

Companion plants and straw mulch reduce cabbage stem flea beetle (*Psylliodes chrysocephala*) damage on oilseed rape

Gaëtan Seimandi-Corda,^{a*}  Julian Winkler,^{b,c} Todd Jenkins,^{a,d} Sascha M Kirchner^b and Samantha M Cook^a 



Abstract

Background: Plant diversification, especially sowing crops with the addition of companion plants has been demonstrated as a suitable practice to increase insect pest control in multiple cropping systems. Since the ban on use of neonicotinoid seed treatments in oilseed rape (OSR), the harvested area has reduced significantly in Europe, mainly because of the damage caused by cabbage stem flea beetle (*Psylliodes chrysocephala*). Several companion plants such as legumes and other species of Brassicaceae have been reported as potential companions for OSR but robust evaluation of their efficiency to reduce cabbage stem flea beetle damage in replicated trials is lacking.

Results: Four field trials were conducted in the UK and Germany to test the effect of different companion plants, or the addition of straw mulch, on cabbage stem flea beetle adult feeding and larval infestation in OSR. We found significant differences in the level of feeding damage between treatments in all experiments. Combinations of OSR with cereal companion plants or with straw mulch showed the strongest reduction in adult feeding damage. A protective effect of legumes was also observed in one trial. Differences in larval infestation were also observed between treatments but were not consistent and might be more related to the OSR plant biomass than to treatments.

Conclusion: This study shows that companion planting can protect OSR crops from cabbage stem flea beetle adult feeding damage. We show for the first time that not only legumes, but also cereals and the application of straw mulch can have a strong protective effect on the crop.

© 2023 The Authors. *Pest Management Science* published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Supporting information may be found in the online version of this article.

Keywords: intercropping; undersowing; mulching; legumes; cereal volunteers; rapeseed

1 INTRODUCTION

The simplification of farming systems, increasing field sizes and the extensive use of pesticides over past decades are considered to be the main drivers of biodiversity loss in agricultural landscapes.^{1–3} Implementation of new practices to increase plant diversity can potentially mitigate this loss of biodiversity while supporting ecosystem services.⁴ An increase in plant diversity can be achieved by the addition of trees (agroforestry) or flowers in or around the field (flower margins), but most often plant diversification is achieved by companion planting in which multiple crops are grown together in the same field.⁵ These plants can provide resources and shelter to the natural enemies of crop pests, which can help to regulate pest populations in the crop.^{4,6} Companion plants can have a direct effect on pest behaviour by being attractive or repellent to insect pests, and can be used as a trap or barrier crop^{7,8}; they can also affect host location behaviour and obfuscate the crop.^{9,10}

Oilseed rape (OSR, *Brassica napus*) is the most important oilseed crop in Europe and is mainly used to produce oil for human consumption and biofuel.¹¹ The crop is attacked by multiple insect pests over the course of its life cycle, making it particularly prone to insect damage.¹² Over recent years the OSR area harvested has reduced drastically in western Europe,¹³ and especially in the UK

* Correspondence to: Gaëtan Seimandi-Corda, Rothamsted Research, West Common, Harpenden, AL5 2JQ, UK. E-mail: gaetan.seimandi@outlook.fr

a Rothamsted Research, Harpenden, UK

b Faculty of Organic Agricultural Science, University of Kassel, Witzenhausen, Germany

c Advisory Team Organic Farming, Landesbetrieb Landwirtschaft Hessen, Witzenhausen, Germany

d Agriculture and Environment Department, Harper Adams University, Newport, UK

where the harvested area fell by 50% between 2012 and 2020.¹¹ This decline is largely because of the impact of the cabbage stem flea beetle (CSFB, *Psylliodes chrysocephala*).¹⁴ Adult *P. chrysocephala* damage the plant by feeding on cotyledons and young leaves early in the autumn, which can threaten crop establishment, and the larval stages feed on the petioles and stem causing reduced vigour and plant survival.¹² Neonicotinoid seed treatments were the main method used to control this insect, but with a ban on their use because of concerns over environmental impact (EU Regulation No. 485/2013)¹⁵ and the increase in CSFB populations resistant to pyrethroids,^{16,17} farmers are left without efficient options to manage this pest.¹⁸

In the UK, farmers have adapted their crop management practices to mitigate the impact of CSFB. Changes in the drilling date or sowing rate are reported as potential ways to manage this insect (reviewed by Ortega-Ramos et al.¹⁸). The use of companion plants sown with the OSR crop is also frequently reported by farmers and researchers as a potential way to reduce CSFB attack.¹⁸ The companion plant species used are diverse but usually have common characteristics; they are not too competitive with the OSR and/or are easily destroyed by herbicide application or by low temperatures in winter.

This study focused on four groups of companion plants: (i) the addition of legumes that are not too competitive such as clover and vetch. These plants can provide nutrients to the crop and improve its growth (particularly N from nitrogen-fixing bacteria that colonize the rhizosphere of leguminous plants)^{19,20} and have also been reported to influence CSFB attack.^{21–24} (ii) The addition of other Brassicaceae species. Some plant species such as turnip rape (*Brassica rapa oleifera*) are known to be preferred by CSFB over OSR and can be used in trap cropping systems.^{25,26} Other plants such as white mustard (*Sinapis alba*) are less preferred by CSFB and could be used as repellents.²⁷ The main issue with the use of Brassicaceae as companions for OSR is that they can compete with the crop and are difficult to destroy selectively. This can be overcome if the companion plants are drilled in strips within the crop or around the perimeter of the cropped area, or if an herbicide-resistant OSR cultivar is used.²⁸ (iii) The presence of cereal volunteers has been reported by farmers to protect OSR plants from CSFB attack.²⁹ It would be possible to delay destruction of the volunteers or drill cereals and destroy them once the peak immigration of CSFB has passed. (iv) Finally, farmers also report that direct drilling in cereal stubble of the previous crop can reduce CSFB attack.³⁰ This approach was tested using addition of straw mulch to simulate cereal trash left on the ground as part of direct drilling which has been demonstrated to reduce pest infestation in other cropping systems.^{31–33}

Despite companion planting making its way into farm practice, farmers lack robust evaluation of the effect of companion plants on CSFB damage caused by both adults and larvae. This is particularly true for systems located in areas where winters are mild and companion plants cannot be destroyed by winter frost. This lack of knowledge limits the development and use of alternative cropping strategies less dependent on synthetic pesticides. In this study we present results from different fields trials conducted in the UK over 3 years and in Germany in 1 year. In these trials, different companion plants and other management practices were tested for their impact on CSFB adult feeding damage, OSR crop plant biomass in autumn, and CSFB larval infestation. Insights into how the different treatments affect CSFB behaviour were also investigated.

2 MATERIALS AND METHODS

2.1 Study sites and treatments

Four experimental field trials were conducted between 2018 and 2021 in the UK at Harpenden (Hertfordshire) and in Germany at Witzenhausen (Hesse). These experiments tested the effect of different companion plants and the addition of straw mulch on CSFB adult feeding damage and larval infestation (summarized in Supporting Information, Table S1). In all the experiments OSR seeds were drilled using a seed drill, whereas companion plants and straw mulch were broadcasted manually (handfuls of seed were sprinkled on the soil at an even rate while steadily walking the length and breadth of the plot in a systematic manner).

2.1.1 Experiment 1

Experiment 1 was sown on 30 August 2018, at Rothamsted farm, Harpenden, UK; the following treatments were used:

- OSR mixed with white mustard (*Sinapis alba* cv. unknown, 150 seeds/m²);
- OSR mixed with Berseem clover (*Trifolium alexandrinum* cv. Tabor, 5 kg/ha);
- OSR mixed with winter wheat (*Triticum aestivum* cv. KWS Siskin, 800 seeds/m²);
- OSR with winter wheat (as above) – simulation of intercrop with plots split in half with one crop on each half;
- OSR surrounded by a 1-m-wide trap crop of turnip rape (*Brassica rapa* cv. Jupiter, 100 seeds/m²);
- OSR monocrop control.

In all treatments the winter OSR cv. PT279CL (Clearfield, Cor-teva) was used at a sowing rate of 70 seeds/m². Treatments were replicated six times in a Latin square with a plot size of 12 m × 12 m.

2.1.2 Experiment 2

Experiment 2 was sown on 19 August 2019, at Rothamsted farm; the following treatments were used:

- OSR mixed with wheat (cv. KWS Siskin, 800 seeds/m²);
- OSR mixed with barley (*Hordeum vulgare* cv. KWS Orwell, 800 seeds/m²);
- OSR mixed with rye (*Secale cereale* cv. Danielio, 800 seeds/m²);
- OSR mixed with oats (*Avena sativa* cv. Mascani, 800 seeds/m²);
- OSR covered with wheat straw mulch applied immediately after sowing (5.5 t/ha) – the chopped straw mulch was spread manually all over the plots to cover the soil homogeneously;
- OSR monocrop control.

In all treatments the winter OSR cv. Barbados was used at a sowing rate of 70 seeds/m². All the cultivars used in this experiment were winter cultivars. Four replicates were sown of each of the six treatments in a randomized complete block design (RCBD) with a plot size of 3 m × 9 m.

2.1.3 Experiment 3

Experiment 3 was sown on 24 September 2020 at Rothamsted farm; the following treatments were used:

- OSR mixed with Berseem clover (cv. Tabor, 5 kg/ha);
- OSR mixed with Berseem clover (cv. Tabor, 4 kg/ha) and vetch (*Vicia sativa* cv. Jose, 4 kg/ha);
- OSR mixed with oats (cv. Mascani, 800 seeds/m²);

- OSR surrounded by a 1-m wide trap crop of turnip rape (cv. Jupiter);
- OSR monocrop with herbicide application similar to the treatments with companion plants (low herbicide);
- OSR monocrop control with a standard herbicide regime (standard herbicide).

In all treatments the winter OSR cv. Barbados was used at a sowing rate of 70 seeds/m². Treatments were replicated six times in a Latin square with a plot size of 24 m × 24 m. To test the potential effect of the change in herbicide regime between plots with experimental treatments with and without companions, a treatment with low herbicide application was added. In this treatment, plots received reduced herbicide applications similar to the treatments with Berseem clover. However, because this experiment was terminated earlier than expected, herbicide was applied only on plots with oat to destroy the companion plant in winter. Consequently, there was no difference in herbicide application between plots with low and standard herbicide.

2.1.4 Experiment 4

Experiment 4 was sown on 9 February 2020 at Witzenhausen, Germany; the following treatments were used:

- OSR mixed with oat (cv. unknown, 800 seeds/m²);
- OSR covered with wheat straw mulch (5 t/ha);
- OSR monoculture control.

In all treatments the winter OSR cv. Armani was used at a sowing rate of 40 seeds/m². Treatments were replicated four times in a RCBD with a plot size of 15 m × 15 m.

After drilling, yellow water traps were placed at the crop edge on each side of the field where trials were located and 25 m inside the crop to monitor the arrival of the CSFB on the experiment.

2.2 Adult CSFB leaf damage

Estimation of adult CSFB feeding damage started once the OSR germinated, and CSFB were detected in the yellow water traps. Typical 'shotgun' holes were observed on the cotyledons and true leaves. The percentage of leaf area lost to feeding was estimated on individual plants with the help of a visual scale (Supporting Information, Fig. S1). In experiment 1, ten plants were chosen randomly on each plot; in experiment 2, five plants per quadrat were sampled in three quadrats (0.25 m²) randomly placed in each plot (15 plants/plot); and in experiments 3 and 4, three plants per quadrat were sampled in five quadrats (0.25 m²) randomly placed in the plot (15 plants/plot). Plants sampled within the quadrats were selected randomly.

The feeding damage was assessed four times in experiments 1 and 3 (September–November) and three times in experiments 2 and 4 (September–October). Each assessment was separated by 1–2 weeks (Supporting Information, Fig. S2).

2.3 Plant cover

The cover of the companion plants in each plot was recorded in the same quadrats and at the same time as adult feeding damage assessments were made on OSR crop plants. The percentage of cover of the different companion plants was recorded in three quadrats (0.25 m²) per plot in experiments 3 and 4; however, data were missing for the last sampling date of experiment 3.

2.4 Plant biomass

For experiments 2, 3 and 4, fifteen OSR plants per plot in the UK and five plants per plot in Germany were randomly sampled in each experiment in October and November (10 February 2019, 30 October 2020 and 11 September 2020, respectively), when plants were at BBCH growth stage 11–16 (one to six true leaves³⁴). These plants were returned to the laboratory and were oven dried overnight at 80 °C. They were then weighed (with a precision of 1 mg).

2.5 CSFB larval infestation

Between the end of November and February, three plants were collected at random from each plot and returned to the laboratory. Plants were then dissected under a binocular microscope and the leaves, petioles and stem were inspected for CSFB larvae. The number of leaves per plant was recorded. Because dissection is time consuming, plants were kept in a cold room (4 °C) for up to 5 days before being dissected. For experiments 1 and 2, one sampling session was conducted at the beginning of winter (12 March 2018 and 25 November 2019, respectively). Two sampling sessions were conducted for experiments 3 and 4, one assessment at the beginning of the winter on 23 November 2020 for experiment 3 and on 24 November 2020 for experiment 4, and another assessment was conducted at the end of the winter on 17 February 2021 for experiment 3 and on 25 February 2021 for experiment 4. In experiments 3 and 4, another set of three plants per plot were collected the following week and dissected to give a total of six plants per plot for each sampling period.

2.6 Data analysis

All analyses were performed using R version 4.1.3³⁵ and the packages lme4,³⁶ car,³⁷ and multcomp.³⁸

2.6.1 Difference in CSFB adult feeding damage, CSFB larval infestation and OSR plant weight between companion crop treatments

The effect of companion plant treatments on CSFB adult feeding damage was analysed using a linear mixed model (LMM) explaining the percentage of leaf damage by treatment, sampling date and their interaction as a fixed factor. The quadrat was nested in the sampling date, nested in the plot, nested in the block, and was used as random factor for the trials with RCBD. The interaction between the quadrat, the sampling date, the row and the column of the trial, as well as the interaction between the row and the column were used as random factors for experiments designed as Latin squares. The percentage of damage was square-root transformed to ensure normality of the residuals. Separate models were built for each experiment. Similar models were used to analyse the numbers of larvae per plant for each sampling assessment. The only difference was that the quadrat random factor was removed because plants were not sampled in quadrats for the larval infestation assessment. The number of larvae was square-root transformed to ensure normality of the residuals. Differences in the OSR plant biomass between treatments were also analysed with the same type of models for each experiment. The plant biomass was log transformed to ensure the normality of the residuals. The significance of the fixed factors was then tested using a Wald χ^2 test and pairwise comparisons were performed on the estimated marginal means for the number of larvae per plant and OSR plant biomass.

2.6.2 Relationship between CSFB adult feeding damage and companion plant cover

To test the relationship between the percentage of leaf area damaged by CSFB adults and the percentage of companion plant cover, data collected in 2020 in experiments 3 and 4 were used. The damage estimations were averaged for each quadrat where an estimation of the plant cover was available. Only the plots with OSR and oats, or OSR and legumes were used to establish this relationship because no companion plants were present in the OSR crop area of plots with treatments using straw mulch and turnip rape trap crop borders. The percentage of leaf area lost to adult CSFB feeding damage and the percentage of companion plant cover were logit transformed. An offset was added to scores of zero when present in the data. The relationship between the two variables was then tested with a LMM for each experiment. The sampling date was used as a fixed factor in the two models; the plot treatment was used as a fixed factor only for experiment 3 because only one treatment (OSR mixed with oats) was used in experiment 4. For experiment 3, the column, row, interaction between the column and row and interaction between the column, row and sampling date were used as random factors. The block and the interaction between the block and sampling date were also used as random factors for the data collected in experiment 4. The significance of the different fixed factors was then tested using a Wald χ^2 test.

2.6.3 Relationship between larval infestation and plant biomass

To test the relationship between the level of CSFB larval infestation and the plant biomass, the average level of larval infestation (number of larvae/OSR plant) and the average OSR plant biomass were computed for each plot in experiments 2, 3 and 4, where those values were available. When two assessments for larval infestation were performed on the same experiment, only the first date, that is the date closest to the weight measurement, was used. The relationship between the average number of CSFB larvae per plant in each plot and the log of the average plant biomass per plot was analysed using a multi-trial LMM allowing separate residual variance terms for each trial, taking account of their different blocking structures (RCBD or Latin squares). The treatment, the experiment, as well as their interactions were also included as explanatory factors. The effects of the different explanatory variables were tested using a Wald χ^2 test.

3 RESULTS

3.1 Effect of companion crops on adult CSFB feeding damage

Significant differences between treatments in the percentage of leaf area loss were observed for each experiment (Fig. 1; experiment 1: $\chi^2 = 144.66$, $df = 5$, $P < 0.001$; experiment 2: $\chi^2 = 62.27$, $df = 5$, $P < 0.001$; experiment 3: $\chi^2 = 240.44$, $df = 5$, $P < 0.001$;

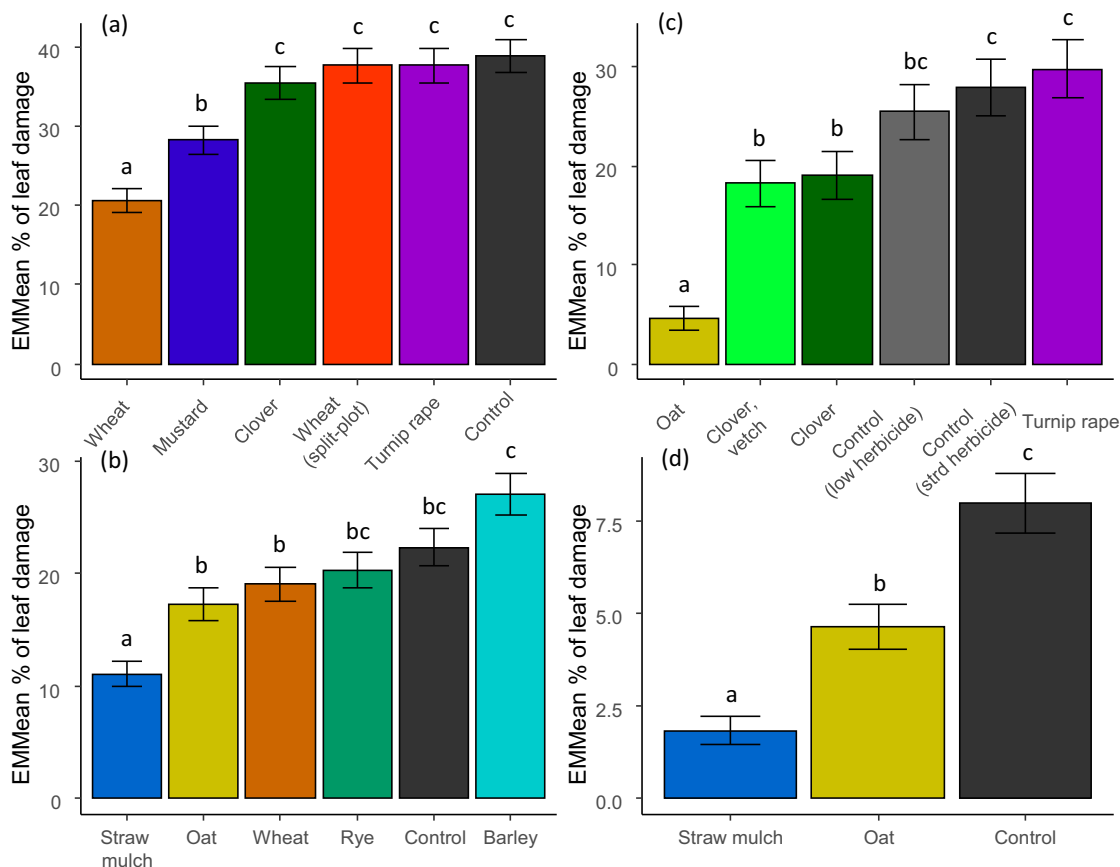


Figure 1. Estimated marginal mean (\pm SE) percentage of leaf area loss caused by adult cabbage stem flea beetle (*Psylliodes chrysocephala*) on oilseed rape in different companion plant treatments. Means are averaged over multiple sampling dates. Significant differences between treatments are indicated with different letters. (a) Experiment 1 conducted at Rothamsted Research, 2018. (b) Experiment 2 conducted at Rothamsted Research, 2019. (c) Experiment 3 conducted at Rothamsted Research, 2020. (d) Experiment 4 conducted at Witzhausen, Germany, 2020.

experiment 4: $\chi^2 = 103.86$, $df = 2$, $P < 0.001$). The OSR plants when sown with cereal companion plants were less damaged than OSR in control treatments of experiments 1, 3 and 4. However, no reduction in damage was observed for plots with wheat–OSR split-plots ('intercrop') compared to control plots. A significant reduction in damage was also observed in plots sown with a white mustard companion in experiment 1. No significant differences between the OSR monoculture control and the treatments with legume companions were found in experiment 1, but significant differences were found in experiment 3. In experiment 3, the differences between the feeding damage observed on OSR plants in the control and legume treatments increased over time (Supporting Information, Fig. S2). In experiments 1 and 3 no significant difference between OSR in plots with a turnip rape trap crop and control plots was observed. Finally, a significant reduction in the level of feeding damage was observed in plots with straw mulch compared with control plots in experiments 2 and 4. Significant differences between sampling dates were also found in all experiments except experiment 4 (experiment 1: $\chi^2 = 20.71$, $df = 3$, $P < 0.001$; experiment 2: $\chi^2 = 18.76$, $df = 2$, $P < 0.001$; experiment 3: $\chi^2 = 71.50$, $df = 3$, $P < 0.001$; experiment 4: $\chi^2 = 0.194$, $df = 2$, $P = 0.907$). An effect of the interaction between the sampling date and the treatment was observed only for experiment 3 (experiment 1: $\chi^2 = 18.53$, $df = 15$, $P = 0.236$; experiment 2: $\chi^2 = 13.64$, $df = 10$, $P = 0.19$; experiment 3: $\chi^2 = 47.12$, $df = 15$, $P < 0.001$; experiment 4: $\chi^2 = 3.631$, $df = 4$, $P = 0.458$).

3.2 Relationship between plant cover and adult CSFB damage

Using the data recorded during the assessment of feeding damage in experiments 3 and 4, it was possible to relate the average damage level per quadrat to the companion plant cover. This relationship is significant and negative in experiment 3 (Fig. 2(a); $\chi^2 = 10.42$, $df = 1$, $P = 0.001$), with an effect of the treatment ($\chi^2 = 32.20$, $df = 2$, $P < 0.001$), the sampling date ($\chi^2 = 6.46$,

$df = 2$, $P = 0.04$) and the interaction between the sampling date and treatment ($\chi^2 = 10.6$, $df = 4$, $P = 0.031$). In experiment 4, no significant effect of plant cover (Fig. 2(b); $\chi^2 = 1.05$, $df = 1$, $P = 0.307$), or the sampling date ($\chi^2 = 1.28$, $df = 2$, $P = 0.526$) was found. The treatment effect was not tested in this experiment as only data on the mixture of OSR with oat were used.

3.3 Effect of companion plants on OSR plant biomass, CSFB larval infestation and the relationship between OSR plant biomass and larval infestation

The number of larvae per plant differed significantly between treatments for each experiment and sampling date (Fig. 3; experiment 1: $\chi^2 = 20.9$, $df = 5$, $P = 0.001$; experiment 2: $\chi^2 = 92.1$, $df = 4$, $P < 0.001$; experiment 3, date 1: $\chi^2 = 46.6$, $df = 5$, $P < 0.001$; experiment 3, date 2: $\chi^2 = 20.9$, $df = 5$, $P = 0.001$; experiment 4, date 1: $\chi^2 = 49.14$, $df = 2$, $P < 0.001$; experiment 4, date 2: $\chi^2 = 37.1$, $df = 2$, $P < 0.001$). For the two trials in which larval infestation was estimated twice, the average number of larvae per plant per plot in autumn was correlated with the number found later in the season (experiment 3: $P < 0.001$, $df = 34$, $r = 0.79$; experiment 4: $P = 0.006$, $df = 9$, $r = 0.76$). In all experiments, OSR plants sown with cereal companions or mulched with straw were significantly less infested by CSFB larvae than the OSR control plants (except experiment 3, winter samples) (Fig. 3). The number of larvae in OSR plants with all other treatments was not consistently significantly reduced. In experiment 2, plots with barley were not sampled because most of the OSR died by the time of the larval sampling assessments.

Significant differences in the dry biomass of OSR plants were observed between treatments in experiment 2 ($\chi^2 = 188.9$, $df = 5$, $P < 0.001$), experiment 3 ($\chi^2 = 11.5$, $df = 5$, $P = 0.041$) and experiment 4 ($\chi^2 = 175.4$, $df = 2$, $P < 0.001$). In experiments 2 and 4, OSR plants grown with cereals had a significantly lower biomass than OSR plants grown with straw mulch or OSR plants in control plots (Supporting Information, Fig. S3). No significant difference in plant dry weight was found between treatments by pairwise comparisons in experiment 3.

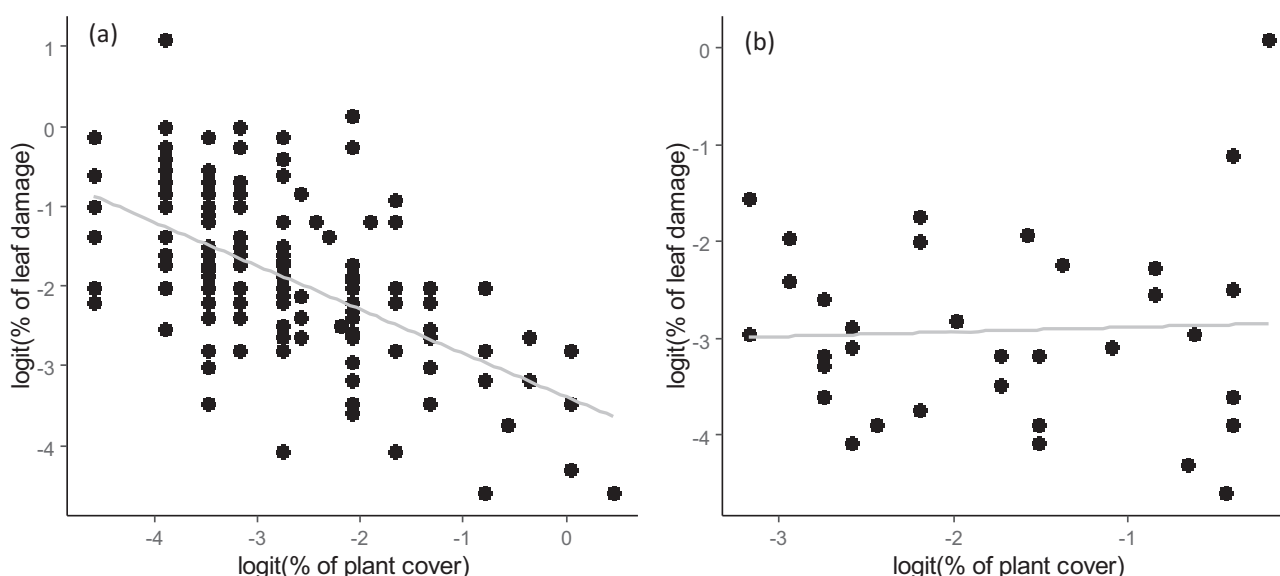


Figure 2. Relationship between the logit of the percentage of oilseed rape leaf area damaged by cabbage stem flea beetles (*Psylliodes chrysocephala*) and the proportion of ground covered by companion plants. Data from (a) experiment 3 conducted at Harpenden in 2020 (including oat, clover and vetch companions) and (b) experiment 4 (oat companions) conducted at Witzhenhausen in 2020. Grey lines represent linear regression between the two variables.

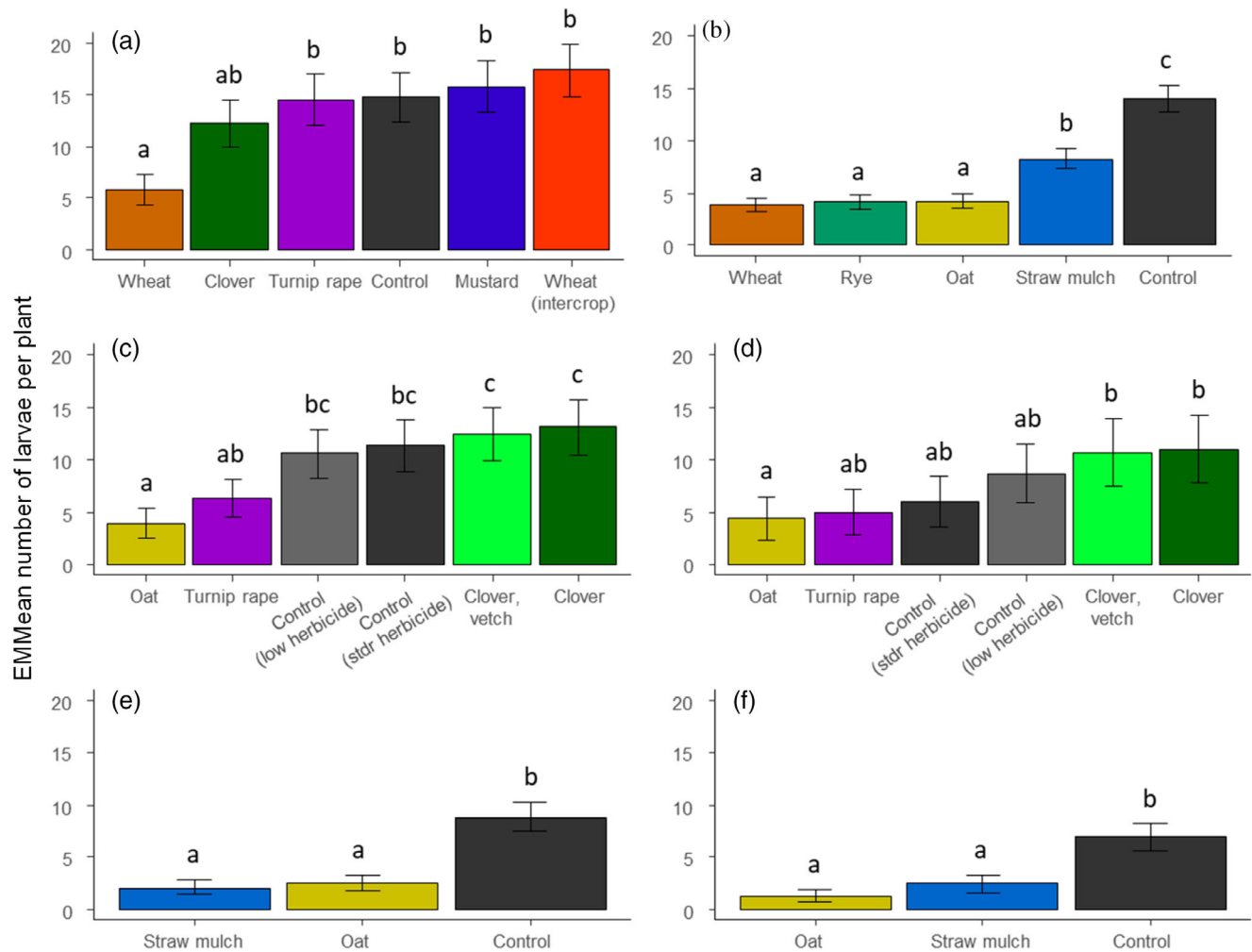


Figure 3. Estimated marginal mean (\pm SE) number of cabbage stem flea beetle larvae (*Psylliodes chrysocephala*) per oilseed rape plant grown with different companion plants. (a) Experiment 1 conducted at Rothamsted Research, 2018. (b) Experiment 2 conducted at Rothamsted Research, 2019. (c,d) Experiment 3 conducted at Rothamsted Research, 2020 (c) autumn sample, (d) winter sample. (e,f) Experiment 4 conducted at Witzenhausen, Germany, 2020 (e) autumn sample, (f) winter sample.

A significant relationship between average plant biomass per plot and average larval infestation was found (Fig. 4; $\chi^2 = 52.13$, $df = 1$, $P < 0.001$). This relationship is affected by the treatment ($\chi^2 = 91.31$, $df = 8$, $P < 0.001$), the experimental trial ($\chi^2 = 48.54$, $df = 2$, $P < 0.001$) and their interaction ($\chi^2 = 25.46$, $df = 3$, $P < 0.001$).

4 DISCUSSION

CSFB is a major pest of OSR in Europe and farmers currently lack reliable options to manage this insect.¹⁸ Combining OSR with companion crops, leaving volunteers or stubble trash in the field (here simulated by the sown cereal treatments and the addition of a straw mulch, respectively) have been proposed as potential ways to reduce CSFB infestation.^{21,23,24,29,30,39} Many farmers are taking up this practice,^{40,41} but there is little evidence to support efficacy. In the current study, four field experiments were conducted to test the effect of different companion plants and other management measures on CSFB attack by both adult and larval stages. Companion planting and straw mulch significantly reduced adult damage and larval abundance, therefore showing

great promise as strategies to control CSFB, but some treatments were more effective than others, and there were inconsistencies between study years.

The primary concern of many farmers is crop establishment; adult CSFB feeding damage can completely devastate newly emerging plants and result in failure to establish and crop loss.⁴² Companion plants that aid establishment by reducing CSFB attack are therefore in high demand by farmers. In the current study, a reduction in CSFB adult feeding damage on OSR plants was observed when companion plants such as mustard, cereals and legumes were grown with the crop plants, or when the crop was mulched with wheat straw. This reduction was strong and observed in most years of the experiment when OSR was combined with cereals (wheat or oat). No significant reductions in damage were observed between plots with companion cereals and OSR only in two situations: (i) in OSR–wheat split-plots that simulated intercropping in experiment 1, suggesting that OSR and the companion plants need to be spatially close or mixed to have a protective effect; and (ii) in experiment 2 when the environmental conditions that year (2019) were locally very dry early in the season (45 mm rain in August) – this is likely to have

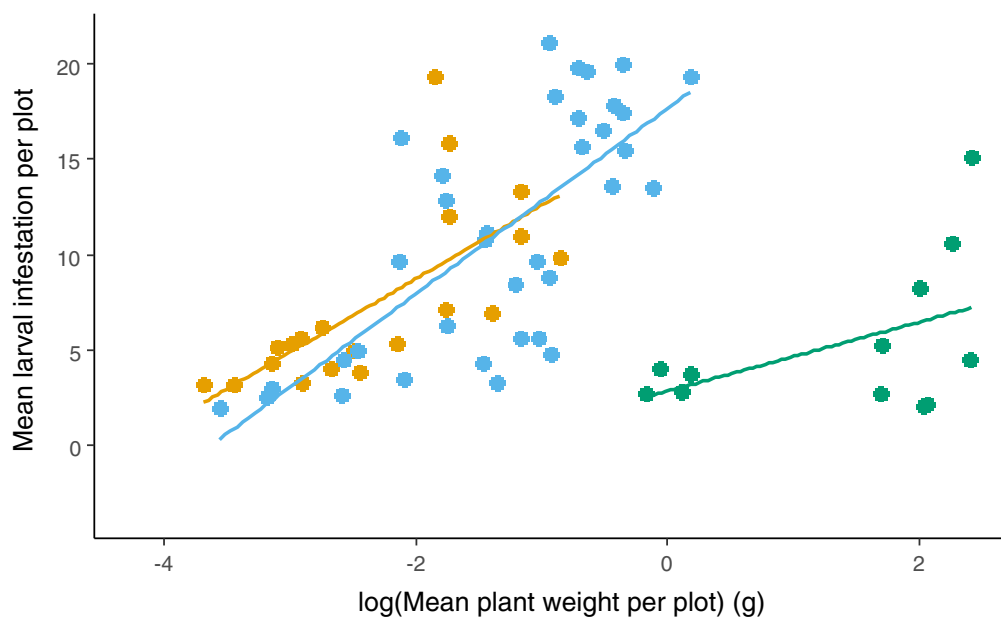


Figure 4. Relationship between the mean number of cabbage stem flea beetle (*Psylliodes chrysocephala*) larvae per oilseed rape plant per plot and the log of the mean plant weight (g) per plot. Lines represent linear regression between the two variables for each experiment. Orange: experiment 2 conducted at Rothamsted Research, UK, 2019; blue: experiment 3 conducted at Rothamsted Research, UK, 2020; green: experiment 4 conducted at Witzenhäusen, Germany, 2020.

strongly constrained crop growth and resulted in the cereal companions competing with the OSR plants for water during establishment. As companion plants, cereals have the benefit of fast establishment, quickly forming a dense cover. These trials are the first demonstration that cereals combined with OSR can reduce CSFB attack. OSR is usually included in a rotation with cereals and combination of OSR with a cereal can be achieved by leaving volunteers to grow early in the season.⁴³ This method has the benefit of being cheap for farmers because no cost is associated with the seeds and sowing of the cereal, but the volunteer density can be spatially uneven. The use of sown cereals such as oat that are cheaper than wheat and barley and easy to destroy by herbicide application can be an alternative to volunteers. Combining cereals and Brassicaceae is not common because the two crops tend to compete with each other.⁴⁴ Consequently, it is important to destroy the cereal companions as soon as the OSR has passed the most susceptible stage; optimization of the timing of this and the seed rate of the cereals to reduce the competition requires further work.

The field trials also demonstrated that the application of straw mulch significantly reduced CSFB adult attack in both trials where this method was tested. The effect of the mulch is in line with results obtained from experiments on potato that showed a strong reduction in Colorado potato beetle (*Leptinotarsa decemlineata*) and aphid infestation in plots treated with mulch compared with controls.^{45–47} The mulch also has the benefit that it does not compete with the crop; part of its success was probably due to its moisture-retaining capacity, helping to maintain soil moisture early in the season. This might explain why the OSR plants grown with straw mulch in experiment 2 in 2019, which was exceptionally dry, had higher biomass than in the other treatments. Addition of mulch to the crop at the rate used in our experiments (5 t/ha) and at a large scale can be challenging and costly (B. Wenzel, pers. comm.) but as part of direct drilling practice, leaving straw and stubble from the previous cereal crop on the soil

surface could help to reduce adult CSFB attack.³⁰ The amount of mulch applied in our experiment was likely to be more than that left as part of direct drilling strategies and further experiments are needed to optimize the amount required.

The effect of the legumes on reducing adult CSFB feeding attack was observed in experiment 3 but not in experiment 1. This is likely to be because of reduced establishment of the Berseem clover in experiment 1 compared with experiment 3. The effect of clover, and especially Berseem clover, on reducing CSFB adult feeding damage has been observed previously^{23,39} and is confirmed by our results. Clover has the benefit of not competing with the OSR (pers. obs.) but takes time to properly establish and create a dense cover. It is interesting to note that the protective effect of the clover observed in experiment 3 increased over time, probably with the growth of the clover during the experiment. This could explain the negative relationship observed in this trial between the companion plant density and the CSFB damage level. To reduce this delayed effect, clover could be sown before the OSR to ensure that a dense companion cover develops to protect the crop during its susceptible establishment phase.

In the only field trial in which OSR was combined with white mustard companion plants, a reduction in adult feeding damage was found which supports observations from a previous study.²⁷ However, because white mustard is a Brassicaceae, like OSR, this strategy is limited by the need to use a specific herbicide-resistant OSR variety (e.g. Clearfield). Because of potential problems of timing removal of the companion, this strategy was not developed further. Contrary to observations in other studies,^{25,27,26} no effect of a trap crop of turnip rape was observed in experiments 1 and 3. This could be because CSFB pressure in autumn 2018 and 2020 was very high and probably higher than the conditions in these previous studies. CSFB in experiments 1 and 3 almost completely destroyed the turnip rape and OSR plants, pointing to the fact that the trap crop effect was not strong enough to control the sizable pest infestation.

The effect of companion plants on CSFB larval infestation has already been reported for legumes.^{23,24,39} Differences between larval infestation in OSR plants in control plots and plots with companions were found in this study, but they are not consistent from one trial to the other. The fact that we found a strong positive relationship between OSR plant biomass and larval infestation suggests that under conditions of high insect pressure, plant biomass can be a limiting factor for the larvae and that larval infestation is more dependent on host plant biomass than the presence or absence of companion plants. However, it is important to note that in experiments 2 and 4 there were significantly fewer larvae in plants in plots with straw mulch than in the control plots, and that no significant difference in plant biomass between these two treatments was observed. The same was observed between the oat and control treatments in experiment 3. This indicates that it might be possible to reduce larval infestation while having a limited impact on plant biomass. Observations conducted by Breitenmoser *et al.* found a protective effect of a legume (*Vicia faba*) on OSR against CSFB larval infestation with an increase in plant biomass.²⁴

The mismatch between the effect of companion plants on adult CSFB attack and larval infestation could be explained by the different ways in which the companion plants affect host plant location and acceptance for feeding and oviposition. Companion plants could mask the cues, both visual and olfactory, used at distance by the insects during the crop colonization phase,⁴⁸ and could explain the difference in feeding damage observed.⁴⁹ Oviposition occurs later, after crop colonization and an initial feeding phase.⁵⁰ The effect of companion plants may not be as efficient at short range and/or may not affect host plant location and acceptance for oviposition in the same way as they do for feeding, thereby explaining the disparity.

The use of companion plants to protect OSR crops from CSFB attack is promising. Here we demonstrated a reduction in adult feeding damage when OSR is combined with cereals, legumes or straw mulch. The addition of straw mulch limited the larval infestation without affecting plant growth. These results demonstrate that this strategy can easily be transferred to farmers, but there is a need for more research to define the best agronomical practices, such as the seed rate of the companions in relation to crop plant density and their sowing date. The addition of companion plants can deliver additional ecosystem services such as a reduction in infestation by other insect pests (e.g. aphids, cabbage root fly, stem mining weevils and pollen beetles),^{24,51–53} increased biological control services from natural enemies,^{54,55} or improved weed management.⁵⁶ By providing a more functionally complex habitat in the crop, companion planting systems by their nature of increased plant diversity can also promote an increased diversity of insects within the field⁴ and can help farmers to farm more sustainably and mitigate the negative effects of food production on the environment.

ACKNOWLEDGEMENTS

We thank Rothamsted Research farm team (UK) and Günter Kellner (Germany) for managing the OSR trials conducted for this study on Rothamsted farm and Mühlendorf Kellner farm, respectively. We are grateful to Alex Dye, Jamie Hall, Tom Hood and Deborah Wright (UK) and Simeon Leisch-Waskönig (Germany) for assisting with sampling, to Chris Hall (UK) for help with desiccating plants, as well as Rainer Wedemeyer and Joachim Deckers (Germany) for technical support. We would like to thank Suzanne

Clark for support with experimental design and statistical analyses. We are indebted to Yoann Bourhis for creating the damage level scale used for these experiments. Rothamsted Research receives grant-aided support from the UK BBSRC. This work was funded by the European Union's Horizon 2020 Research and Innovation programme as part of the project EcoStack (Grant Agreement no. 773554). SMC is part funded by EcoStack and AgZero+ (NE/W005050/1, jointly supported by UK NERC and BBSRC).

DATA AVAILABILITY STATEMENT

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

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

- Gámez-Virués S, Perović DJ, Gossner MM, Börschig C, Blüthgen N, de Jong H *et al.*, Landscape simplification filters species traits and drives biotic homogenization. *Nat Commun* **6**:8568 (2015).
- Gagic V, Holding M, Venables WN, Hulthen AD and Schellhorn NA, Better outcomes for pest pressure, insecticide use, and yield in less intensive agricultural landscapes. *P Natl Acad Sci USA* **118**: e2018100118 (2021).
- Brühl CA and Zaller JG, Biodiversity decline as a consequence of an inappropriate environmental risk assessment of pesticides. *Front Environ Sci* **7**:177 (2019).
- Wan N-F, Zheng X-R, Fu L-W, Kiaer LP, Zhang Z, Chaplin-Kramer R *et al.*, Global synthesis of effects of plant species diversity on trophic groups and interactions. *Nat Plants* **6**:503–510 (2020).
- Hufnagel J, Reckling M and Ewert F, Diverse approaches to crop diversification in agricultural research. A review. *Agron Sustain Dev* **40**:14 (2020).
- Letourneau DK, Armbrrecht I, Rivera BS, Lerma JM, Carmona EJ, Daza MC *et al.*, Does plant diversity benefit agroecosystems? A synthetic review. *Ecol Appl* **21**:9–21 (2011).
- Cook SM, Khan ZR and Pickett JA, The use of push-pull strategies in integrated pest management. *Annu Rev Entomol* **52**:375–400 (2007).
- Shelton A and Badenes-Perez F, Concepts and applications of trap cropping in pest management. *Annu Rev Entomol* **51**:285–308 (2006).
- Morley K, Finch S and Collier RH, Companion planting—behaviour of the cabbage root fly on host plants and non-host plants. *Entomol Exp Appl* **117**:15–25 (2005).
- Finch S and Collier R, Host-plant selection by insects—a theory based on “appropriate/inappropriate landings” by pest insects of cruciferous plants. *Entomol Exp Appl* **96**:91–102 (2000).
- FAOSTAT, 2022. <https://www.fao.org/faostat/en/#home> [accessed 13 October 2022].
- Williams IH, The major insect pests of oilseed rape in Europe and their management: an overview, in *Biocontrol-Based Integrated Management of Oilseed Rape Pests*, ed. by Williams IH. Springer, Dordrecht, pp. 1–43 (2010).
- Ortega-Ramos P, Cook S and Mauchline AL, How contradictory EU policies led to the development of a pest: the story of oilseed rape and the cabbage stem flea beetle. *GCB Bioenergy* **14**:258–266 (2022).
- Andert S, Ziesemer A and Zhang H, Farmers' perspectives of future management of winter oilseed rape (*Brassica napus* L.): a case study from North-Eastern Germany. *Eur J Agron* **130**:126350 (2021).
- Wood TJ and Goulson D, The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013. *Environ Sci Pollut Res* **24**: 17285–17325 (2017).
- Højland DH, Nauen R, Foster SP, Williamson MS and Kristensen M, Incidence, spread and mechanisms of Pyrethroid resistance in European populations of the cabbage stem flea beetle, *Psylliodes chrysocephala* L. (Coleoptera: Chrysomelidae). *PLoS One* **10**:e0146045 (2015).

- 17 Willis CE, Foster SP, Zimmer CT, Elias J, Chang X, Field LM *et al.*, Investigating the status of pyrethroid resistance in UK populations of the cabbage stem flea beetle (*Psylliodes chrysocephala*). *Crop Prot* **138**:105316 (2020).
- 18 Ortega-Ramos PA, Coston DJ, Seimandi-Corda G, Mauchline AL and Cook SM, Integrated pest management strategies for cabbage stem flea beetle (*Psylliodes chrysocephala*) in oilseed rape. *GCB Bioenergy* **14**:267–286 (2022).
- 19 Lorin M, Jeuffroy M-H, Butier A and Valantin-Morison M, Undersowing winter oilseed rape with frost-sensitive legume living mulches to improve weed control. *Eur J Agron* **71**:96–105 (2015).
- 20 Cadoux S, Sauzet G, Valantin-Morison M, Pontet C, Champolivier L, Robert C *et al.*, Intercropping frost-sensitive legume crops with winter oilseed rape reduces weed competition, insect damage, and improves nitrogen use efficiency. *OCL* **22**:D302 (2015).
- 21 Trotin V and Ginestiere Y, Colza associé a des plantes de service: comparaison d'espèces et d'itinéraires techniques dans un réseau de parcelles, December 2012 <https://agriculture-de-conservation.com/sites/agriculture-de-conservation.com/IMG/pdf/colza-associe-poitou.pdf> [accessed 12 October 2022].
- 22 Pickering F, White S, Ellis S, Collins L, Corkley I, Leybourne D *et al.*, Integrated pest management of cabbage stem flea beetle in oilseed rape. *Outlooks Pest Manag* **31**:284–290 (2020).
- 23 Breitenmoser S, Steinger T, Hiltbold I, Grosjean Y, Nussbaum V, Bussereau F *et al.*, Effet des plantes associées au colza d'hiver sur les dégâts d'altises. *Rech Agron Suisse* **11**:16–25 (2020).
- 24 Breitenmoser S, Steinger T, Baux A and Hiltbold I, Intercropping winter oilseed rape (*Brassica napus* L.) has the potential to lessen the impact of the insect pest complex. *Agronomy* **12**:723 (2022).
- 25 Barari H, Cook SM, Clark SJ and Williams IH, Effect of a turnip rape (*Brassica rapa*) trap crop on stem-mining pests and their parasitoids in winter oilseed rape (*Brassica napus*). *Biocontrol* **50**:69–86 (2005).
- 26 Coston DJ, Breeze TD, Clark SJ, Field LM, Potts SG, Kightley S *et al.*, Companion planting as a method of reducing pest pressure from *Psylliodes chrysocephala* on winter oilseed rape (*Brassica napus*). *Bulletin IOBC/wprs* **157**:120–130 (2022).
- 27 Coston DJ, Quantifying the impacts of the neonicotinoid restriction on oilseed rape pest control and productivity [dissertation] University of Reading (UK) (2021).
- 28 Pfenning M, Kehler R and Bremer H, New perspectives for weed control in winter oilseed rape due to the introduction of the Clearfield® system. *Julius-Kuhn-Arch* **2**:435–442 (2012).
- 29 Karley A, Synthesis report on national stakeholder meetings (Report, Public) Deliverable 1.1 (D1) (2018). <http://plant-teams.org/wp-content/uploads/2021/03/D1-Deliverable-1.1-National-stakeholder-meetings-ORC.pdf> [accessed 12 October 2022].
- 30 White S, Ellis S, Pickering F, Leybourne D, Corkley I, Kendall S *et al.*, Integrated pest management of cabbage stem flea beetle in oilseed rape, Project Report No. 623 ADHB (2020) (2020).
- 31 Brust GE, Natural enemies in straw-mulch reduce Colorado potato beetle populations and damage in potato. *Biol Control* **4**:163–169 (1994).
- 32 Vincent C, Hallman G, Panneton B and Fleurat-Lessard F, Management of agricultural insects with physical control methods. *Annu Rev Entomol* **48**:261–281 (2003).
- 33 Schmidt MH, Thewes U, Thies C and Tschamtk T, Aphid suppression by natural enemies in mulched cereals. *Entomol Exp Appl* **113**:87–93 (2004).
- 34 Lancashire PD, Bleiholder H, Boom TVD, Langelüddeke P, Stauss R, Weber E *et al.*, A uniform decimal code for growth stages of crops and weeds. *Ann Appl Biol* **119**:561–601 (1991).
- 35 R Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing Vienna, Austria (2022).
- 36 Bates D, Mächler M, Bolker B and Walker S, Fitting linear mixed-effects models using lme4 *arXiv preprint arXiv:1406.5823* (2014).
- 37 Fox J, Friendly M and Weisberg S, Hypothesis tests for multivariate linear models using the car package. *R J* **5**:39 (2013).
- 38 Hothorn T, Bretz F, Westfall P, Heiberger RM, Schuetzenmeister A, Scheibe S *et al.*, Package “multcomp,” simultaneous inference in general parametric models project for statistical computing Vienna, Austria (2016).
- 39 Emery SE, Anderson P, Carlsson G, Friberg H, Larsson MC, Wallenhammar A-C *et al.*, The potential of intercropping for multifunctional crop protection in oilseed rape (*Brassica napus* L.). *Front Agron* **3**:782686 (2021).
- 40 Could companion cropping be the key to successful OSR crops? Farmers Weekly, 30 April 2020 <https://www.fwi.co.uk/arable/osr/could-companion-cropping-be-the-key-to-successful-osr-crops> [accessed 28 October 2022].
- 41 Tips on growing successful oilseed rape companion crops Farmers Weekly, 17 May 2022 <https://www.fwi.co.uk/arable/osr/tips-on-growing-successful-oilseed-rape-companion-crops> [accessed 28 October 2022].
- 42 Dewar AM and Walters K, BCPC pests and Beneficials group inaugural review meeting—can we continue to grow oilseed rape? *Outlooks Pest Manag* **27**:65–69 (2016).
- 43 Hegewald H, Wensch-Dorendorf M, Sieling K and Christen O, Impacts of break crops and crop rotations on oilseed rape productivity: a review. *Eur J Agron* **101**:63–77 (2018).
- 44 Pridham JC and Entz MH, Intercropping spring wheat with cereal grains, legumes, and oilseeds fails to improve productivity under organic management. *Agron J* **100**:1436–1442 (2008).
- 45 Stoner K, Ferrandino F, Gent M, Elmer W and Lamondia J, Effects of straw mulch, spent mushroom compost, and fumigation on the density of Colorado potato beetles (Coleoptera: Chrysomelidae) in potatoes. *J Econ Entomol* **89**:1267–1280 (1996).
- 46 Kirchner S, Hiltunen L, Santala J, Döring T, Ketola J, Kankaala A *et al.*, Comparison of straw mulch, insecticides, mineral oil, and birch extract for control of transmission of potato virus Y in seed potato crops. *Potato Res* **57**:59–75 (2014).
- 47 Saucke H and Döring TF, Potato virus Y reduction by straw mulch in organic potatoes. *Ann Appl Biol* **144**:347–355 (2004).
- 48 Williams IH and Cook SM, Crop location by oilseed rape pests and host location by their parasitoids, in *Biocontrol-Based Integrated Management of Oilseed Rape Pests*, ed. by Williams IH. Springer Netherlands, Dordrecht, pp. 215–244 (2010).
- 49 Ben-Issa R, Gomez L and Gautier H, Companion plants for aphid pest management. *Insects* **8**:112 (2017).
- 50 Bonnemaïson L and Jourdhueil P, L'altise d'hiver du colza (*Psylliodes chrysocephala* L.). *Ann Épiphyties* **4**:345–524 (1954).
- 51 Theunissen J, Booij C and Lotz L, Effects of intercropping white cabbage with clovers on pest infestation and yield. *Entomol Exp Appl* **74**:7–16 (1995).
- 52 Finch S and Kienegger M, A behavioural study to help clarify how undersowing with clover affects host-plant selection by pest insects of brassica crops. *Entomol Exp Appl* **84**:165–172 (1997).
- 53 Hooks C, Hinds J, Zobel E and Patton T, Impact of crimson clover dying mulch on two eggplant insect herbivores. *J Appl Entomol* **137**:170–180 (2013).
- 54 Booij C, Noorlander J and Theunissen J, Intercropping cabbage with clover: effects on ground beetles. *Biol Agric Hortic* **15**:261–268 (1997).
- 55 Prasifka JR, Schmidt N, Kohler KA, O'neal ME, Hellmich RL and Singer J, Effects of living mulches on predator abundance and sentinel prey in a corn-soybean-forage rotation. *Environ Entomol* **35**:1423–1431 (2006).
- 56 Verret V, Gardarin A, Pelzer E, Médiène S, Makowski D and Valantin-Morison M, Can legume companion plants control weeds without decreasing crop yield? A meta-analysis. *Field Crop Res* **204**:158–168 (2017).