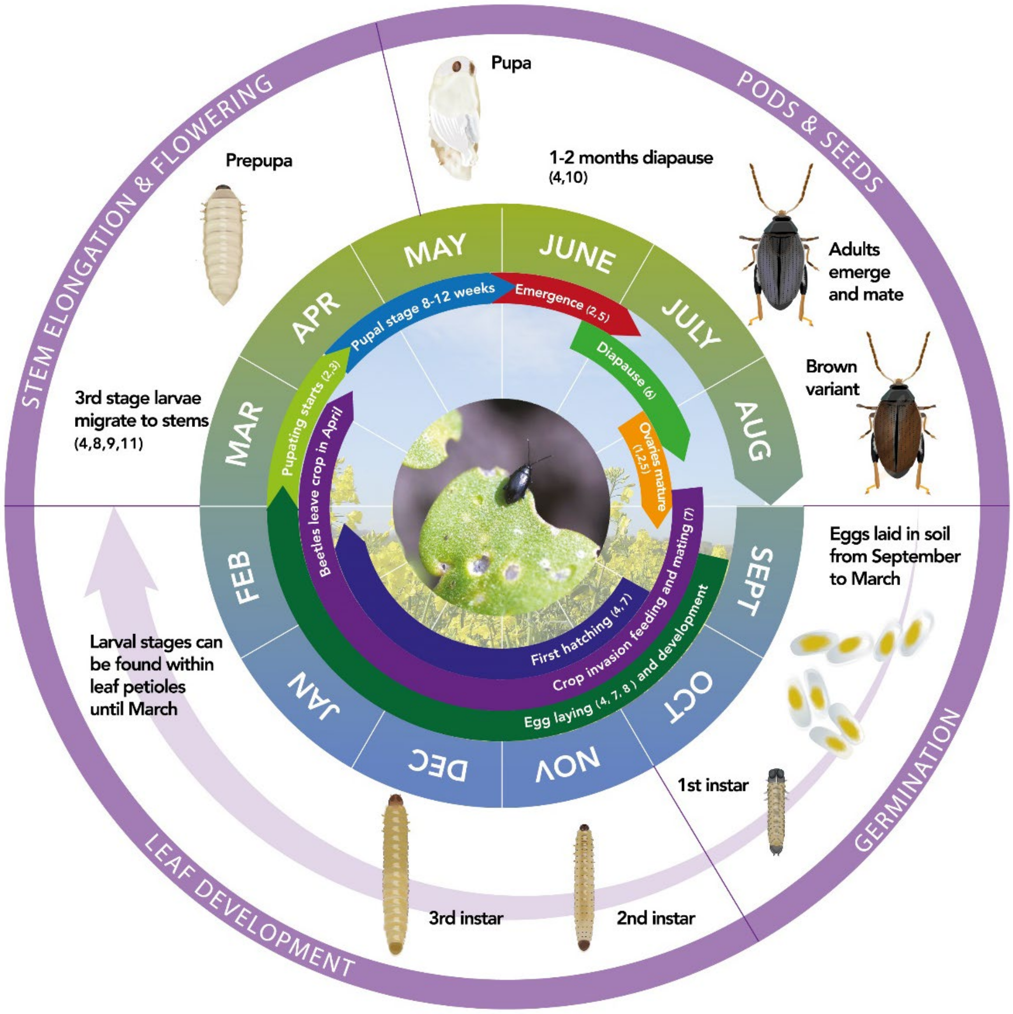

Figure 1. Life cycle of the cabbage stem flea beetle, relative to oilseed rape development (Source: Penny Greeves). Figures in brackets represent the following references: 1: Börner and Blunck, 1920; 2: Kaufmann, 1941; 3: Godan, 1951; 4: Ebbe-Nyman, 1952; 5: Williams & Carden, 1961; 6: Bonnemaison, 1965; 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 et al., 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., 1994).
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 (Graham and Alford, 1981; Nilsson, 2002, 1990; Williams and Carden, 1961; Winfield, 1992).

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 et al., 2010a; 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, 2013). 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., 2015). Overuse of pyrethroid insecticides also threatens biological diversity in and around the fields, for example by killing natural enemies and thus compromising biological control (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 South East 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, 2021a).

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 et al., 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 et al., 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 et al., 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 et al., 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 et al., 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, 1996; Vänninen et al., 1989; Zec-Vojinovic et al., 2006). However, some species of EPF can be effective when applied in an inundative strategy (Reddy et al., 2014).

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 (de Barros et al., 2015; Khachatourians and Qazi, 2008). 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 et al., 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., 2014).

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, 2021b). *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 et al., 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 et al. (2007a, 2007b) 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 et al., 2010).

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

4.1.2 Entomopathogenic nematodes (EPN)

EPN 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, EPN 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).

EPN are commonly used as short-term inundative biological control agents (Grewal et al., 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, n.d.; Koppert UK, n.d.). 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/cm² reduced larval populations from 38 to 84%, with the most affected instar being the third instar larvae. Hou et al. (2001) applied 1.75 x 10^9 *S. carpocapsae* nematodes/hm², 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 et al., 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 pest. 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, EPN 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 et al., 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. More recently, laboratory bioassays completed at Harper Adams University testing the efficacy of *Btt* against adult CSFB reached the same conclusion. Here, three different commercial formulations (*Btt* strain SA-10, and two Btt strains undisclosed) were applied to oilseed rape leaves, that were then given as food to the beetles for 12 days. These treatments did not result in beetle mortalities of more than 45% and recorded beetle mortalities were not significantly different from controls (Hoarau, unpublished data). 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 et al., 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 (d'Errico et al., 2019; Keren-Zur et al., 2000; Mendoza et al., 2008; Terefe et al., 2009). 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 et al., 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 et al., 2010a). For the conservation of parasitoid population, the authors of the review suggested adapting the choice of insecticide and rate applied (Ulber et al., 2010a). For example, the pyrethroid tau-fluvalinate has been proven to be less harmful than another pyrethroid, lambda-cyhalothrin, to natural enemies (Ulber et al., 2010a). Applying pyrethroids at half the recommended rate is also effective in protecting population of parasitoids in crops (Ulber et al., 2010a). 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 et al., 2010a). 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 the 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 et al., 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., 2013) 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. Conclusion

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, 2021b), 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 & 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 (Gul et al., 2014; Shapiro-Ilan et al., 2006).

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 our unpublished results indicate that this biopesticide is not effective against the 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 et al., 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 et al., 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 et al., 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 we stated earlier in this paper, there are ways to protect entomopathogens against UV radiations 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 et al., 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 et al., 2004) and can be used as biocontrol agents against pests such as insects and pathogens (Brum et al., 2012; Mantzoukas and Eliopoulos, 2020; Mejía et al., 2008; Zhang et al., 2014). 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 et al., 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, we have seen that 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

We have seen that several studies have attempted to combine biocontrol agents together, such as two species of EPF (Reddy et al., 2014), two species of EPN (Antwi and Reddy, 2016), EPN with a conventional insecticide (Antwi and Reddy, 2016), or azadirachtin with EPN (Yan et al., 2013). 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 Pickering et al. (2020), Ortega-Ramos et al. (2021) and Blake et al. 2021. 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 pass 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.

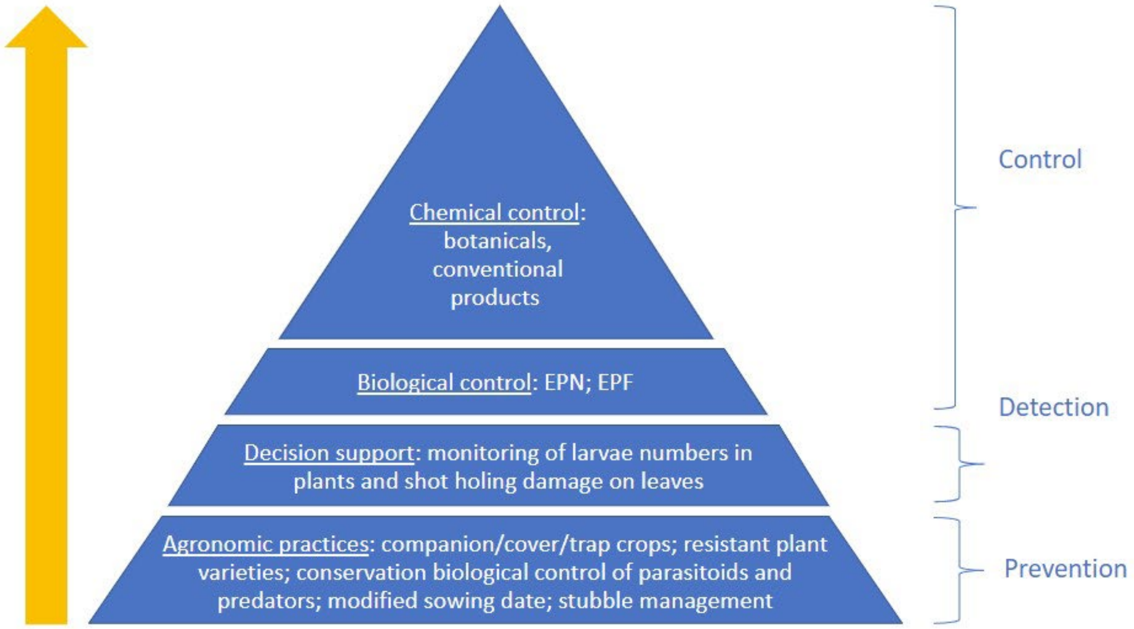

Figure 2. Integrated Pest Management pyramid for the control of cabbage stem flea beetle in oilseed rape (EPN: entomopathogenic nematodes; EPF: entomopathogenic fungi).

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., 2010b), 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.

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

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 & Carden, 1961; 6: Bonnemaison, 1965; 7: Alford, 1979; 8: Alford et al., 2003; 9: Nilsson, 1990; 10: Cox, 1998; 11: White, 2015.

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