Assessing the potential of biopesticides to control cabbage stem flea beetle Psylliodes chrysocephala

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Research Article

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Assessing the potential of biopesticides to control the cabbage stem flea beetle *Psylliodes chrysocephala*

Claire Stéphanie Véronique Price,* Heather Campbell and Tom William Pope

Abstract

BACKGROUND: 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.

RESULTS: Fatty acids were effective, with up to 100% CSFB mortality after 24 h. 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.

CONCLUSION: Entomopathogenic fungi and fatty acids could potentially be used to control CSFB as part of an integrated pest management 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 the formulation and application methods, and to assess the impact on nontarget organisms. Finally, efficacy under field conditions must be determined to understand the influence of environmental variables.

Keywords: oilseed rape; fatty acids; azadirachtin; entomopathogens; biological control; integrated pest management

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.[1,2] Young adults begin to emerge in late spring–early summer after around 2–3 months pupating in the soil.[2,3] After completing a summer diapause, adult CSFB damage young seedlings when they invade the crop from early August onwards, where they feed, mate and lay eggs.[1] Larvae hatch from eggs laid in the soil from late September onwards and climb up young oilseed rape plants before boring into leaf petioles,[1] and then through the winter and spring larvae move into the main stem of infested plants.[4] CSFB larvae pupate in the soil after completing their development inside the plant.[1] Adult damage, known as shot-holing,[1] can kill young plants if feeding pest pressure is high.[5] In the spring, stem mining by mature larvae can lead to stem wilting, delayed flowering or even total plant collapse.[3,6] A more detailed description of the CSFB life cycle can be found in recent reviews.[7,8]

Until recently, CSFB was effectively controlled by neonicotinoid insecticides.[2] 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.[9] 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.[10–15] In the UK, populations of CSFB where 100% of beetles are resistant to the pyrethroid lambda-cyhalothrin have been recorded recently.[11] 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

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33% in 2018 to 56% in 2019.[11] Similarly, in a recent French study, knockdown resistance to pyrethroids, also known as *kdr*, was found in 94% of CSFB populations studied.[16] Difficulty in effectively controlling CSFB has been closely associated with a reduction in the area of oilseed rape grown in Europe.[17] In the UK, for example, the area of oilseed rape was 756 000 ha in 2012 before the ban on neonicotinoid seed treatments but had reduced to 307 000 ha in 2021.[18] 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.[19] It has also been shown that numbers of larvae found in oilseed rape plants in the UK have increased following the neonicotinoid ban in 2013.[20] In Germany, oilseed rape yields have decreased from 4.27 t ha^{-1} between 2010 and 2015 to 3.57 t ha^{-1} between 2016 and 2019.[21] 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.[21]

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.[2] It is therefore necessary to find alternative 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[22] such as botanicals, entomopathogens, and physically acting products. Botanical biopesticides are chemical compounds extracted from plants that can have both lethal and sublethal effects.[23,24] Widely used examples of botanical biopesticides include pyrethrum, a substance obtained from the flower of *Tanacetum cinerariifolium* (Asteraceae),[25] which has the same mode of action and quick knockdown effect as synthetic pyrethroids, but with reduced persistence in the environment.[26] Another widely used example is azadirachtin, a tetranotriterpenoid obtained from the neem tree (*Azadirachta indica* A. Juss., Meliaceae)[27] that has both lethal[23,28] and sublethal effects, including reduced insect growth, longevity, fertility, reproduction, oviposition and feeding.[23,24,29] In addition, there is a wide range of essential oils components, such as limonene (extracted from citrus oil),[30] which may kill the pest but that also has repellent properties.[31]

Entomopathogens are species of bacteria, virus, nematodes or fungi that are pathogenic to insects and can be used as control agents of pest species.[32] Other studies have focused on the potential of entomopathogenic nematodes against CSFB.[33,34] 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.[35,36] The fungus grows into the haemocoel, then the rest of the body of the host insect, which is typically killed in 4–6 days by physical damage and secretion of fungal metabolites.[37] Spores are 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).[38] When it sporulates, *Bt* produces a bipyramidal protein crystal composed of δ-endotoxins that are lethal to insects when ingested but are not toxic to vertebrates.[39,40] 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.[41] 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 48 h after ingestion as a result of septicemia.[42] Two *Bt* subspecies are known to kill coleopteran insects: *Bt* subsp. *tenebrionis*[43] was shown to be up to 100% effective against the larvae of the white-spotted rose beetle *Oxythyrea funesta* (Poda) (Coleoptera: Cetoniidae)[44] and subsp. *san diego*[45] was shown to be effective against larvae of the Colorado potato beetle *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) and is commercially available in the USA.[46]

Physically acting products may be defined as having a nonspecific mechanical or physical mode of action.[47] 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.[48] Fatty acids are another widely used example. The active substances of fatty acids products are unsaturated carboxylic acids (e.g., C14–C20, potassium salts[49]). Fatty acids affect the insect by removing the waxy layer covering the cuticle and penetrating the cuticle, disrupting cellular membranes and leading to cytolysis. Treated insects become dehydrated as a result of water loss, feeding is disrupted and death typically follows soon after.[49–51]

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[52]) 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 (30 × 30 × 30 cm) in a growth room (Fitotron® SGR 122; Weiss Technik UK Limited, Loughborough, UK) at a constant 20 °C temperature and 60% relative humidity (RH) and fed by placing potted oilseed rape plants (variety Mirakel) grown under glasshouse conditions to growth stage 12 (BBCH (Biologische Bundesanstalt Bundessortenamt und Chemische Industrie) system[53]) into each cage. Potted oilseed rape plants were replaced every 2 weeks. Insect populations were kept under these conditions for up to 9 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 that had reached a minimum growth stage of 12 (BBCH system[53]). 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, active ingredients, rates tested and number of replicates. Biopesticide efficacy was compared to water, which was used here as a negative control.

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 (0.5, 1.0 and 1.4 mL L^{-1}). 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 (17 × 11 × 5cm) and an oilseed rape leaf was fully immersed for 5 s and then left to dry. Incubation chambers (cylindrical plastic containers, 12 cm/7 cm 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 (one disc per leaf) were cut from the soaked leaves, and one 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 [4 cm diameter opening, mesh aperture 1 × 1 mm, with an open area of 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) with a 16/8 h day/night photoperiod, 20 °C temperatures and 60% RH. Mortality was assessed every day for 8 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 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.[46] 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 10 mL L^{-1}, 2.5 g L^{-1} and 1.25 g L^{-1} 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 Section 2.2. Tap water was used as the control and 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 Section 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 described in Section 2.2. Mortality was assessed every day for 12 days by counting the number of dead CSFB in each chamber. The bioassay was completed in December 2020.

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

The efficacy of Botanigard WP (entomopathogenic fungus *B. 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 1.26 g L^{-1}] and tap water was used as a control. Each concentration and control was replicated six times. Solutions of Botanigard WP were prepared by

Table 1. Product name, manufacturer, active ingredients, application concentrations and number of replicates of products used in laboratory bioassays against the adult cabbage stem flea beetle (*Psylliodes chrysocephala*)

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

Note: A water control was tested alongside each product, except for the bioassay with lambda-cyhalothrin for which the control was acetone.

diluting the product in tap water. Each solution of Botanigard WP or tap water was poured separately into a 200-mL 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, placed in tubes (10 insects per tube, unsexed) and 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 Section 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 0.10 mL 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 lid, as described in Section 2.2.

The 24 incubation chambers were placed randomly inside a plant growth room (model MLR-351H; Sanyo, Osaka, Japan) with a 16/8 h day/night photoperiod, constant 20 °C temperature and 85% RH. Mortality was assessed every 2 days for 14 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 32 mL L^{-1} for FLiPPER and 10, 20 (field rate) and 40 mL L^{-1} 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 Section 2.2, and insects were prepared and treatments applied as described in Section 2.4. The 21 incubation chambers were placed randomly and kept under the same conditions as described in Section 2.4. Mortality was assessed every day for 4 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 ×2000 magnification using a scanning electron microscope (Cambridge Instruments Stereoscan 200, UK).

2.6 Lambda-cyhalothrin (pyrethroid) glass vial bioassay

The lambda-cyhalothrin bioassay was carried out using the Insecticide Resistance Action Committee (IRAC) susceptibility test method 031 (https://irac-online.org/methods/weevils-and-flee-beetles/) with technical-grade lambda-cyhalothrin (Fluka™ Honeywell). Glass vials [6 cm high (h) and 1.25 cm radius (r)] were selected and their surface area (SA, cm^2) calculated using 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 the SA by 0.0375 μg cm^{-2} (50% of field dose), 0.015 μg cm^{-2} (20% dose) and 0.003 μg cm^{-2} (4% dose) to give the doses 1.95 μg, 0.78 μg and 0.16 μg 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 placed in each vial and the lids were secured. Vials were kept in a controlled environment cabinet (as described in Section 2.2). Mortality was assessed after 24 h. 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*) was 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 (*B. bassiana*) and after the fatty acid treatments FLiPPER and Neudosan, however, were analysed using mixed-effect models from the package *lme4*[54] 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*[55] and *lsmeans*,[56] or using the *HSD.test()* function included in the package *agricolae*.[57] Box plot graphical illustrations were made with the *boxplot* function from the package *graphics*[58] after the data had been tidied with the *mutate* function from the package *tidyverse*.[59]

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 Fig. 1. There were no significant differences in CSFB mortality between the water control and each azadirachtin application rate: 0.5 mL of azadirachtin/L [$z = -0.373$, hazard ratio (HR) = 0.752, 95% confidence interval (CI) = 0.168–3.360, $P = 0.709$], 1 mL of azadirachtin/L ($z = -0.795$, HR = 0.502, 95% CI = 0.092–2.743, $P = 0.427$) and 1.4 mL of 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 (1 mL L^{-1}) than for the control (4.8% leaf area eaten) or when leaves were treated with the highest dose (1.4 mL L^{-1}, 3.7% leaf area eaten, $F = 1.172$, residual degrees of freedom (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

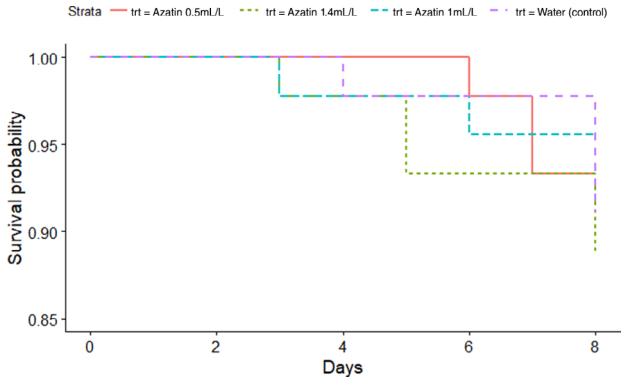

Figure 1. Survival curve of cabbage stem flea beetle (*Psylliodes chrysocephala*) after application of different rates of azadirachtin and water (control).

usually used against smaller, soft-bodied insects such as whiteflies and aphids.[60] 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.[61] 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).[62] 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 to understand which combination would be the most effective against CSFB in the field.

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 Fig. 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.[63] The only other study investigating the use of *Btt* against adult flea beetle is a patent in which reduced feeding activity of the adult crucifer flea beetle (*Phyllotreta cruciferae*) was reported after they were exposed to treated leaves, but no mortality was reported.[64] 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.

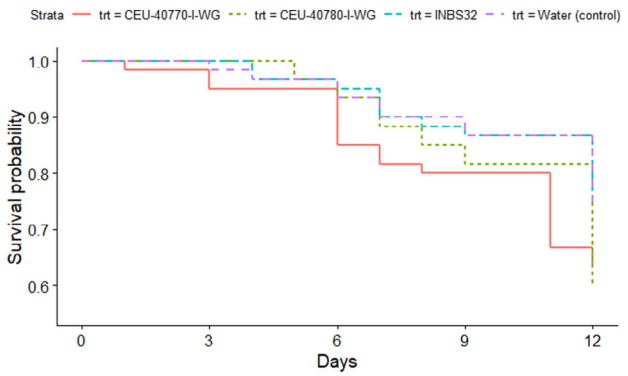

Figure 2. Survival curve of cabbage stem flea beetle (*Psylliodes chrysocephala*) after application of different strains of *Bacillus thuringiensis* sbsp. *tenebrionis* and water (control).

3.3 *B. bassiana* strain BHA product whole-leaf bioassay

Adult CSFB mortality increased significantly over time ($t = 8807$, df = 143, $P < 0.001$), but only the application of double the field rate (1.26 g L^{-1}, equivalent to 5.5×10^7 spores/mL) of *B. bassiana* strain GHA significantly increased mortality compared to the control ($t = 5.628$, df = 20, $P < 0.001$), as shown in Fig. 3. Application of the field rate (0.63 g L^{-1}, equivalent to 2.7×10^7 spores/mL) resulted in mortality similar to the control ($t = 0.743$, df = 20, $P = 0.466$), and application of half the field rate (0.32 g L^{-1}, equivalent to 1.4×10^7 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 *B. bassiana* against adult flea beetles. For example, in one study, 15 isolates were tested using a concentration of 1×10^7 spores/mL against CSFB and a maximum mortality of 47% after 14 days was recorded when isolate V55 was used.[65] In another study, 14 isolates of *B. bassiana* were tested at a concentration of 1×10^8 spores/mL against crucifer flea beetle (*P. cruciferae*) adults. Here mortality varied between 50% and 90%, 7 days after treatment.[66] In the field, Menzler-Hokkanen *et al.* unpublished (cited in Hokkanen *et al.*[67]) reported that spray application and soil incorporation of *Metarhizium anisopliae* (strain/isolate unidentified) led to reductions in adult *Phyllotreta*

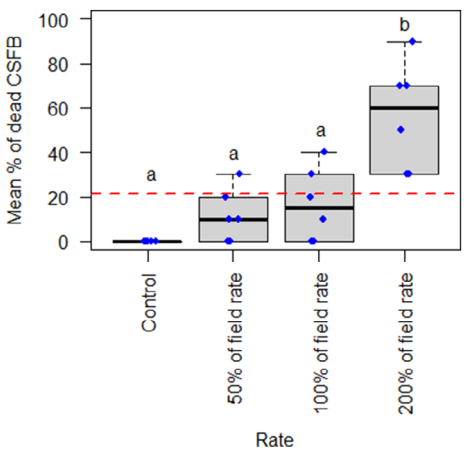

Figure 3. Percentage of dead cabbage stem flea beetle (CSFB) (*Psylliodes chrysocephala*) after 14 days of contact with entomopathogenic fungi *Beauveria bassiana* strain GHA and water (control). The dotted red line represents the overall mean of the data. Different letters indicate significant differences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

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 is not effective against this species.[68,69] 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.[62] 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.[62] 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,[70,71] entomopathogens are short-lived in the field and multiple applications allow 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, the 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,[65,72] so more research is needed, under both laboratory and field conditions, to test the efficacy of a wider range of combinations of fungal species and strains, and isolates.

3.4 Physically acting products whole-leaf bioassay

CSFB mortality results are illustrated in Fig. 4. 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 24 h. Mortality did not increase further over time ($F = 2.4554$, df = 62, $P = 0.122$) and increasing the

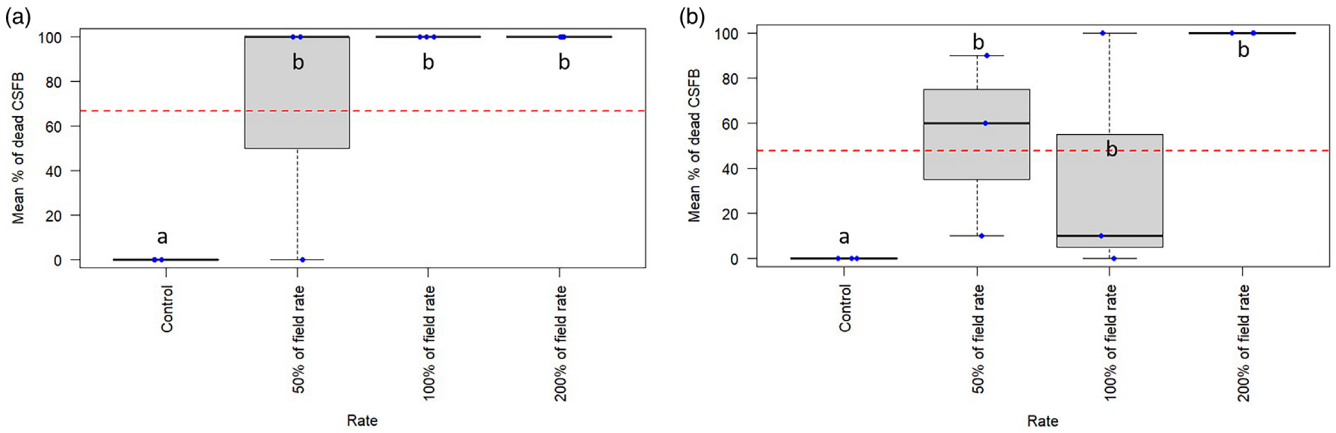

Figure 4. Percentage of dead cabbage stem flea beetle (CSFB) (*Psylliodes chrysocephala*) after 4 days of contact with fatty acid products FLiPPER (a) and Neudosan (b). The dotted red line represents the overall mean of the data. Different letters indicate significant differences ($P < 0.05$).

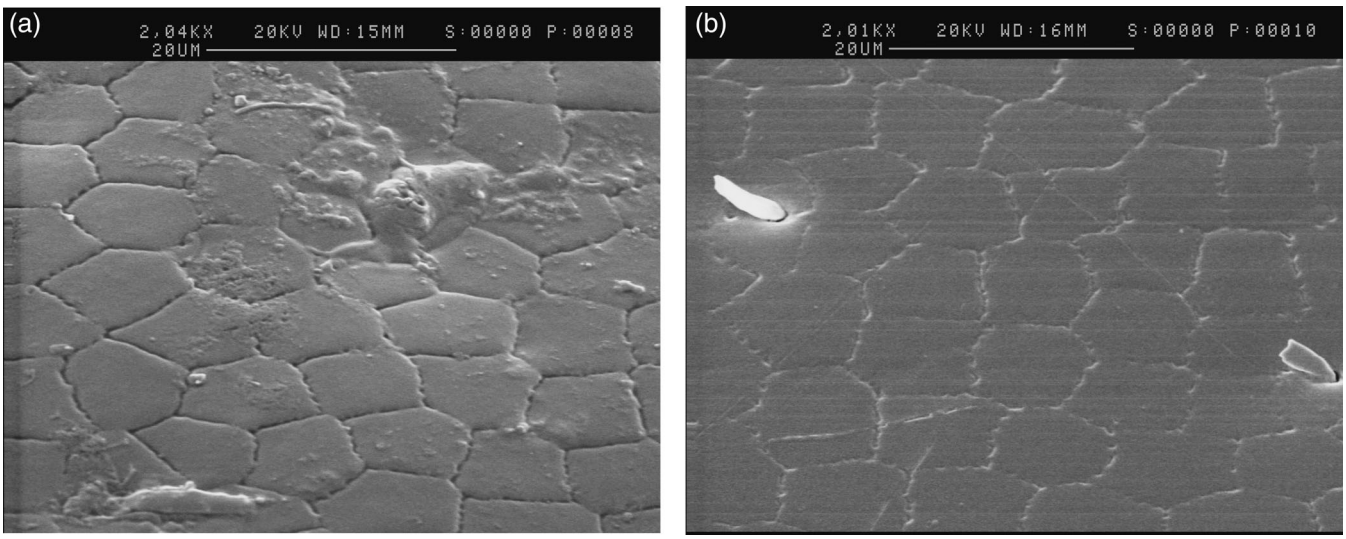

Figure 5. Cabbage stem flea beetle (*Psylliodes chrysocephala*) elytra cuticle observed through a scanning electron microscope (×2000) after treatment with fatty acids (a) and water (b).

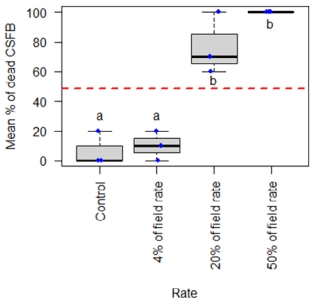

Figure 6. Percentage of dead cabbage stem flea beetle (CSFB) (*Psylliodes chrysocephala*) 1 day after treatment with lambda-cyhalothrin (pyrethroid) and control. The dotted red line represents the overall mean of the data. Different letters indicate significant differences ($P < 0.05$).

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*,[50,51] the aphid *Aphis gossypii* and the mealybug *Planococcus citri*.[50] Future work should focus on testing these physically acting products under field conditions.

Analysis of the CSFB elytra cuticle with scanning electron microscopy showed differences in the structure of CSFB elytra when treated with FLiPPER compared with the water control (Fig. 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[50,51] 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.

3.5 Lambda-cyhalothrin (pyrethroid) glass vial bioassay

CSFB mortality results are illustrated in Fig. 6. The mortality of CSFB differed with lambda-cyhalothrin concentration, with the two highest concentrations causing higher mortality than the lowest concentration and the control ($F = 40.07$, df = 3, $P < 0.001$). According to the IRAC protocol,[73] a mortality lower than 90% at 20% of the field rate indicates 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.[11]

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 *B. 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 the available literature suggests that this botanical biopesticide may be effective when combined with other biopesticides.

Further work is required to investigate the potential nontarget effects of the products tested here, as biopesticides have a range of attractive properties that make them good components of integrated pest management (IPM) programmes,[22] but it is important to consider the potential negative impacts of these products on nontarget organisms. There is, for example, uncertainty as to how safe azadirachtin is to nontarget organisms, with some studies concluding that it is safe,[74,75] while others have questioned this conclusion.[76–82] 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).[83] 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 (e.g., the use of adjuvants) and application techniques can improve the efficacy and reliability of products under field conditions. 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, which 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.[84] The work presented here is an important first step in identifying potentially effective tools that may be included in future IPM programmes. Biopesticides may then form one part of an IPM pyramid[7] that would also include other tools for the management of CSFB such as crop rotation, stubble management, seed rate, companion cropping, organic amendments and resistant or tolerant varieties[8,85] alongside monitoring and the use of natural enemies, to enable CSFB to be managed in a sustainable way.

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CONFLICT OF INTEREST

The authors declare that beyond the funding provided by Certis Belchim BV they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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