

*Modelling the factors affecting the spatiotemporal distribution of cabbage stem flea beetle (*Psylliodes chrysocephala*) larvae in winter oilseed rape (*Brassica napus*) in the UK*

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# Modelling the factors affecting the spatiotemporal distribution of cabbage stem flea beetle (*Psylliodes chrysocephala*) larvae in winter oilseed rape (*Brassica napus*) in the UK

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## Abstract

**BACKGROUND:** Cabbage stem flea beetle (CSFB; *Psylliodes chrysocephala* L.) management in oilseed rape (*Brassica napus* L.) has become an urgent issue in the absence of permitted and effective insecticides. Understanding the meteorological and management factors affecting their population dynamics has become critical to the development of pest management strategies.

**RESULTS:** The spatio-temporal changes in CSFB larval populations were assessed both in autumn and spring, in the UK from 2003 to 2017 (a period encompassing pre- and post-neonicotinoid insecticide restriction). After the neonicotinoid ban in 2013, the number of larvae both in autumn and spring increased 10-fold in the UK. When neonicotinoids were available, later sown crops contained fewer larvae than early sown crops, and bigger fields had fewer larvae than smaller fields, whereas after the ban, bigger fields tended to have more larvae than smaller fields. Wet and mild/hot Septembers were related with higher numbers of larvae when neonicotinoids were available and with lower larval numbers after the neonicotinoid ban. Low temperatures in December and January combined with high rainfall were related with high numbers of larvae in spring both before and after the neonicotinoid ban.

**CONCLUSION:** This study will help to produce decision support systems that allow future predictions of regional CSFB population changes and will help growers and consultants to adjust their management methods to reduce the risk of high infestations. © 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:** pest control; insecticides; integrated pest management; decision support systems

## 1 INTRODUCTION

The cabbage stem flea beetle, *Psylliodes chrysocephala* L. (CSFB, Coleoptera: Chrysomelidae) is the most important pest in winter oilseed rape (OSR, *Brassica napus* L.) in Europe.<sup>1</sup> Adult beetles migrate into the newly sown crop in autumn.<sup>2</sup> The beetles feed on the cotyledons and young leaves of plants and after 2–3 weeks they start to mate and oviposition begins.<sup>3,4</sup> Adult feeding gives rise to characteristic ‘shot-holing’ symptoms. Severe and sustained feeding damage can lead to the death of plants, especially if the hypocotyl is eaten, thus threatening crop establishment.<sup>5</sup> Eggs hatch from September onwards.<sup>6,7</sup> The neonate larvae tunnel into the petioles and stems of the plants where they feed and develop throughout the winter and into late spring.<sup>8</sup> The larval damage can reduce plant vigor and increase risk of frost damage and disease, increasing crop losses in winter and causing stem splitting, plant stunting and delayed flowering in the spring.<sup>3,9,10</sup> Further details about its life cycle have been reviewed in Ortega-Ramos *et al.*<sup>11</sup>

The CSFB, and especially the feeding damage caused by adult beetles, was traditionally controlled by using neonicotinoid insecticide

seed treatments, however, following the concerns regarding their effects on non-target organisms<sup>12,13</sup> the European Union banned the use of these treatments in 2013 (European Commission, 2013 [EU] No 485/2013). After the ban, pyrethroids became the only permitted synthetic insecticide available for controlling CSFB. The lack of alternatives and a high spraying frequency has led to increasing problems of resistance against pyrethroids in CSFB populations.<sup>14–17</sup> This lack of control coincided with warm winters, which favor extended oviposition and larval development, giving rise to a major

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increase in CSFB populations, especially in the UK and northern France.<sup>18</sup> Consequently, there has been a substantial decrease in the area of OSR grown in the EU due to farmers protecting against further crop losses,<sup>1,19</sup> forcing the EU to rely on imports of OSR to cover the domestic demand.<sup>18</sup> Moreover, it is expected that if no alternative pest control methods are available, crop protection costs could increase by 10–20% due to the increased number of pesticide applications and changes in pest management practices, for example increasing sowing densities and fertilizer usage.<sup>20,21</sup> This situation has led to an urgent need for new integrated pest management (IPM) strategies to control CSFB, thereby reducing dependence on synthetic insecticides. IPM means careful consideration of all the plant protection methods for the growth of a healthy crop with the least possible effects on agro-ecosystems.<sup>22</sup> However, to develop these strategies, we need to better understand different aspects of the ecology and development of CSFB, as well as the effects of management practices and meteorological factors on their population dynamics and crop damage.

The infestation and level of CSFB damage on OSR crops shows temporal and spatial variation. In the UK, for example, annual differences in infestation levels have been detected over a 14-year survey; peak larval numbers were registered in autumn in 2015 compared with previous years (2002–2014) and with the following year 2016.<sup>23</sup> The UK also has regional variation in CSFB numbers; the Eastern region of England has been identified as the area with consistently the most CSFB damage observed in crops from adult and larval stages<sup>24,25</sup> while crops in the west of England rarely suffer above-threshold damage levels.<sup>25</sup> Although the factors behind these spatio-temporal changes are unknown, the effect of some weather factors and management conditions have been reviewed.<sup>11</sup> The migration time, start of oviposition and larval survival have been shown to be affected by autumn temperatures<sup>2,6,26</sup> and moisture.<sup>3</sup> In relation to crop management, early sowing reduces crop vulnerability to CSFB adults,<sup>27</sup> but increases the risk of larval damage.<sup>24,28</sup> Other management factors affecting the number of CSFB in the field are proximity of the crop to the previous year's OSR crops,<sup>9,27</sup> and plant density.<sup>29</sup> Although there is some evidence about the effects of weather, management and landscape factors on CSFB populations,<sup>30</sup> there are no peer reviewed quantitative models available for CSFB in the UK that show the relationships between CSFB population density and these factors or the interaction between them using long term and large-scale observations.

This study assesses spatio-temporal changes in CSFB larval populations in the UK and whether these changes are affected by weather and/or agronomic practices – including pesticide application. The spatio-temporal changes in CSFB larval populations in autumn and spring from 2003 to 2017 in the UK were analyzed using statistical modelling approaches to identify the key weather and management factors, and/or their interactions, associated with high numbers of CSFB larvae (hotspots) across the UK.

## 2 MATERIALS AND METHODS

### 2.1 Larval infestation data

Long-term data on CSFB larval abundance supplied by the UK National Survey of CSFB larvae, funded by the UK Department of environment, food and rural affairs (Defra)<sup>23</sup> was used. This dataset contains annual data on the number of CSFB larvae recorded in winter OSR crops from 2003 to 2017. Fields sown to OSR were selected at random from a list of farms in England derived from annual returns to Defra Census Branch. Each year, plants from

80–100 OSR crops were assessed in the autumn (plants collected in early November) and a subset of 40–54 of the same fields were revisited in spring (plants collected in mid-March). From each crop, 25 plants were randomly selected along a linear transect into the crop starting 3–4 m from the edge of the OSR field. Plants were dissected using a standard protocol detailed in Collins<sup>23</sup> and the total number of CSFB larvae from 25 plants was recorded. Data from each site was grouped into regions to facilitate data interpretation. The assignment of counties to regions is provided in Supporting Information, Fig. S1.

### 2.2 Agronomic factors

For each OSR crop from which CSFB larvae were sampled, additional data on crop variety, sowing date, previous crop and field area were collected as well as the crop growth stage at the time of the plant assessment. Data on the number of insecticide applications, product/s used, date and rate applied were also collated.

Crop variety was grouped according to the breeding system of the cultivars used: open pollinated and restored hybrid (produced *via* genic male sterility (GMS) or cytoplasmic male sterility (CMS) techniques). Each OSR variety was also classified by traits selected from the AHDB recommended lists based on their potential to influence CSFB damage, either directly by affecting larval feeding and development (stem stiffness, shortness of stem) or indirectly by affecting crop growth (earliness of flowering, earliness of maturity). The type of insecticide applied to the crops was categorized according to chemical composition (Pyrethroids: Cypermethrin, lambda-cyhalothrin, Alpha-cypermethrin, Deltamethrin, zeta-cypermethrin, Organochloreines: 1,3-Dichloropropene; Pyridines: pymetrozine, neonicotinoids: thiacloprid). The time between sowing and first insecticide application was also calculated. We assumed that neonicotinoid seed treatments were used in all sites sown before 2014, at which point their use was restricted, and they were not used at any site after that year. In 2015, a derogation for neonicotinoid dressed seed was approved in the high-risk counties of Suffolk, Cambridgeshire, Bedfordshire and Hertfordshire (Derogation Area). However, no data on the actual use of neonicotinoid treated seed in these areas were obtained, so we assumed from 2014 onwards that none of the fields were sown using neonicotinoid treated seeds. The agronomic variables analyzed are summarized in Tables 1 and 2.

### 2.3 Weather data

Weather data were obtained from the UKCP09: Met Office gridded land surface climate observations CEDA archive (Centre for Environmental Data Analysis) (for 2003 to 2016)<sup>31</sup> and HadUK-Grid Gridded Climate Observations (for 2017).<sup>32</sup> Daily summaries of the minimum, maximum and mean temperature (°C) and accumulated rainfall (mm) were obtained from August to mid-April at each site every year. Temperature was expressed as monthly means and as day-degrees ( $(\sum (\text{daily maximum} + \text{daily minimum})/2) - \text{base temperature or threshold}$ ). A different threshold was used for each time period based on the effect of temperature on adult reproduction, larval survival and development. For the period between mid-August to October a threshold of 4 °C was used.<sup>33</sup> Day-degrees in October to November and December to February were calculated using a threshold of 8 °C and 0 °C, respectively, taking into account the estimated larval developmental and larval mortality thresholds described by Vig<sup>34</sup> and Mathiasen *et al.*<sup>27</sup> and Mathiasen *et al.*<sup>35</sup> respectively. Rainfall was calculated as monthly total (in mm) from August to

**Table 1.** Summary of the agronomic variables used in analysis to understand the main factors affecting spatial and temporal differences in the abundance of cabbage stem flea beetle (CSFB) larvae per oilseed rape plant in areas across the UK

Variable type	Variable name	Description	Variable categories
CSFB larval number	Larvae25p	Number of CSFB larvae found in 25 plants	Min: 0.00 Max: 118.0 Mean: 3.506
Year	Year	Year in which the plants were collected	2003–2017
Geographical drivers	X, Y	Geographical coordinates (eastings and northings)	
Field management drivers	Previous crop	Previous crop	Cereals (barley + other cereals), fallow, OSR, winter wheat, other (grass+beans and peas)
	Field area	Field area in m <sup>2</sup>	Min: 0.6600 Max: 80.00 Mean: 13.72
	Date sown	Date the crop was sown in julian days	Min: 192.0 Max: 242.3 Mean: 284.0
	GH-BBCH	Growth stage in the BBCH scale (Lancashire <i>et al.</i> , 1991)	Autumn: 10 to 19 = leaf production (LFP); >19 = side shot development (SSD) Spring: Spring: 19 = leaf production (LFP), 30–39 = stem elongation (SD), 50–60 = flower buds development (FBD)
	Pestic_app	Number of pesticides applications before the collection of the plants	1–6 (as factor)
	n_products	Number of different products applied	1–4 (as factor)
	Chemical family	Active ingredients sub-group	Cypermethrin, lambda-cyhalothrin, Alpha-cypermethrin, Deltamethrin, zeta-cypermethrin
	Dafter_sow	Days from sowing to the first pesticide application	Min: –19.00 Max: 87.00 Mean: 35.39

Source: Defra Census Branch.

**Table 2.** Summary of the variables related to the oilseed rape varieties used in analyses to understand the main factors affecting spatial and temporal differences in the abundance of cabbage stem flea beetle larvae per plant in areas across the UK

Variable type	Variable name	Description	Variable categories
OSR variety drivers	breeding_var	Breeding system used to create each variety	Open pollinated, restored hybrid
	stem_stiff2	Stiffness of the stem at the pod development stage	Very high (9); high (8); medium (6, 7).
	stem_short2	Crop height presented in centimeters taller (+) or shorter (–) than the mean of all varieties listed.	Very short (9); short (8); medium (6, 7); tall (4, 5)
	early_flower2	Earliness of flowering presented as number of days earlier or later than the mean of all varieties listed	Early (8, 9); medium (6, 7); late (4, 5)
	early_mat	Earliness of maturity presented as number of days earlier or later than the mean of all varieties listed	Very early (9); early (7, 8); medium (6); late (4, 5)

Source AHDB recommended lists (2003–2017).

April; and monthly rainy days (number of days when rainfall >1 mm) was calculated for the same months.

## 2.4 Statistical analysis

### 2.4.1 Variograms and kriging

To create maps of larval densities, variograms from all data points for each year and each season were estimated and modelled. Ordinary Kriging was used to predict the larval numbers across Great Britain at points on a 10 km grid and were contoured and

clipped to the area where OSR was grown in 2016 and 2017 (determined using the CEH Landcover + crops map for 2016 and 2017 with a 10 km buffer applied around each parcel of land) in ArcMap (ESRI). In the case of CSFB larval counts, where the distribution was skewed, a log transformation was used before estimation of the variogram. However, distribution still did not conform to the assumption of normality, and so the method of Cressie and Hawkins<sup>36</sup> was used for a more robust estimation of the variogram. Variograms were modelled using an exponential function



as this provided the best fit to all the data. Whilst this function does not provide a finite range as it approaches its sill asymptotically, it does still allow an estimate of the effective range over which 95% of the variance is reached. All the spatial analyses were performed using GenStat.<sup>37</sup>

#### 2.4.2 Random Forest and decision tree

Because many of the variables, especially the weather variables, were different measurements or combinations of the main variables and therefore highly correlated, a Random Forest (RF) analysis was performed to assess the relative importance of the explanatory variables to include in the regression models. The number of larvae were categorized into four groups [absent (0 larvae), low (1–10), medium (11–50), high (>50)]. All variables mentioned in Tables 1 and 2 were included in the RF model. When modelling the spring larval data, the number of larvae in the previous autumn samples were also included as a variable as it could affect the number of larvae later in the season. A total of 29 and 40 variables were included in autumn and spring analyses, respectively. Each model run was allowed to generate 5000 trees<sup>38</sup> and was run 10 times (creating 50 000 trees) to obtain an estimate variation in variable importance. The Mtry value (number of available predictor variables at each split) was set to be  $\sqrt{p}$ , as recommended for classification.<sup>39,40</sup> The Mean Decrease Gini, (total decrease in node impurities from splitting on the variables averaged over all trees) was used to select the most important variables for estimating the number of CSFB larvae across all the trees that make up the forest.<sup>41</sup> Then, a decision tree was constructed with the selected variables.

Random Forest analyses were performed in R version 3.6.2<sup>42</sup> using the 'Random Forest' package<sup>43</sup> and based on the script from Coulthard *et al.*<sup>38</sup> The packages 'rpart',<sup>44</sup> 'rpart.plot',<sup>45</sup> and 'party-kit'<sup>46</sup> were used to build a decision tree as a result of the consensus RF model run.

#### 2.4.3 Regression analysis

Before all analyses, data were checked for normal distribution and homo/heteroscedasticity. The choice of distribution was based on Akaike information Criterion and overdispersion parameters (Poisson vs negative binomial).<sup>47</sup> Generalized linear mixed models (GLMM) assuming a negative binomial distribution were used to assess the effects of management and weather factors on the number of CSFB larvae. A subset of the explanatory variables with the highest relative importance obtained in the RF analysis were included in the model as fixed effects. For the autumn data, date sown, field area, mean August temperature, mean September temperature, day-degrees above 8 °C during October–November, accumulated rainfall in August, September, October and November were included as main fixed effects. For the spring GLMMs, date sown, field area, mean temperature and accumulated rainfall in August, September, October, November, December, January, February, March and April were included as main fixed effects. Additionally, the interactions between mean temperatures and accumulated rainfall within the same months were included in the model as fixed effects in both models. As neonicotinoid use (yes/no) was selected in the RF as an important variable, the autumn and spring data sets were divided into pre-(2003–2013) and post-neonicotinoid (2014–2017) ban and analyzed separately. Year, region, and insecticide applications were included as random effects in the models. Despite clear spatial autocorrelation within years, we found little evidence for spatial autocorrelation at the dataset level and so there was no need to account for this further (in addition to region) in the random

effects of the model. Terms were selected using backwards elimination according to the largest *p*-value given by an approximate *F*-test when that term was dropped.<sup>48</sup> The final predictive model was chosen when all remaining terms gave significant values ( $P \leq 0.05$ ) in the *F* test. Graphical interpretations of the interactions effects were tested using the 'emmeans' package.<sup>49</sup>

GLMM analyses were performed in R version 3.6.2<sup>42</sup> using the lmer and glmer functions in the 'lme4'<sup>50</sup> and the 'lmerTest' packages.<sup>51</sup> All GLMM models were simplified by sequentially removing non-significant terms variables in turn.<sup>52</sup> Plots of all models were obtained using 'ggplot2' package.<sup>53</sup>

## 3 RESULTS

### 3.1 Variograms and kriging

Numbers of CSFB larvae per plant varied greatly by region and year (Fig. 1). Larval numbers per plant were low across the country until autumn 2014, *i.e.*, the first crop planted without neonicotinoids, when CSFB populations started to increase, reaching a peak in 2015/16. Between 2003/4 and 2013/4 the average number of larvae per 25 plants in autumn was  $3.51 \pm 0.32$  (mean  $\pm$  SE) which translates to 0.14 larvae per plant. Whereas in 2014/15–2016/17 it increased to  $34.39 \pm 3.31$  (mean  $\pm$  SE) larvae per 25 plants, or 1.37 larvae per plant. In 2016 larval numbers were lower, both in autumn and spring, compared with the previous 2 years (Fig. 1). Overall CSFB larval numbers in spring were higher than in autumn, as would be expected as oviposition would have continued after the autumn samples in mild conditions. However, regions with the highest mean numbers in autumn did not necessarily present the highest numbers in the following spring. For example, there was a drastic peak in larval population in autumn 2015, especially in the East region, whereas in spring 2016 the highest mean number of larvae occurred in the North.

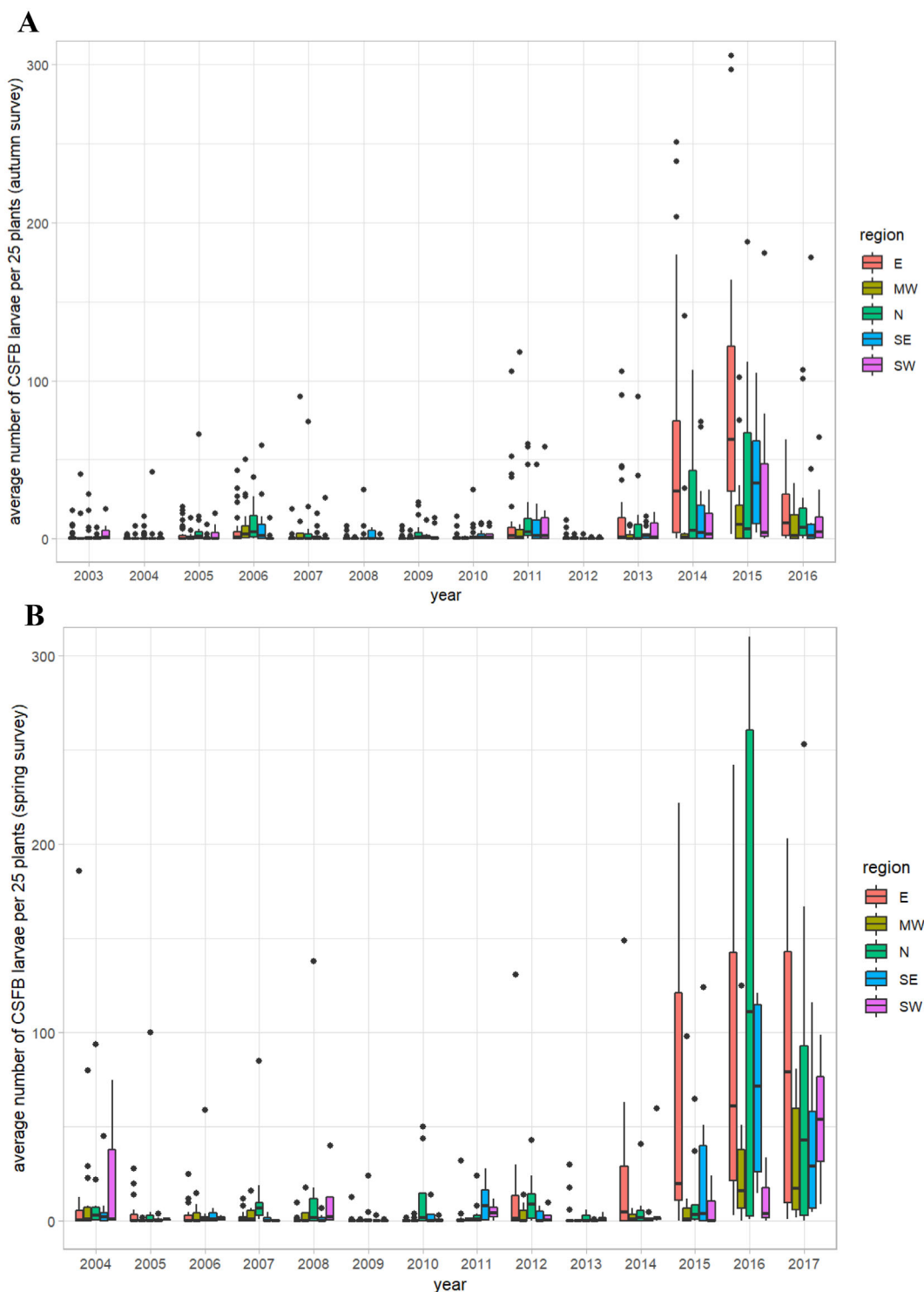
The spatial distribution of CSFB larval abundance varied through years and had a strongly skewed distribution to the East and South East Regions (Figs 2 and 3). The increase in the number of larvae per plant after autumn 2014 was observed across all regions, however it was most notable in the 'East' region in autumn and in the 'North', 'East' and 'South East' in spring. The larval population increase was less remarkable in the 'Midlands and West' region in the autumn and in the 'Midlands and West' and 'South West' regions in spring. In contrast to the other regions, the number of larvae in the 'South West' increased in spring 2016 compared with previous years. We also saw an increase in the effective range of the modelled variograms following autumn 2014 from approximately 70 km to over 350 km.

### 3.2 Random forest

#### 3.2.1 Number of larvae in autumn

The out of bag (OOB) error rate for the last tree fitted was 43.6%, with successful prediction of larval numbers of zero on 85.1% of occasions. Prediction of large numbers of larvae was not so good, with an accuracy of only 28.4%, 0.76% and 40.4% for larval numbers between 1–10, 11–50 and more than 50, respectively. Sowing date was identified as the most important variable in predicting the number of larvae (Fig. 4). Following this, day degrees above 8 °C in October and November (day degrees > 8 Oct–Nov), mean temperature in November, October, and September, field area and mean temperature in August were also top predictors (Fig. 4).

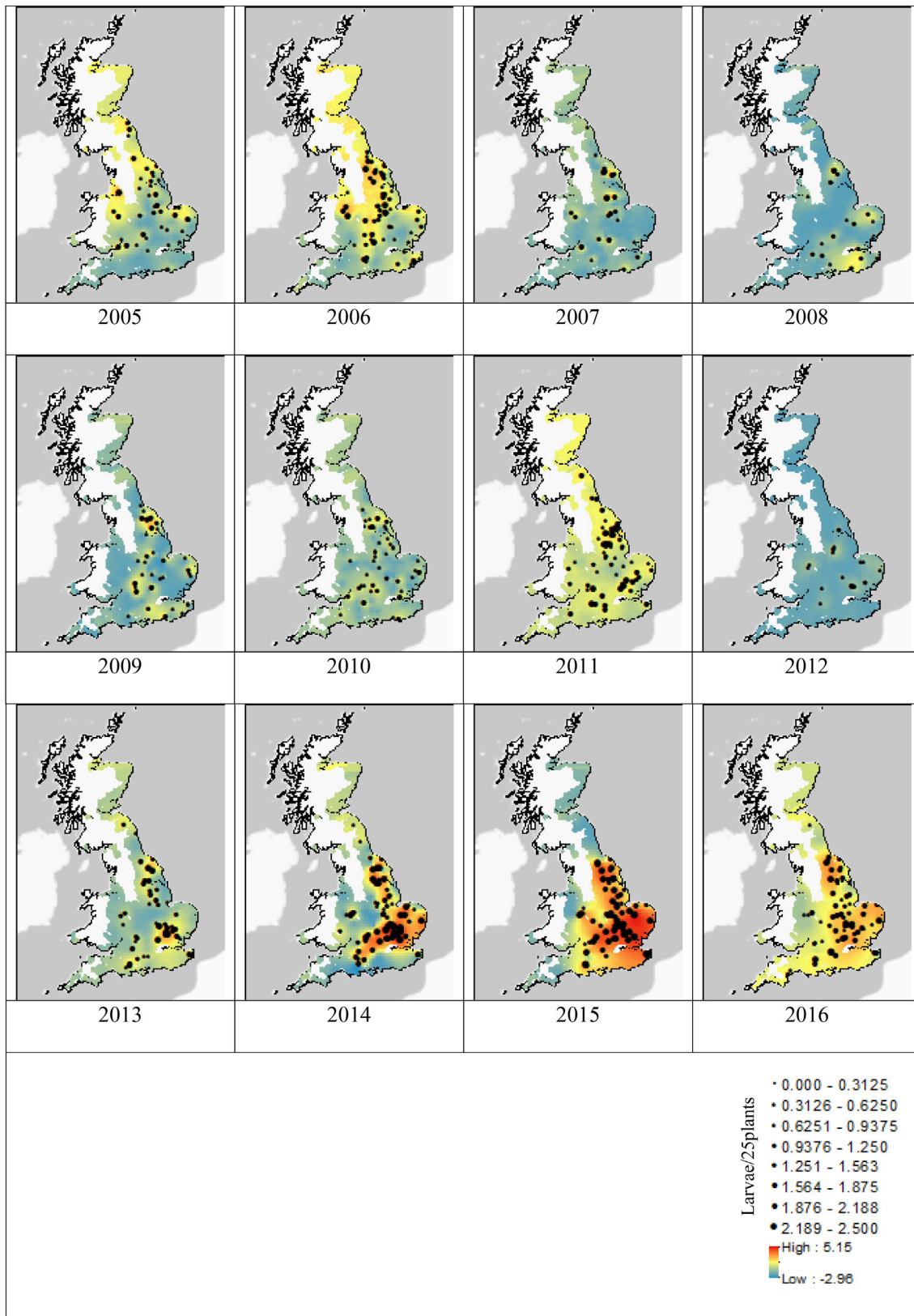
Decision trees created using the variables from the RF revealed that neonicotinoid seed treatments, day degrees above 8 °C in October and November, field area, sowing date, mean temperature



**Figure 1.** Mean number of cabbage stem flea beetle larvae per 25 oilseed rape plants by UK region 2004–2017: (A) autumn survey, B) spring survey. Regions: E = East, MW = Midlands and West, N=North, SE = South East, SW = South West. [Boxplots show distribution of the data and range from the lower (1st) first quartile to the upper (3rd) quartile, representing the middle 50% of scores, the black line shows the median. The whiskers show the lower 25% and upper 25% of scores to the minimum or maximum. The black dots represent outliers.

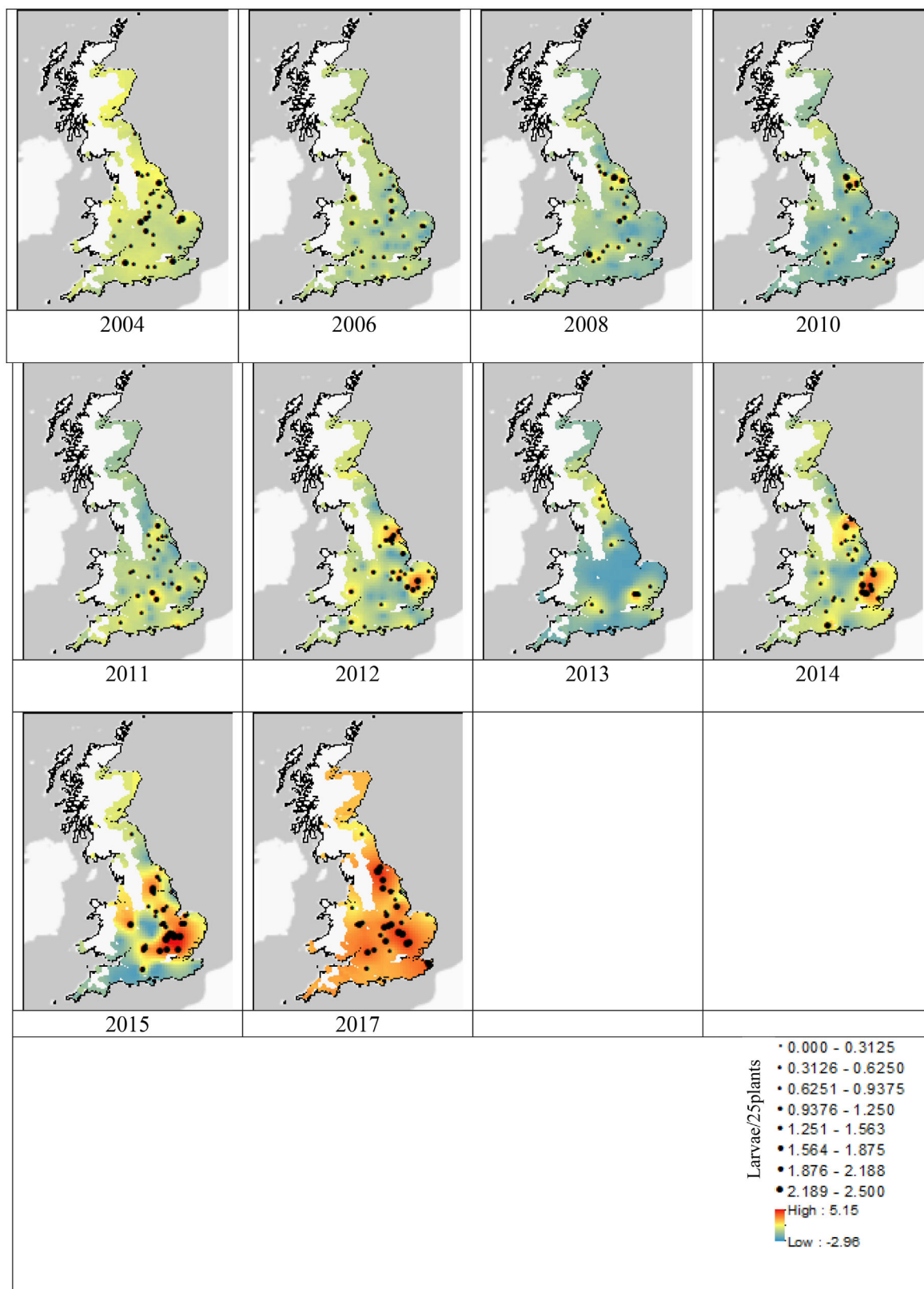
in August, accumulated rainfall in November, accumulated rainfall in August and accumulated rainfall in September were the most important factors in determining the number of CSFB larvae (Fig. 5). In node 4, which explained the largest amount of the data (37%), fields with no CSFB larvae found in OSR plants tended to be

fields where neonicotinoid seed treatments were used, where day degrees above 8 °C during September and October were < 121 and the crop was sown later than 25th August (day 238 in Julian date). Node 23 explains that fields with high numbers of larvae (>50 larvae per 25 plants, 6% of the data) tend to be fields that

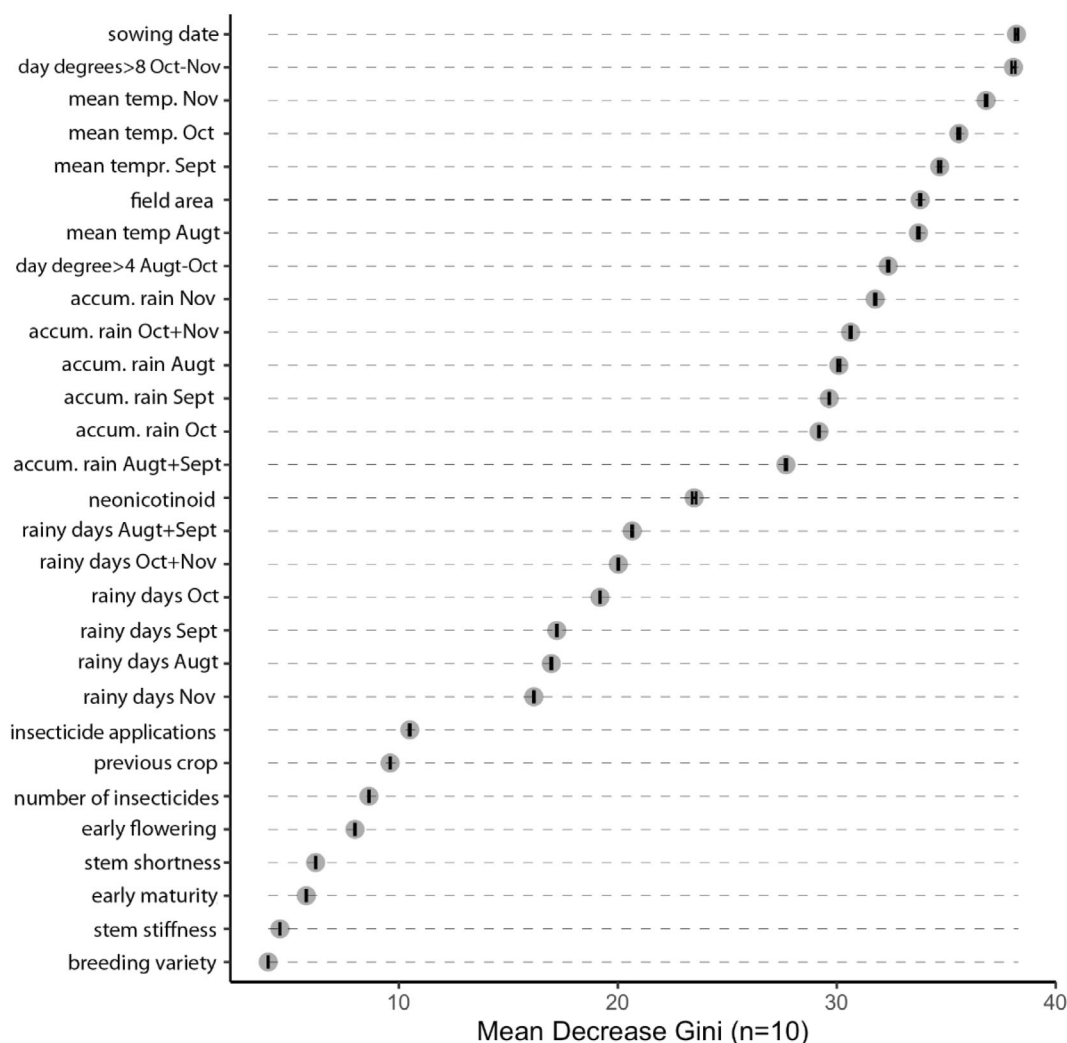


**Figure 2.** Maps showing the sampling locations (black circles) of oilseed rape (OSR) crops across UK in Autumn 2005–2016. The circle size represents the observed number of cabbage stem flea beetle larvae (natural logarithms) per 25 OSR plants. Kriged predictions (natural logarithms) are shown as colored fill on a log scale with sites predicted to have no larvae colored blue and the highest larval numbers predicted in red (173 larvae). The Kriging was done using ordinary Kriging based on the variogram fitted to that year's data. Predictions are only given for the OSR growing areas of UK (determined using the CEH Landcover +crops map for 2016 and 2017 with a 10 km buffer applied around each parcel of land). Kriging variances were particularly high in areas where there were no observations (e.g., Scotland) and so there will be large errors associated with predictions at those locations.





**Figure 3.** Maps showing the sampling locations (black circles) of oilseed rape (OSR) crops across UK in Spring 2004–2017. The circle size represents the observed number of cabbage stem flea beetle larvae (natural logarithms) per 25 OSR plants. Kriged predictions (natural logarithms) are shown as colored fill on a log scale with sites predicted to have no larvae colored blue and the highest larval numbers predicted in red (173 larvae). The Kriging was done using ordinary Kriging based on the variogram fitted to that year's data. No kriged maps were produced for years 2005 and 2007 as the data had no spatial structure. Predictions are only given for the oilseed rape growing areas of UK (determined using the CEH Landcover +crops map for 2016 and 2017 with a 10 km buffer applied around each parcel of land). Kriging variances were particularly high in areas where there were no observations (e.g., Scotland) and so there will be large errors associated with predictions at those locations.



**Figure 4.** Random Forest variable importance plot explaining the number of cabbage stem flea beetle larvae per 25 oilseed rape plants in Autumn using mean ( $\pm$ SE) decrease in Gini using  $N = 10$  ensemble of trees (50 000 trees). Higher values of mean decrease Gini indicates variables that are more important to the classification.

were not treated with neonicotinoids bigger than 12 ha and that were sown before 29th August (day 242). Medium and high numbers of larvae ( $>10$  larvae per 25 plants) were always associated with no neonicotinoid seed treatment (node 22 and 23). With regards to low numbers of larvae (between 1 and 10 larvae per 25 plants) (nodes 9, 15 and 16) classification was more complicated and separation between low numbers and zero larvae is less clear. Most cases with low numbers were fields where neonicotinoid seed treatment was used and that had warm autumns (day degrees above  $8^{\circ}\text{C}$  during September and October were  $> 121$ ). Because the use of neonicotinoids was selected as the most important variable (node 1) dividing zero to low and medium to high larval numbers, autumn larval data was divided into two periods: pre- and post- neonicotinoid ban to perform the GLMM analysis.

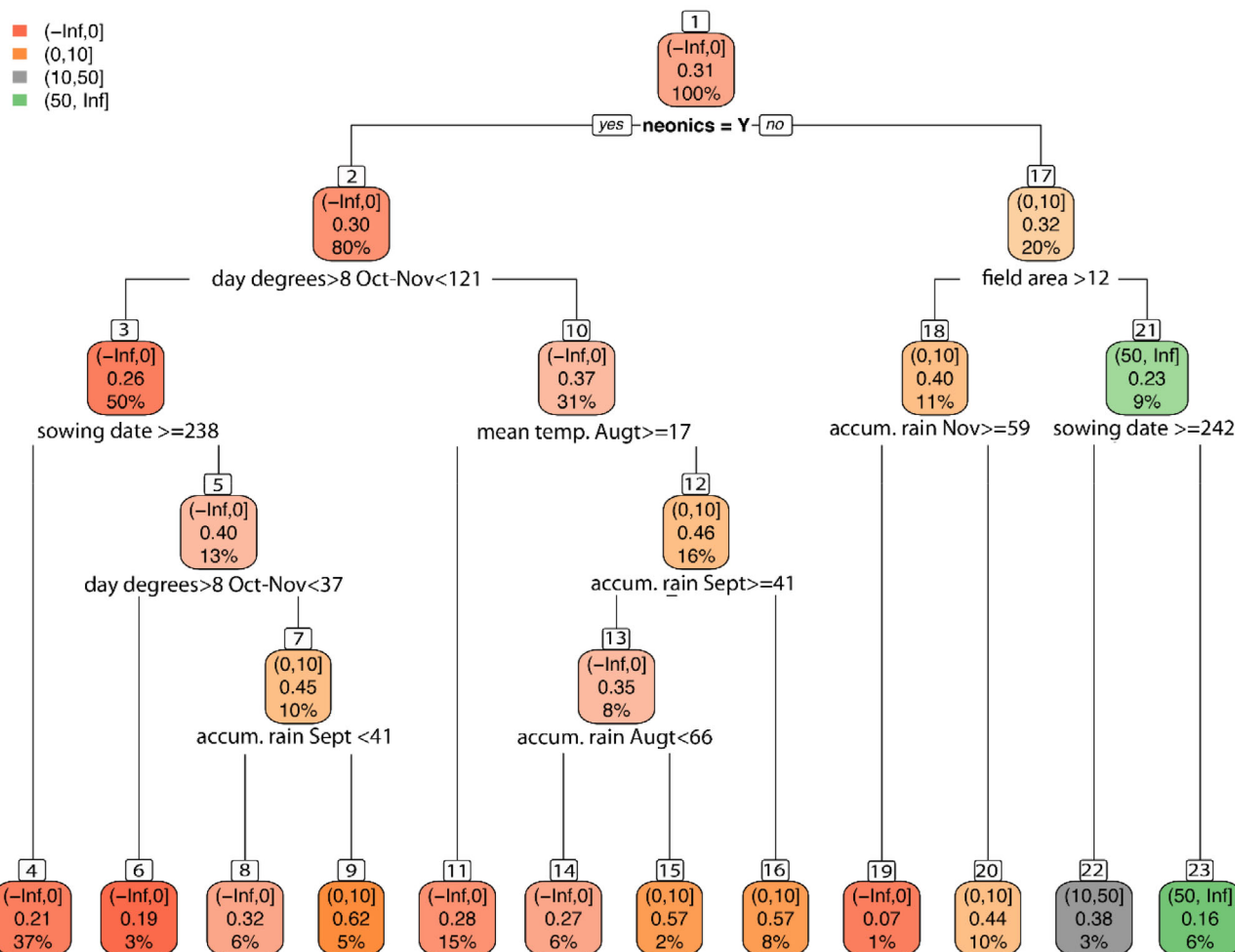
### 3.2.2 Number of larvae in spring

The OOB error rate for spring RF classification analysis was 48.7%, with successful prediction of larval numbers of zero on 75.8% of occasions. Prediction of large numbers of larvae had lower precision, with an accuracy of only 30.9%, 11.6% and 53.1% for larval

numbers between 1–10, 11–50 and more than 50, respectively. Mean temperature in January and December were identified to be the most important variables in predicting the number of larvae (Fig. 6). Following from this, field area, accumulated rainfall in October, April, November, March, January, were also top predictors (Fig. 6).

Although not included in the final RF analysis, number of larvae in autumn was included at first in the analysis for the spring larval data. This variable was selected as the most important variable both by the RF classification and decision tree. When dropping this variable out of the analysis, the RF classification remained the same, but the decision tree changed slightly. However, as the aim of the study was to identify the key meteorological and management factors affecting spring larval numbers; including number of larvae in autumn could have masked the effect of other variables and therefore, it was not included.

Decision trees created using the variables from the RF revealed that, as in autumn, use of neonicotinoid treated seed was the most important factor determining the number of CSFB larvae separating zero and low numbers (0–10 larvae per 25 plants) from medium-high numbers ( $>10$  larvae per 25 plants) (Fig. 7). Other



**Figure 5.** Decision tree based on the Random Forest analysis of the number of cabbage stem flea beetle larva per 25 oilseed rape plants collected in Autumn from sites across the UK (2005–2016) (categorized as: 0, 1–10, 11–50, >50 larvae) according to weather and oilseed rape crop management factors. Each node of the tree (1–23; node number shown on top of each colored square), shows larval number category with the biggest proportion on each node, the Gini impurity measure used to split the node and the proportion (percentage) of data that meet those requirements. The higher the Gini coefficient, the more different instances within the node. Color of each node shows the larval number category with the biggest proportion:  $(-\text{inf}, 0]$  = 0 larvae;  $(0, 10]$  = between 1 and 10 larvae;  $(10, 50]$  = between 11 and 50 larvae;  $(50, \text{Inf}]$  more than 50 larvae.

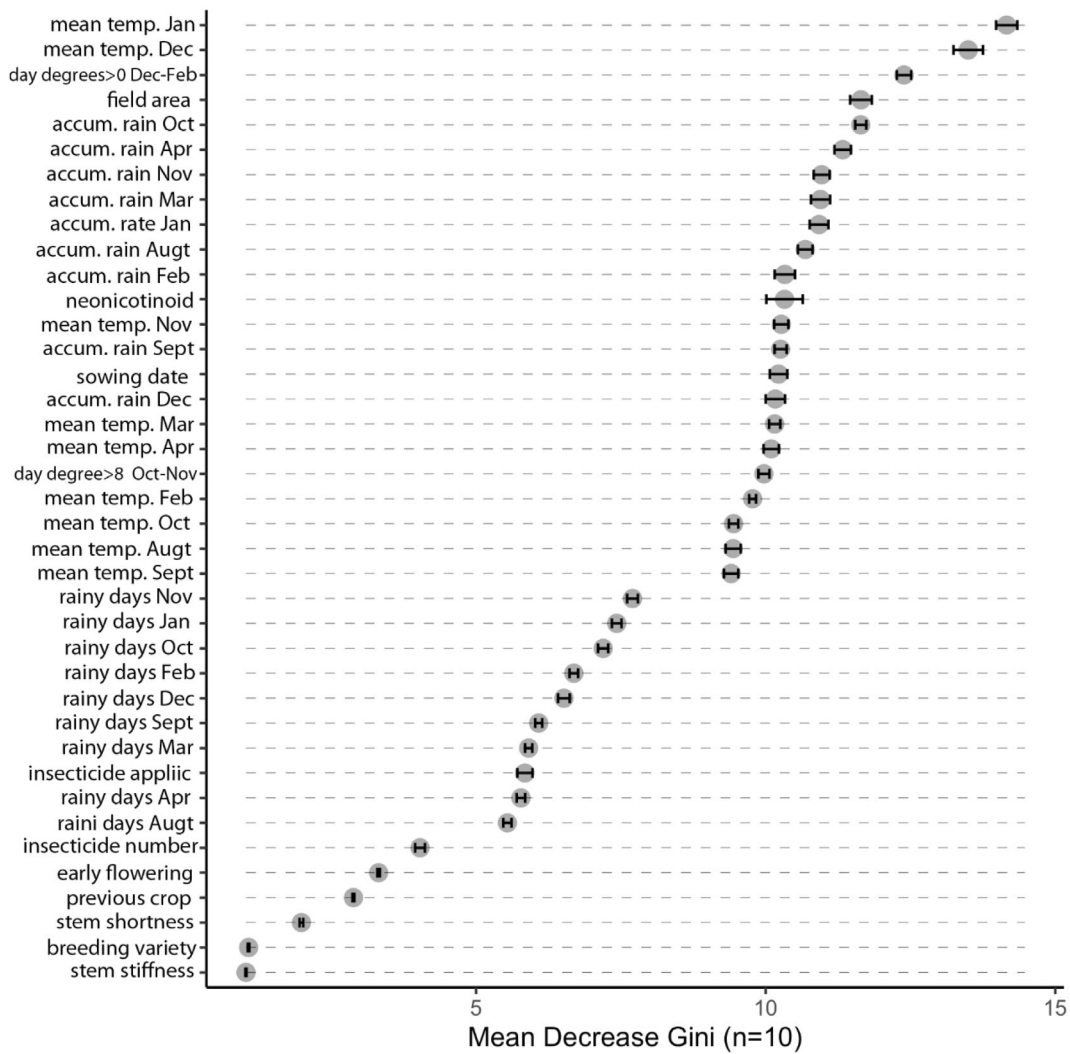
factors such as mean temperature in January and November, field area and accumulated rainfall in December and October were also important factors in determining the number of CSFB larvae (Fig. 7). Field area was the most important variable (after neonicotinoid use) in determining high numbers of larvae in spring. Node 33, which explained 15% of the data, shows that fields with high numbers of larvae (>50 larvae/25 plants) tend to be large fields with an area more than 7.9 ha. Most fields with zero larvae (node 3, explaining 17% of the data) were fields using neonicotinoid treated seed in years when mean temperature in January was below 3 °C. Fields with low numbers of larvae (<10 larvae per 25 plants) were fields sown using neonicotinoid treated seed in years when the mean temperature in January was above 7.2 °C and accumulated rainfall in December was above 47 mm (node 28, 24 and 26).

As with the autumn data, the use of neonicotinoids was selected as the most important variable (node 1) dividing low and high larval numbers in the Spring dataset, therefore the larval data were divided into pre- and post- neonicotinoid ban to perform the GLMM analyses.

### 3.3 GLMM models

The GLMM revealed that the number of CSFB larvae in autumn 2003–2013 (pre-neonicotinoid ban) was positively affected by the interaction of temperature and accumulated rainfall in September (Table 3). This interaction suggests that the relationship between temperature and number of larvae varies according to the level of accumulated rainfall. Results from the graphic interpretation of the interaction suggested that the positive effect of temperature increases with high rainfall and *vice versa* (Supporting Information, Fig. S2). Sowing date and field area were shown to have a negative effect on the number of CSFB larvae in autumn 2003–2013 (Table 3). Therefore, in fields treated with neonicotinoids the bigger the fields and the later the crop was sown, the fewer the CSFB larvae per plant in autumn.

For autumn 2014–2017 (post-neonicotinoid ban), the models showed a positive effect of field area on the number of larvae and a negative effect of the interactions between mean temperature and accumulated rainfall in August, mean temperature and accumulated rainfall in September, and day degrees above 8 °C in October and November and the accumulated rainfall in



**Figure 6.** Random Forest variable importance plot explaining the number of cabbage stem flea beetle larvae per 25 oilseed rape plants in Spring using mean ( $\pm$ SE) decrease in Gini using  $N = 10$  ensemble of trees (50 000 trees). Higher values of mean decrease Gini indicates variables that are more important to the classification.

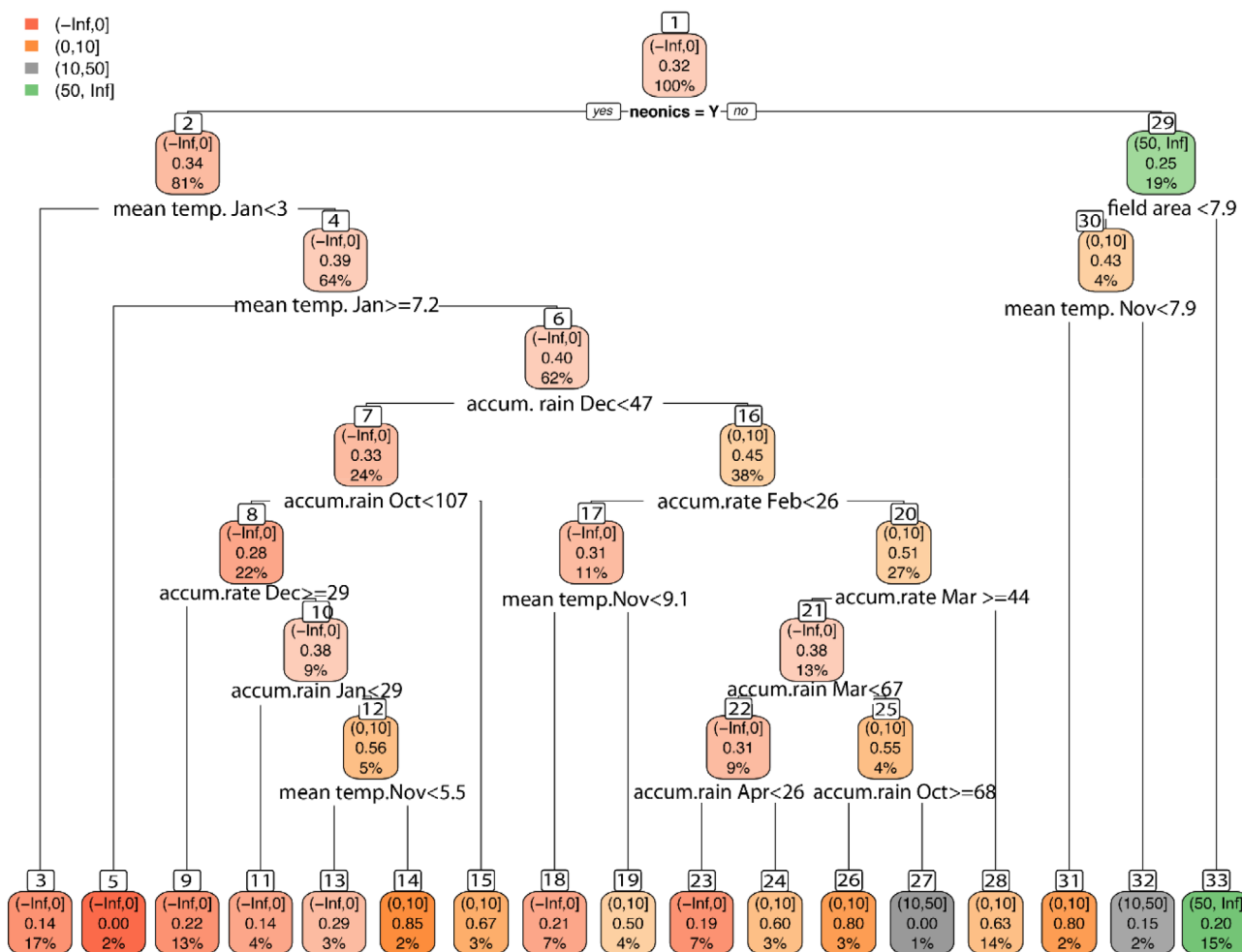
October (Table 3). Mean temperature in August had a positive effect on the number of larvae; this effect was significantly greater when rainfall in August was low compared with high rainfall (Supporting Information, Fig. S3). High rainfall and low temperatures ( $< 13\text{ }^{\circ}\text{C}$ ) in September had a positive effect on larval numbers in autumn (negative effect of mean temperature in September was bigger when rainfall was high and *vice versa*; Supporting Information, Fig. S3). High temperatures (day-degrees above  $8\text{ }^{\circ}\text{C} > 150$ ) in October and November together with low rainfall in October negatively affected the number of larvae. Temperature in October and November had a different effect on the number of larvae depending on rainfall; in wet conditions (high and medium rainfall), temperature had a negative effect on the number of larvae and *vice versa* (with low rainfall and temperature having a positive effect on the number of larvae) (Supporting Information, Fig. S3).

Models for spring 2003–2013 data (pre-neonicotinoid ban) showed negative effects of sowing date, mean temperature in February and the interaction between mean temperature and accumulated rainfall in December (Table 3). Accumulated rainfall in January and the interaction between mean temperature

and accumulated rainfall in September had a positive effect on the number of CSFB larvae per OSR plant. As before, these interactions suggest that the relationship between temperature and number of larvae varies with the level of accumulated rainfall. The effect of temperature in September on the number of larvae was positive when accumulated rainfall was high, but negative when accumulated rainfall was low (Supporting Information, Fig. S4). The interaction between temperature and rainfall in December had a significant effect on the number of larvae; the effect of temperature increased with high accumulated rainfall (there was no effect of temperature on the number of larvae when accumulated rainfall was low) (Supporting Information, Fig. S4).

Different variables were found to be as significant in spring 2014–2017 (post-neonicotinoid ban). Larval numbers in spring 2014–2017 were negatively affected by mean temperature in November, accumulated rainfall in February and April and the interactions between mean temperature and accumulated rainfall in September and January (Table 3). Mean temperature in October and April had a positive effect on the number of larvae. Both interactions have the same effect; the negative impact of





**Figure 7.** Decision tree based on the Random Forest analysis of the number of cabbage stem flea beetle larvae per 25 plants collected in Spring from sites across UK (categorized as: 0, 1–10, 11–50, >50 larvae) according to weather and oilseed rape crop management factors. For each node of the tree (node number shown on top of each colored square), it shows larval number category with the biggest proportion on each node, the Gini impurity measure used to split the node and the proportion (percentage) of data that meet those requirements. The higher the Gini coefficient, the more different instances within the node. Color of each node shows the larval number category with the biggest proportion: (-inf, 0] = 0 larvae; (0, 10] = between 1 and 10 larvae; (10, 50] = between 11 and 50 larvae; (50, Inf] more than 50 larvae.

mean temperature in September and January was bigger with high accumulated rainfall and smaller with low accumulated rainfall in each respective month (Supporting Information, Fig. S5).

#### 4 DISCUSSION

Mean numbers of CSFB larvae in OSR plants in autumn and spring were influenced by a combination of meteorological and management factors, and their relative importance changed between pre- and post-neonicotinoid restrictions. There appears to be no cyclical pattern unlike the 8-year population cycles that have been reported in Sweden<sup>30</sup> and northern Germany.<sup>54</sup>

This could be because our data was limited to 14 years and that neonicotinoid had a massive effect on the population numbers. Other factors that had a consistent effect on CSFB larval numbers in most models were sowing date, field area and the interaction between temperature and rainfall in autumn and winter, especially in September, October, December, and January. The fact that many of the interactions between temperature and rainfall

were statistically significant reflects the complexity of the effects of weather on this pest, evidencing the need to study further how these interactions affect adult and larval populations.

Spatial analysis of the larval count variograms revealed that sites close to each other were more similar in their larval count than sites which were far apart, especially following the neonicotinoid ban. Although the number of larvae in one field will differ from the number of larvae in other fields in the same area, nearby fields will be influenced by similar weather conditions that will have similar effects on CSFB populations in each field. Also, it is likely that the surrounding landscape, for example the presence of woodland around the field or the surrounding area under OSR, will have an effect on CSFB populations.<sup>29,55</sup>

##### 4.1 Effect of agronomic practices on CSFB larval numbers

The number of larvae both in autumn and spring increased 10-fold from 2013/14 to 2014/15, coinciding with the neonicotinoid restrictions<sup>56</sup> and the first detection of pyrethroid resistance in the UK.<sup>17</sup> Although larval numbers increased after the neonicotinoid ban, the mean number of larvae per plant (1.36 and 2.4



**Table 3.** Estimates from generalized mixed models testing the effect of weather and agronomic variables on the number of cabbage stem flea beetle larvae in oilseed rape plants in autumn and spring from 2003–2013 and 2014–2017

		Fixed effects	Estimate	Std. error	z value	P
Pre-neonic ban (2003–2013)	Autumn	(Intercept)	21.286	3.826	5.564	<0.001
		sowing date	–16.169	2.142	–7.548	<0.001
		field area	–0.295	0.149	–1.981	<0.05
	Spring	Temp * rain Sept	5.869	2.685	2.185	<0.05
		(Intercept)	14.747	7.339	2.009	<0.05
		sowing date	–13.800	3.460	–3.989	<0.001
		Temp Nov	–3.043	1.984	–1.534	0.125
		Temp Jan	1.695	0.997	1.7	0.089
		Temp Feb	–2.420	1.156	–2.093	<0.05
		Rain Jan	1.275	0.524	2.433	<0.05
		Temp * rain Sept	10.555	4.031	2.619	<0.05
		Temp * rain Oct	–1.105	2.377	–0.465	0.642
		Temp * rain Dec	–2.884	1.015	–2.841	<0.05
Temp * rain March	–2.891	1.931	–1.497	0.134		
Post-neonic ban (2014–2017)	Autumn	(Intercept)	–38.683	9.499	–4.072	<0.001
		field area	0.600	0.227	2.637	<0.05
		Temp * rain Aug	–29.734	6.921	–4.296	<0.001
		Temp * rain Sept	–9.369	4.139	–2.264	<0.05
	Spring	DD > 8 °C Oct–Nov * rain Oct	–2.905	1.089	–2.667	<0.05
		(Intercept)	–44.134	16.301	–2.708	<0.05
		Temp Sept	–3.194	15.582	–0.205	0.838
		Temp Oct	54.806	14.236	3.85	<0.001
		Temp Nov	–28.129	7.908	–3.557	<0.001
		Temp April	17.418	7.588	2.295	<0.05
		Rain Feb	–3.650	1.221	–2.988	<0.05
		Rain April	–3.336	0.767	–4.348	<0.001
		Temp * rain Sept	–25.800	10.986	–2.349	<0.05
Temp * rain Jan	–22.113	7.268	–3.042	<0.05		

Statistically significant *P* values are shown in bold. Interactions between variables are represented with an asterisk. Models include the main effects of the variables that were used to compute the interaction terms, although these are not shown in the table.

larva per plant in autumn and spring, respectively) did not exceed the UK economic threshold for CSFB (5 larvae per plant).<sup>57</sup> Only a 7.7% of the fields studied in autumn exceeded the economic threshold, 85% of these fields were in the East region.

The RF analysis showed neonicotinoid use to be the most important predictor of the number of larvae, associating the use of neonicotinoids with low larval numbers (< 10 larvae/25 plants) and *vice versa*. This is clear evidence of the impact of the pesticide restriction policies on pest numbers faced by farmers in the years after the ban.<sup>18</sup> The low number of larvae when neonicotinoids were available make it difficult to statistically detect significant differences between fields with different management practices or assess the impact of meteorological factors. Also, because there is only data for 3 years after the ban – not enough to draw strong conclusions, caution is required in the interpretation of these results.

Sowing date was an important predictor of the number of larvae pre-neonicotinoid ban; early sown crops had more larvae than late sown crops. Results from the RF showed that crops sown earlier than 29th August were associated with very high larval numbers in autumn (>50 larvae/25 plants). These results support previous studies finding that early sown crops had higher number of larvae and significant yield losses than late sown crops.<sup>28,29,58</sup> The earlier the crop is sown the longer the plants are susceptible to larval infestation due to increased duration of opportunity for

reproduction and oviposition in the field. Crops sown in September may also emerge after the peak migration flights have occurred; once the CSFB have arrived into early sown crops, they gradually lose their ability to fly and are likely to remain there to and lay eggs.<sup>2</sup> Therefore, late sowing seems to be an appropriate cultural method to control CSFB larval numbers especially when used in combination with neonicotinoid treated seeds. However, this practice also implies some risk; if late sowing coincides with late CSFB adult migration into the crops, feeding attacks can destroy the crop completely before it is even established or result in slow development and high plant mortality over-winter.<sup>58–60</sup> However, no effect of sowing date was found in autumn or spring larval numbers after the neonicotinoid ban. With no available control method, the adult CSFB population became a major threat to OSR growers and early sowing practices became popular; by sowing early, farmers wanted to get the crop established before adult CSFB migration, reducing crop vulnerability to feeding attack. After the neonicotinoid ban the mean sowing date took place earlier and the range of sowing dates was reduced: from 11th July to 10th October before the ban, from 6th August to 28th September after the ban. These changes in the sowing date could explain the lack of effect on the number of larvae, because there may be fewer differences in the larval numbers if most of the crops were sown before September.

Before the neonicotinoid ban, the number of larvae in autumn was affected by field area with bigger fields (>12 ha and > 7.9 ha in autumn and spring, respectively) having a smaller number of larvae than smaller fields whereas, after the ban, bigger fields had higher larval numbers. In bigger fields under a low beetle pressure, larval numbers per plant were lower probably due to a dilution effect where larval infestation per plant is significantly reduced in areas of high plant density because larvae are spread over more plants.<sup>29,61,62</sup> However, when beetle pressure is high (post-neonicotinoid ban), large OSR fields no longer enjoy a dilution effect. Furthermore, large fields can be associated with reduced natural enemy activity and higher pest loads as the beneficial effects of natural enemy decrease as the distance to the field edges increase.<sup>63,64</sup>

There was no relationship between OSR variety or any of their characteristics (stem stiffness, stem shortness, early flowering, and early maturity) and the number of larvae. However, these characteristics were based on scores from the AHDB recommended lists and none of them were directly measured in the field. Therefore, there might have been large variation in the actual expression of these traits in the field at each site and therefore inaccuracy in the trait classification on our models. Also, the analyzed data set was not specifically set up to test the effect of OSR variety and/or plant characteristics; the data available for each characteristic was sometimes very unbalanced making it difficult to reveal statistically significant differences.

#### 4.2 Effect of weather on CSFB larval numbers

Results from this study showed that autumn and winter conditions were the most important weather variables defining the number of larvae both in autumn and spring. Dry and hot conditions in August had a positive effect on the number of larvae in autumn after the neonicotinoid ban. These results contradict previous reports that found autumn larval populations decreasing with increasing sum of day degrees >3.2 °C in August.<sup>28</sup> Although it is known that high temperatures can reduce CSFB longevity,<sup>26</sup> dry conditions in August could increase CSFB flight activity, favoring early crop invasion (crops were sown earlier after the neonicotinoid ban) and extending the reproduction and oviposition period.

The interaction between temperature and rainfall in September was the only significant variable across all the models. However, the effect of this interaction was opposite in the pre- and post-neonicotinoid data sets; wet and mild/hot Septembers were related with higher numbers of larvae when neonicotinoids were available and to lower numbers of larvae after the neonicotinoid ban. Other studies have found that CSFB reproduction is highly affected by temperature; higher temperatures increase the total number of eggs, and daily oviposition rate increase with temperature<sup>4,26</sup> and reduced the pre-oviposition time.<sup>26</sup> Therefore, higher temperatures during the pre-oviposition and egg laying phases will increase the number of larvae found in the stems in autumn and spring. However, if temperatures during this period are above 25 °C, eggs could be destroyed (desiccated) and female lifespan significantly reduced.<sup>26,34</sup> A somewhat unexpected result is the negative effect of high temperature and rainfall in September on the number of larvae after the neonicotinoid ban. Although high temperatures during the egg-laying phase usually increases larval numbers, high temperatures in September could extend the aestivation period,<sup>6</sup> delaying migration into the field and reducing the reproduction and oviposition period. Also, high temperatures in

autumn could also increase the activity of natural enemies, thus affecting both the adult and larval CSFB populations.<sup>65</sup>

High temperatures in October and November together with low rainfall negatively affected the number of larvae in autumn after the neonicotinoid ban. High temperatures in November had the same effect on the number of larvae in spring. However, higher temperatures in October had a positive effect on the number of larvae in spring. It has been widely shown that warm autumn conditions lead to continuous egg laying and development, as well as increasing egg hatching.<sup>6,26,34</sup> Therefore, it is likely that warm October and November conditions will result in high numbers of larvae hatching in the autumn and infesting the plants.

Winter conditions significantly affected the number of larvae in spring; the decision tree showed that fields with no larvae were associated with low mean temperatures in January (below 3 °C). This threshold is very close to Alford's,<sup>6</sup> Johnsen's *et al.*<sup>7</sup> and Mathiasen's *et al.*<sup>35</sup> egg developmental threshold estimations of 3.2 °C, 4 °C and 5.1 °C, respectively. It has also been shown that larval mortality increases with increasing exposure time to low temperatures; if temperatures drop under 0 °C for an extended period larval development will be slowed and larval mortality increased.<sup>35</sup> Therefore, low temperatures in winter can have a negative effect on egg and larval development, larval survival and hence the number of larvae in spring. However, high temperatures in December and January combined with high rainfall were related with high number of larvae in spring (pre- and post- neonicotinoid ban). Also, when rainfall was low (< 30 mm) there was almost no effect of temperature and the number of larvae remained low. Although the effect of rainfall has been included in commercial phenological models to predict CSFB larval development,<sup>7</sup> the model and estimate behind this effect on CSFB has not been published. While there is no evidence of the direct effect of rainfall on CSFB larval development and/or survival, there are other effects of rain that could indirectly affect the number of larvae. For example, rainfall may dissolve and translocate the active ingredients or wash-off the pyrethroid insecticides reducing the efficacy of these pesticides up to 28% within 2 days after spraying.<sup>66</sup>

Further research is needed to confirm the importance of the significant variables (sowing date, field size, interaction between temperatures and rainfall in late summer and autumn) and to understand their interactions on larval infestation in OSR. There is also a need to explore further explanatory factors like rotation, tillage regime, proximity to other OSR fields, percentage of OSR in the surrounding fields and other landscape variables and how they are related year to year. These data could be used to inform farmers on which management practices can best reduce CSFB damage and could be used to build phenological models to predict CSFB population outbreaks. This could help to reduce prophylactic insecticide use, reducing the negative effects on non-target species and reducing the risk of resistance development.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from Fera Science limited. Restrictions apply to the availability of these data, which were used under license for this study. Data are available from the author(s) with the permission of Fera Science limited.

#### SUPPORTING INFORMATION

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

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