Research Article

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Relative efficiency of methods to estimate cabbage stem flea beetle (Psylliodes chrysocephala) larval infestation in oilseed rape (Brassica napus)

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Abstract

Background: The cabbage stem flea beetle (CSFB; *Psylliodes chrysocephala*) is an important pest of winter oilseed rape (OSR) crops in Europe. Damage is caused by adults feeding on young leaves and by larvae mining in the leaves and stems. The most common method to estimate the severity of CSFB larval infestation is by plant dissection, which is time consuming and labour intensive. Two alternative methods have been proposed: extraction of larvae from desiccating plants and counting the percentage of leaves with scars left by larvae on plant petioles. These methods are easy to implement and less time consuming than plant dissection but have not been properly validated.

Results: OSR plants were sampled in five different experiments and the two alternative methods tested; assessment of the total number of scars per plant as a predictor of the number of larvae was also tested. The number of larvae remaining in plants following various periods of desiccation was checked via plant dissection. We found that the desiccation method is efficient, giving reliable results after 7 days (76% of the total larvae extracted) with good accuracy in estimating the number of larvae per plant (±0.38 larvae per plant). The total number of scars also gives a reliable estimation of the number of larvae ($r = 0.76$, accuracy of ±1.01 larvae per plant).

Conclusion: OSR plant desiccation and the total number of scars per plant are reliable methods to estimate CSFB larval infestation and will facilitate monitoring of this cryptic pest stage.
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Keywords: stem miners; leaf scars; Berlese method; plant dissection; rapeseed; threshold detection

1 INTRODUCTION

The cabbage stem flea beetle (CSFB, *Psylliodes chrysocephala*) is one of the most important pests of winter oilseed rape (OSR) in Europe.[1] With the European Union (EU) ban on use of neonicotinoid insecticides, CSFB management is becoming increasingly difficult, especially in the United Kingdom (UK) and northern Europe.[2] Due to severe infestations in recent years, farmers are struggling to grow OSR which is leading to a reduction in OSR area grown in the main producer countries (i.e. France, Germany, UK).[3,4] The adult stage is particularly damaging to the crop early in autumn when plants are establishing (have less than four leaves),[5] but once the crop is established, larvae have the capacity to cause severe yield losses with control thresholds in Europe ranging from one to five larvae per plant.[2,6] CSFB adults lay eggs on or near the plant in the soil and after hatching larvae move into the plant and feed by mining the plant's leaf veins, petioles, and stem.[7] Larvae can reduce OSR overwintering survival by increasing susceptibility to frost and diseases but can also affect plant architecture by destroying the growing tip and this results in production of many side shoots, which in turn results in delayed flowering.[8–10] The EU ban on neonicotinoid seed treatments and development of resistance to pyrethroids in CSFB throughout Europe[11,12] led to an unprecedented increase in the population, with the control threshold of five larvae per plant frequently observed, making larval infestation more and more problematic for farmers.[2,13]

New management practices are needed to mitigate the effect of both CSFB adults and larvae and there are several in the research

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pipeline.[2,14] However, to test the efficiency of new practices and products to control CSFB, standardized and accurate methods to quantify the level of pest infestation or damage are needed. These methods must be developed for farmers and agronomists to enable them to better evaluate the infestation of their crop and better adapt their practices, but are also needed by researchers and developers who need methods that can be used at large scale to guide their research. Tests of visual estimation of herbivore leaf damage show a good degree of accuracy[15] and have been easily implemented in commercial practise to estimate damage of other species of flea beetle[16]; automated leaf area loss applications are also starting to emerge,[17] but estimating larval infestation is more problematic as larvae develop inside the plant (i.e. are cryptic). The standard method to estimate the number of larvae per plant is to dissect the plant.[18] This method is time consuming (*c.* 10 min per plant on average, depending on plant growth stage – personal observation), needs specialist equipment (i.e. binocular microscope) and good taxonomy skills to find and identify the larvae, which are small (1.5–8 mm), and can be mistaken with other pests present on or in the plant at the same time, e.g. *Ceutorhynchus pallidactylus*, *Ceutorhynchus picitarsis* or *Delia radicum*. These limitations make the plant dissection method difficult to implement by both farmers and researchers.

Two alternative methods to quantify CSFB larval infestation, which are potentially faster and easier to implement than dissection, have been proposed. The first we will refer to here as the 'desiccation method'. This method is adapted from the Berlese funnel method where samples, usually soil or leaf litter, are placed in a funnel over a container with preservative liquid located under a heat source, usually a lamp.[19] The heat desiccates the samples and arthropods present inside fall into the container. This method was adapted to extract CSFB larvae and differs from the original method by the fact that no heat source is used.[20,21] This method is recommended by agronomists in France[22] and was tested in a preliminary study[20] but lacks robust validation of the efficiency and accuracy of the method. The benefits of this method include the ease of implementation and the fact that no specialist equipment is needed. The funnel can be replaced by tubs, trays or plastic yellow bowls used for OSR pest monitoring making the method affordable for any farmer. However, it takes up to 30 days to extract the larvae[21] which is not suitable for a farmer or agronomist who need to be informed of the infestation level as soon as possible to know if the control threshold is breached. The second method proposed to quantify CSFB larval infestation is to count the percentage of leaves with scars.[23] This method is based on the observation that larvae leave characteristic scars on the petioles when they move between petioles for feeding.[2,7] This method reported a good level of accuracy and is rapid and easy to implement.[23] However, this method was tested before the neonicotinoid ban when larval infestation levels were lower than those observed post-ban.[24] With high infestation, the percentage of leaves with scars could easily reach 100%, making this method unsuitable to accurately quantify the infestation. The count of the total number of scars on the plant might be a more suitable way to estimate the larval infestation but this method has never been tested.

The objectives of this study were to compare the desiccation and scar-counting methods with plant dissections to:

(1) Test the efficiency (percentage of the total number of larvae extracted) and accuracy (variability of the number of larvae found in the plant) of the desiccation method (compared with plant dissection).
(2) Identify the optimal desiccation duration.
(3) Identify if larvae dying in the plant due to desiccation limits the efficiency of the desiccation method.
(4) Test the relationship between the percentage of OSR leaves per plant with scars characteristic of CSFB larvae and the number of scars.
(5) Test the accuracy of using the number of scars to estimate the number of larvae per plant (compared with plant dissection).

2 MATERIALS AND METHODS

2.1 Methods to estimate larval infestation

In the different experiments presented later, the number of larvae per plant was estimated using different methods:

- *Plant dissection*. Plants were dissected under a binocular microscope in the laboratory. Leaf petioles, stems, and leaf veins were opened using a scalpel and sharp forceps. The number of larvae and larval instar of each larva was recorded (L1–L3)[8] and whether it was alive or dead. The location of each larva (leaf veins, petioles, or stem) was also recorded.
- *Percentage of leaves with scars*. The number of leaves on the plant and the number of leaves with scars characteristic of CSFB (Fig. 1(a)) were counted to calculate the percentage of leaves with scars.

Figure 1. Methods to assess the number of cabbage stem flea beetle (*Psylliodes chrysocephala*) per oilseed rape plant: (a) Counting leaf scars: oilseed rape leaf showing scarring damage caused by cabbage stem flea beetle larvae along the petiole (two scars indicated by red arrows); (b) Desiccation method: showing water traps (seed trays filled with water and detergent) with plants suspended above traps on chicken wire to enable larvae to drop from the plant as it dries; larvae are then collected and counted.

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- *Total number of scars on the plant*. The number of scars characteristic of CSFB larvae (Fig. 1(a)) was counted on the whole plant.
- *Plant desiccation*. Plants were placed in a glasshouse (mean temperature = 16.6 °C), individually on a piece of chicken wire over a black plastic seed tray (37 cm × 23 cm) filled with water and detergent (Fig. 1(b)). Larvae exiting the plant dropped into the water; trays were checked daily and any larvae found were collected into alcohol. The CSFB larvae were counted and their instar recorded (L1–L3)[8] in the laboratory.

2.2 Plant sampling sites and assessments

For this study we used larval infestation data collected from plants sampled from five different experiments conducted in the UK during the 2020–2021 growing season. Each experiment was conducted at a different location and had a specific design to test intervention methods for CSFB not considered further in this paper. These experiments were sampled to try to ensure a wide range of larval numbers from plants of different growth stages to enable robust analysis of the different methods tested to estimate larval number. We briefly describe later the design of these experiments and the different measurements conducted for each assessment (a summary of the latter is presented in Supporting Information Table S1). In general, OSR plants were sampled from the field and divided into four groups (t0, t3, t7 and t14). The number of leaves, number of leaves with scars characteristic of CSFB damage and the total number of CSFB scars on each plant were recorded for all plants (see Section 2.1 earlier). The plants assigned to t0 were immediately used for the plant dissection method (controls); they were dissected under a binocular microscope as described earlier (Section 2.1). The remaining plants were transferred to a glasshouse and set up for the desiccation method (see Section 2.1). After 3 days of desiccation plants in the t3 group were returned to the laboratory and the number of larvae remaining in each plant were recorded as per the dissection method. This process was repeated after 7 days (t7) and 14 days (t14).

Experiment 1: OSR plants were sampled from a crop (3.8 ha) located on Rothamsted experimental farm, Harpenden, Hertfordshire, UK on three different occasions: 12 August 2020, 29 January 2021, and 22 February 2021 when the OSR crop was at the growth stage BBCH 12, 14 and 14, respectively.[25] No insecticides were applied. On each occasion OSR plants were collected from 43 different points located on a grid with 5 m between each point. At each point a set of four plants were selected at random (approximately 1 m apart for treatments), placed in labelled bags and returned to the laboratory and stored at 5 °C. One plant from each point was dissected straight after collection (t0) and the number of CSFB larvae counted. The next day, the three remaining plants from each sampling point were placed in a glasshouse to test the desiccation method. Plants were assigned to 3-, 7- or 14-day desiccation periods (t3, t7 and t14, respectively) before being dissected. For this experiment, the desiccation was stopped after 7 days on the first sampling occasion because plants were very small and completely dried after a week of desiccation (Table S1). The set of plants assigned for dissection after 14 days of desiccation (t14) was not used in the analysis.

Experiment 2: OSR plants were sampled from a crop (4 ha) located on a commercial farm in Harpenden, Hertfordshire. This field was divided into two areas: one sprayed with biodigestate fertiliser and the other conventionally managed with synthetic fertilisers; neither side was treated with insecticides. Sampling was carried out on two occasions: 12 April 2020 and 15 March 2021, when plants were at the growth stage BBCH 17 and 20, respectively.[25] On both occasions, ten sets of four plants were sampled from each area along a 30 m transect in the centre of each area; plants were selected at random with *c*. 3 m between each set. Plants were processed as in Experiment 1 and left 3, 7 or 14 days to desiccate. However, the plant dissection for t0 was conducted only on the second sampling occasion (Table S1).

Experiment 3: Plants were sampled from an OSR crop (24 ha) located in Caxton, Cambridgeshire, UK. On this field 24 m-wide tramlines were sown with OSR (control) or OSR sown with different companion plants: berseem clover, and a mix of berseem clover and white clover. Each of these treatments were replicated five times. Plant sampling was carried out on one occasion (12 July 2020) with plants at growth stage BBCH 16; a set of three plants was uprooted on three different points along each of the 700 m tramlines, with points separated from each other by 300 m. A total of 135 plants were sampled. Each of the three plants was left 3, 7 or 14 days to dry as in Experiment 1 with the exception that plants were not dissected at t0, and scars were not counted (Table S1).

Experiment 4: Sampling was conducted at two locations in OSR crops (8.5 and 6.5 ha) located at Rothamsted experimental farms in Harpenden (Hertfordshire) and Broom's Barn (Suffolk, UK), respectively. This experiment aimed to test the effect on CSFB infestation of different farming systems (soil amendment, tillage, crop cultivar, and rotation history). Each farm location contained 16 OSR plots (24 m × 12 m) and nine plants were randomly sampled in each plot site. Plants were sampled on 3 June 2021 in Harpenden and were at the growth stage BBCH 16, and on 3 August 2021 in Broom's Barn with plants at the growth stage BBCH 17. The crop failed to establish in some plots, so it was not possible to sample all the plots. A total of 144 plants from 16 plots were collected in Broom's Barn, and 45 plants from five plots in Harpenden were sampled. The two locations were considered as different experiments in the statistical analyses. Three plants from each plot were dissected immediately following collection (t0) the six others were assigned to 7- and 14-day desiccation periods (three plants in each) (Table S1).

Experiment 5: Sampling was conducted in an OSR crop (9.4 ha) located in Harpenden, Hertfordshire. This field trial comprised 36 plots (24 m × 24 m) arranged in a Latin Square design sown with six different companion crop treatments (OSR and turnip rape, OSR and berseem clover, OSR and berseem clover and vetch, OSR and oat, OSR control with low and high herbicide spray). Six plants were randomly sampled from each plot on 23 November 2021 and 17 February 2021 when plants were at the growth stage BBCH 14 and 16, respectively. These plants were all dissected (i.e. no plants were desiccated). The number of scars was counted on both occasions; the number of leaves with scars was counted only on the second date.

2.3 Data analysis

2.3.1 Optimal desiccation duration

Using the data collected for each experiment where plants were left for 14 days to desiccate, we estimated the number of days needed to collect more than 90% of the total number of larvae found using this method. The proportion of the total number of larvae collected each day was calculated for each plant and averaged for each experiment. This proportion was also calculated for each larval instar (L1–L3).

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2.3.2 Efficiency of the desiccation method

From Experiments 1–4 we assessed the number of larvae collected by the desiccation method and the number of additional larvae found during the dissection of the plant post-desiccation. The proportion of larvae collected by desiccation of the total (number found during the desiccation period + number found by dissection post-desiccation) was calculated for each plant. A simple linear model was built explaining the proportion of larvae extracted by desiccation, according to the desiccation duration and the sampling date nested in the experiment. The effect of the different factors was tested using an *F*-test and pairwise comparisons of the estimated marginal means (EMMs) were used to test differences between desiccation durations. The difference in the efficiency of the desiccation method for different larval instars was tested using linear mixed models explaining the proportion of the total number of larvae collected by desiccation according to the larval instar, and the sampling date nested in the experiment as fixed effects. The identity of the plant where the larvae were collected from was included as a random factor. One model was built for each desiccation period (3, 7 and 14 days) and the effects of the different factors tested using a Wald χ^2 test.

2.3.3 Accuracy of the desiccation method

Simple linear models were built for each of the three desiccation durations to explain the total number of larvae (desiccation + dissection post-desiccation) according to the number of larvae collected with the desiccation method. The experiment and the sampling date were nested in the experiment and used as explanatory variables. The coefficients of determination (adjusted R^2) were computed for these models to define the strength of the link between the response variable and the predictors. An *F*-test was then performed on these models to test the effect of the different predictors.

To test the accuracy of the models, data were split and one-third of the data were used as a training dataset and two-thirds were used as a test set. Because we are interested in having a tool that is easy to use by farmers, the model was simplified by removing the experiment and the sampling date as explanatory variables. Models were built with the data collected after 7 and 14 days of desiccation because of the poor relationship between the variables observed after only 3 days. The coefficients of determination of these models were computed to ensure that a strong relationship existed between the two variables and these models were used to predict the number of larvae per plant based on the test dataset. Pearson's correlations between the data predicted and observed were used to estimate the capacity of the model to predict the data.

2.3.4 Dead larvae in the plants

Two simple linear models explaining the number and the proportion of dead larvae per plant (i.e. found after desiccation) according to the experiment factor, the sampling date nested in the experiment and the desiccation duration were built. The number of dead CSFB larvae per plant was square root transformed to meet the normality assumption. The effect of the different factors was tested using an *F*-test and pairwise comparisons on EMMs were used to test differences between desiccation durations for each model.

To test if the proportion of dead larvae per plant varies between larval instars, a linear mixed model was built for each desiccation duration. This model explained the proportion of dead larvae according to the larval instar as a fixed factor and the site, the sampling date nested in the site and a unique plant identifier nested in the sampling date and site as random factors. The effects of the fixed factor were tested using a Wald χ^2 test and pairwise comparisons on EMMs were used to test differences between instars for each model.

2.3.5 Relationship between the number of scars and the percentage of leaves with scars

Data on the number of scars per plant and the percentage of leaves with scars were available from three experiments, totalling 287 plants. To test the relationship between them, a simple linear model explaining the percentage of leaves with scars according to the number of scars, the experiment, and the sampling date nested in the experiment was built. The coefficient of determination (adjusted R^2) was computed, and the effect of the different factors was tested using an *F*-test.

2.3.6 Relationship between the number of larvae and the percentage of leaves with scars

A simple linear model explaining the number of CSFB larvae found during plant dissection according to the percentage of leaves with scars, the experiment, and the sampling date nested in the experiment was built. The coefficient of determination (adjusted R^2) was computed, and the effect of the different factors tested using an *F*-test.

2.3.7 Relationship between the number of CSFB larvae and number of scars

A simple model explaining the number of CSFB larvae in the plant according to the number of scars per plant, the experiment, and the sampling date nested in the experiment was built. The coefficient of determination (adjusted R^2) was computed and the effect of the different factors tested using an *F*-test. Similarly to the desiccation method, the accuracy of the models was tested by splitting the data between a training and test dataset containing one-third and two-thirds of the data, respectively. The model was simplified to include only the number of larvae explained by the number of scars. The coefficients of determination of this model were computed to ensure that a strong relationship exists between the two variables and the model was used to predict the number of larvae per plant based on the test dataset. Pearson's correlations between the data predicted and observed were then used to estimate the capacity of the model to predict the data.

All statistical tests were performed using R software 4.1.1, the R-packages car,[26] and emmeans,[27] lme4,[28] and multcomp.[29] Model residuals were plotted against fitted values and in Q-Q plots to check if model assumptions were fulfilled.

3 RESULTS

3.1 Level of infestation and plant growth stage

The different plant samples showed diverse CSFB larval infestation in a year of particularly high larval infestation in southern UK areas. The mean number of CSFB larvae found per plant dissection on t0 samples ranged from 1.6 to 33.2 depending on the experiment (Table S2). The control threshold commonly used in UK (five larvae per plant[30]) was reached in all the experiments except for Experiments 1 and 4 in Harpenden. Most of the larvae found in the plants were L1 (47% of the total found), with fewer L2 (31%) and L3 (21%) observed. Plants were sampled at different times from November to March and exhibited different growth stages ranging from two to ten leaves per plant (Table S2).

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3.2 Efficiency and accuracy of plant desiccation method

3.2.1 *Optimal desiccation duration*

The number of days of desiccation needed to collect more than 90% of the total number of larvae extracted was on average 5.7 days (see Fig. 2 for results of each experiment). We observed that most of the larvae rapidly dropped from the plants and that the number plateaued after a few days of desiccation (Fig. 2). The rate of larval drop varied slightly between larval instars (Supporting Information Fig. S1), with third instars generally dropping more slowly than first and second instars, taking *c.* 1 day longer to reach 90% observed. This difference might be due to the low abundance of third instar larvae present.

3.2.2 *Efficiency of the desiccation method*

The efficiency of the desiccation method significantly varied between the three desiccation periods ($F_{2,588}$ = 68.213, $P < 0.001$). After 3 days of desiccation 51.25 ± 0.02% of the larvae present in the plants were extracted. This number was significantly lower than the efficiency at 7 and 14 days reaching 76.67 ± 0.02 and 82.4 ± 0.02%, respectively. The proportion of larvae extracted from plants by desiccation also varied according to the experiment ($F_{4,588} = 2.838$, $P = 0.024$), and the sampling date nested in the experiment ($F_{3,588} = 30.997$, $P < 0.001$). No significant differences of efficiency between larval instars after different desiccation periods were observed (Fig. S2; 3 days: χ^2 = 2.91, df = 2, P = 0.233; 7 days: χ^2 = 5.28, df = 2, P = 0.071; 14 days: χ^2 = 4.17, df = 2, P = 0.124). Most of the larvae found during the dissection post-desiccation were found in the leaf petioles (81%). The rest of the larvae were found in the leaf veins (11%) or the stem (8%). This distribution did not change according to desiccation time.

3.2.3 *Accuracy of the desiccation method*

A significant relationship between the number of larvae extracted by desiccation and the total number of larvae in the plant was found after 3, 7 and 14 days (3 days: $F_{1,207}$ = 106.11, $P < 0.001$; 7 days: $F_{1,264}$ = 5280, $P < 0.001$; 14 days: $F_{1,223}$ = 13 532.84, $P < 0.001$). A significant effect of the experiment was found after 7 and 14 days but not after 3 days (3 days: $F_{2,207} = 2.37$, $P = 0.096$; 7 days: $F_{4,264} = 12.15$, $P < 0.001$; 14 days: $F_{4,223} = 6.82$, $P < 0.001$). No effect of the sampling date was observed (3 days: $F_{3,207}$ = 1.24, P = 0.296; 7 days: $F_{3,264}$ = 1.58, P = 0.195; 14 days: $F_{2,223}$ = 1.96, P = 0.143). However, the model built with the data collected after 3 days of desiccation has an R^2 adjusted value of 0.44; this increased to 0.98 and 0.99 after 7- and 14-days desiccation, respectively (Fig. 3). The correlation between the number of larvae predicted from the number caught in the water trap from desiccating plants and the actual number of larvae observed (desiccation + dissection post-desiccation) is high after 7 days (r = 0.99, df = 91,

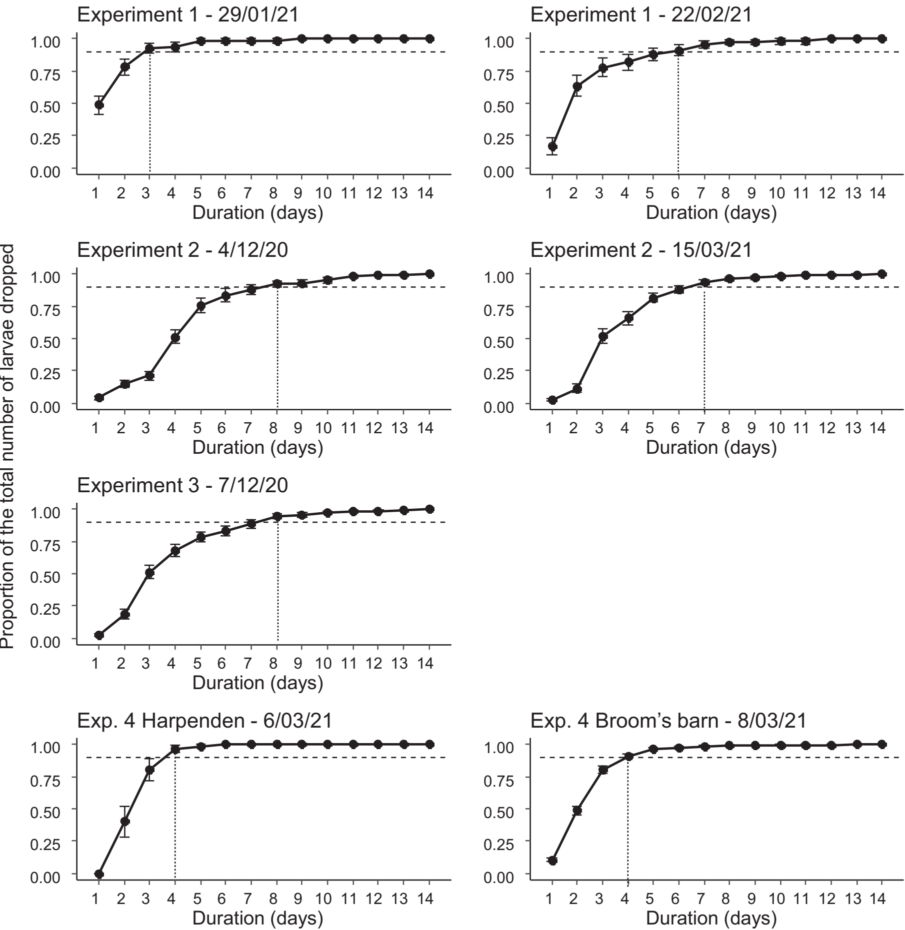

Figure 2. Mean (± standard error) proportion of the total number of cabbage stem flea beetle (*Psylliodes chrysocephala*) larvae that dropped from desiccating oilseed rape plants per day for four different experiments and sampling dates. Horizontal dashed lines represent the 90% threshold. Vertical dashed lines represent the day when the 90% threshold is reached.

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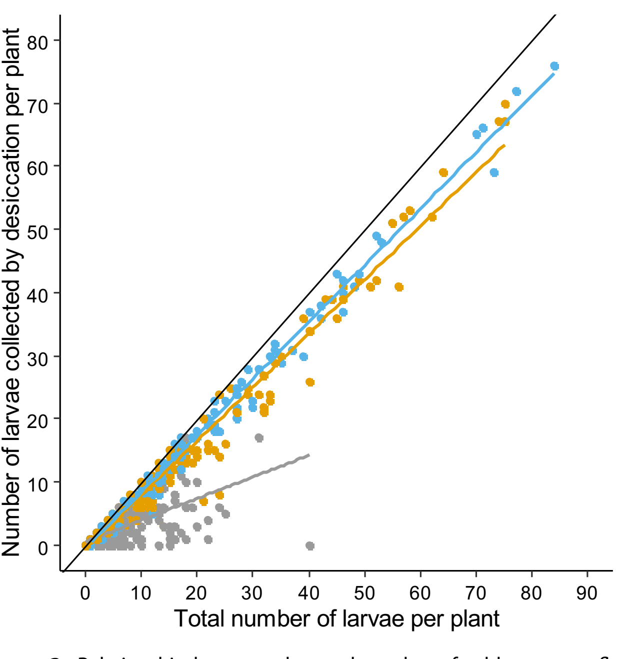

Figure 3. Relationship between the total number of cabbage stem flea beetle (*Psylliodes chrysocephala*) larvae per oilseed rape plant and the number of larvae extracted per plant after the following number of days of plant desiccation: 3 days (grey), 7 days (yellow) and 14 days (blue). The coloured lines represent the linear relationship between the variables and the black line the 1:1 relationship between the two axes. Total number of larvae in the plant = number of larvae extracted via plant desiccation plus number recovered via plant dissection post-desiccation.

$P < 0.001$), and 14 days ($r = 0.99$, df = 77, $P < 0.001$) (Fig. S3). The equations of the relationships at 7 and 14 days are:

$$\text{number larvae per plant} = -0.244 + \text{number larvae extracted 7 days} \times 0.868 \quad (1)$$

$$\text{number larvae per plant} = -0.51 + \text{number larvae extracted 14 days} \times 0.896 \quad (2)$$

Using these equations, it is possible to estimate the number of larvae extracted after 7 or 14 days of desiccation that equate to the control threshold of five larvae per plant. This value is 3.71 ± 0.38 larvae [confidence interval (CI) 95%] after 7 days and 3.97 ± 0.29 (CI 95%) after 14 days.

3.2.4 *Dead larvae in the plant*

The number of dead larvae found during the plant dissection significantly increased between 3 and 14 days of desiccation (Fig. 4(a), $F_{2,463} = 7.648$, $P < 0.001$). This is in line with the results of the proportion of dead larvae (Fig. 4(b), $F_{2,463} = 74.531$, $P < 0.001$). At t0 the average proportion of dead larvae was very low (0.017% ± 0.007 dead larvae per plant) suggesting that larvae died because of the desiccation of the host plant. This increase in the proportion of dead larvae was observed for all the instars except the third instar where the proportion of dead larvae was lower after 7 days than 3 days (Fig. S4). The proportion of dead larvae was significantly less great for third instars than first and second instars for each desiccation duration, except after 3 days of desiccation where more dead first instar larvae were found than second and third instars (Fig. S4).

3.3 Estimation of larval infestation using leaf scars

3.3.1 *Relationship between the number of scars and the percentage of leaves with scars*

For the four dates where both data on the number of scars and percentage of leaves with scars were available, we found a significant relationship between the variables ($F_{1,282} = 105.63$, $P < 0.001$). The relationship varied between sampling sites ($F_{2,282} = 15.17$, $P < 0.001$) and dates ($F_{1,282} = 14.58$, $P < 0.001$). However, it is important to note that the relationship is limited by the threshold of 100% for the percentage of leaves with scars. This value was reached by 40% of the plants collected in Experiment 5. The relationship had a low adjusted R^2 (0.36) (Fig. 5).

3.3.2 *Accuracy of the percentage of leaves with scars measurement*

When pooling all the data available we found a significant relationship between the number of larvae per plant and the percentage of leaves with scars ($F_{1,282} = 60.72$, $P < 0.001$; Fig. 6(a)). However, this relationship was weak (adjusted $R^2 = 0.20$) and was significantly affected by the experiment ($F_{2,282} = 3.25$, $P = 0.04$) and the sampling date nested in the experiment ($F_{1,282} = 11.16$, $P < 0.001$).

3.3.3 *Accuracy of the total number of scars per plant measurement*

A strong and significant positive relationship was found between the number of larvae per plant and the total number of scars on the plant (adjusted $R^2 = 0.67$, $F_{1,587} = 852.919$, $P < 0.001$, Fig. 6(b)). The relationship was significantly affected by the experiment ($F_{4,587} = 13.267$, $P < 0.001$) and sampling date nested in the experiment ($F_{3,587} = 7.356$, $P < 0.001$). We found a strong and significant correlation between the predicted number of larvae and the actual observed data indicating the robustness of the model ($r = 0.79$, df = 201, $P < 0.001$) (Fig. S5). Using this model, it was possible to estimate the number of larvae based on the number of scars:

$$\text{number larvae plant} = 0.053 + \text{number scars} \times 0.944 \quad (3)$$

Using this equation, 4.77 ± 1.01 (CI 95%) scars per plant would correspond to a threshold of five larvae per plant.

4 DISCUSSION

The CSFB is a major pest of OSR in Europe[2] and the development of new strategies to manage this insect, especially the impact of larval infestation, is constrained by methodological aspects related to monitoring. Current methods to estimate larval infestation in the plant are time consuming and their reliability questionable. In the present study different methods to estimate CSFB larval infestation were tested on plants collected in different field trials in the UK. These trials had a wide range of plant infestation levels (ranging between < 2 to more than 33 CSFB larvae per plant) and growth stages (from BBCH 12–20) allowing robust methodological tests to be conducted.

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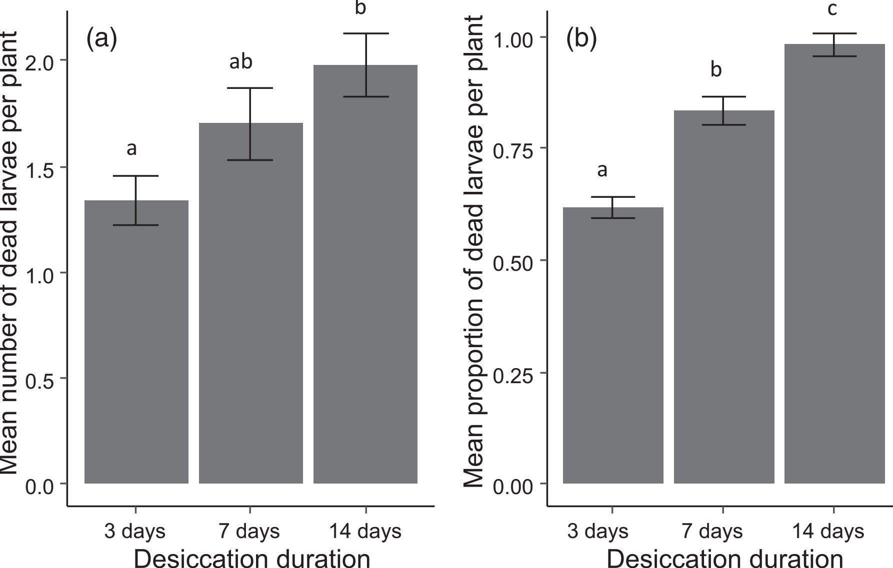

Figure 4. Estimated marginal mean (± standard error) of the number of dead cabbage stem flea beetle (*Psylliodes chrysocephala*) larvae per oilseed rape plant (a) and the proportion of dead larvae per plant (b) found after 3-, 7-, and 14-day desiccation periods of the plants. Different letters indicate significant differences ($P < 0.05$).

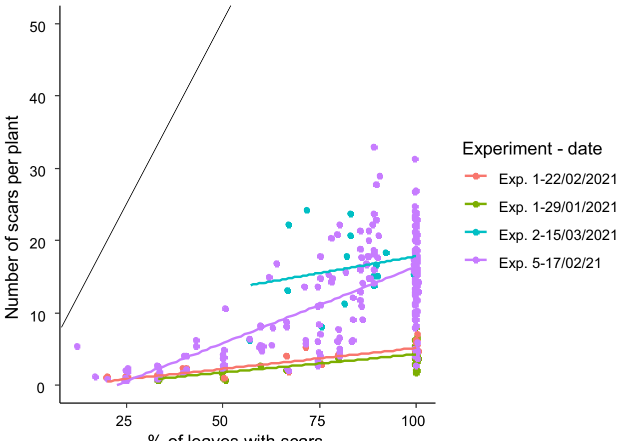

Figure 5. Relationship between the total number of scars characteristic of cabbage stem flea beetle (*Psylliodes chrysocephala*) infestation per oilseed rape plant, and the percentage of leaves with scars on the plant. Lines represent the linear regression based on the data from samples collected in four sampling dates over three experiments. Noise was added to show overlapping points. The black line represents the 1:1 relationship between axes.

Our results show that the desiccation method, whereby CSFB larvae evacuate desiccating plants and are collected in water traps, is an effective and accurate technique to estimate CSFB larval infestation in OSR plants. After 7 days on average 76% of the larvae present in plants were extracted using this method and this number increased to 82% after 14 days. This efficiency is the same for all larval instars. Variability of < 1 larva per plant (0.38 and 0.29 after 7 and 14 days, respectively) was found for a plant reaching the control threshold of five larvae. A small underestimation was observed which is mainly due to the fact that some larvae die within the plant during the desiccation period and therefore do not evacuate. However, these numbers were low, between one and two larvae per plant (on average between 15–20% of all larvae in the plant) and did not strongly affect the results. The data collected also show that the desiccation period can be reduced compared to that suggested in other studies.[20,21] We found that after only 7 days of desiccation a good level of efficiency is reached, which is a shorter period than presented in another comparable study (i.e. 21 days)[20]; contrary to our experiment this study was conducted in a climate chamber with seven plants exposed in each funnel where conditions could have slowed the desiccation. As suggested by our data the speed of the desiccation process, and therefore the optimum time for larval evacuation, could vary depending on the plant size but other factors such as temperature could affect the desiccation. Consequently, it is important to ensure that plants are completely dry at the end of the assessment period rather than stopping the procedure after a specific number of days. Desiccation could be facilitated by removing part of the leaves' lamina, where larvae are rarely found (personal observation[7]) to reduce the biomass that needs to be dried. The desiccation method is efficient, easy to perform, and equipment requirements are low, but a 7-day period before results are acquired may be too slow to inform some spray decisions. In our experiments we measured larval evacuation per plant, but groups of plants could be desiccated at the same time over larger tubs or buckets and a mean number taken. This makes the method accessible for farmers and advisors who could desiccate plants in a barn, but the space requirements of this method may be problematic for researchers who may need to sample many hundreds of plants to conduct their experiments.

Our data show the limitation of using the percentage of leaves with scars to estimate the number of larvae. This method was previously proposed as a fast way to estimate larval infestation and was shown to be efficient in the past,[23] but it seems to be less accurate now, possibly because populations of beetles are much higher now than 20 years ago[24] and conditions are now different with warmer winter periods leading to prolonged periods of oviposition and infestation. As expected, the percentage of leaves with scars correlates with the total number of scars on the plant, and this second measurement seems more

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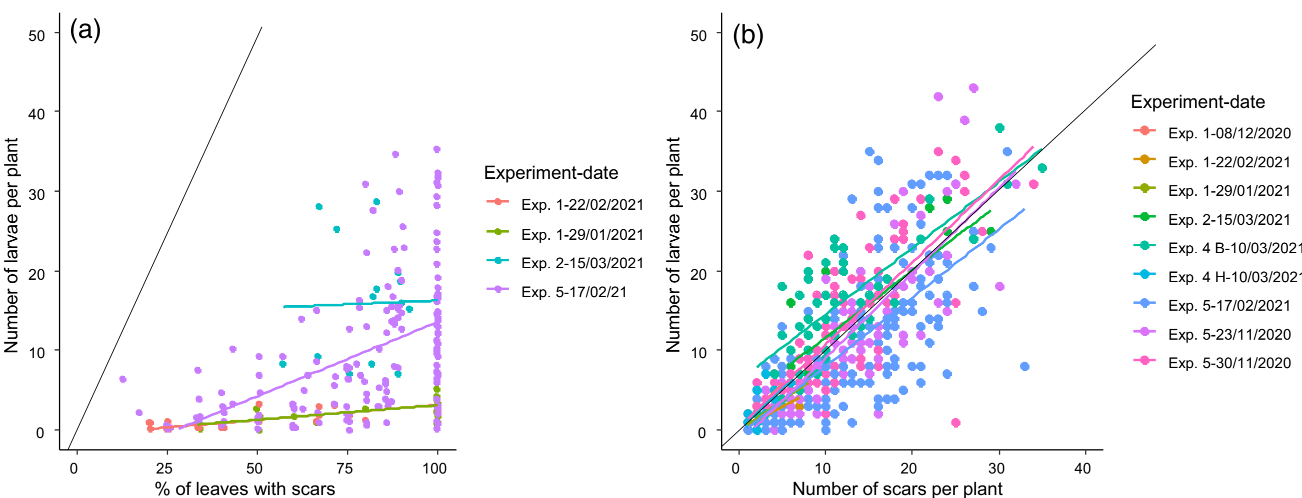

Figure 6. (a) Relationship between the percentage of leaves with scars characteristic of cabbage stem flea beetle (CSFB; *Psylliodes chrysocephala*) on oilseed rape plants and the number of larvae. Plants were sampled on four occasions from three different experiments; noise was added to show overlapping points. (b) Relationship between the total number of scars characteristic of CSFB infestation and the number of CSFB larvae per oilseed rape plant on plants sampled on eight occasions from four different experiments. Coloured lines are the regression lines of the data from each sample. Black lines are the 1:1 relationship between axes.

promising to estimate the potentially large numbers of larvae infesting each plant. A strong relationship between the number of scars and the number of larvae was found allowing determination of action thresholds (4.7 scars relating to five larvae per plant, with a variability of accuracy of ±1 larva). A previous study found that the relationship between the percentage of leaves with scars and the number of larvae in the plant is less stable in winter.[23] This might be due to the fact that some third instar larvae move from the petioles to the stem during the winter.[8] However, no difference in the relationship between the number of scars and the number of larvae was found between samples collected in autumn and winter, but only a few larvae were found in the stem (8%). In a situation where larval infestation in the stem is preve and where frost destroys some of the leaves in winter, the efficiency of counting the total number of scars might be affected. Compared to the desiccation and dissection methods, counting scars has the benefits of being simpler and faster and is also not destructive. This is a particularly important point for researchers, as it would allow the larval infestation level to be related to plant yield. This may help to establish a more accurate economic threshold for CSFB larval infestation. The current UK threshold of five larvae per plant was established and revised multiple times in the UK[30–32] but varies in different countries on the European continent[2,6] and is mainly based on the economics of the insecticides more than the response of the plant to the infestation. Experiments with potted plants seem to confirm this threshold[33] but field experiments are needed to validate it and examine how larval infestation and yield effects vary with cultivar and agronomic practices. However, one of the drawbacks of the number of scars method is that larvae are not directly observed and that the relationship between the number of scars and number of larvae can vary between fields and dates. Consequently, farmers could hesitate to take decisions based only on a proxy of the infestation and in this case this method could thus be combined with the dissection or the desiccation of a small number of plants to support the estimations using scar counting.

Published research dealing with CSFB larvae are rare, probably because of methodological limitations. The methods presented here could facilitate studies of larval infestation and identification of new farming practices that better manage this pest.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available upon request.

SUPPORTING INFORMATION

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

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