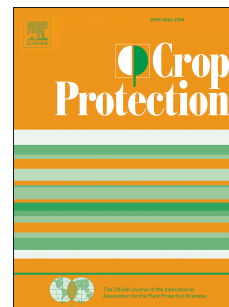


Journal Pre-proof

Investigating the status of pyrethroid resistance in UK populations of the cabbage stem flea beetle (*Psylliodes chrysocephala*)

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1 **Investigating the status of pyrethroid resistance in UK populations of the**
2 **cabbage stem flea beetle (*Psylliodes chrysocephala*)**

3
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14

15 **ABSTRACT**

16 The cabbage stem flea beetle, *Psylliodes chrysocephala* L. is a major pest of winter oilseed
17 rape in several European countries. Traditionally, neonicotinoid and pyrethroid insecticides
18 have been widely used for control of *P. chrysocephala*, but in recent years, following the
19 withdrawal of neonicotinoid insecticide seed treatments, control failures have occurred due to
20 an over reliance on pyrethroids. In line with previous surveys, UK populations of *P.*
21 *chrysocephala* were found to exhibit high levels of resistance to the pyrethroid lambda-
22 cyhalothrin. This resistance was suppressed by pre-treatment with the cytochrome P450
23 inhibitor PBO under laboratory conditions, suggesting that the resistance has a strong
24 metabolic component. The L1014F (kdr) mutation in the voltage-gated sodium channel,
25 which confers relatively low levels (10-20 fold) of resistance to pyrethroids, was also found
26 to be widespread across the UK regions sampled, whereas the L925I (s-kdr) mutation was
27 also present but much less common. The current survey also suggests that higher levels of

28 pyrethroid resistance have spread to the North and West of England, and that resistance levels
29 continue to remain high in the South East.

30

31 **Keywords:** cabbage stem flea beetle; oilseed rape; pyrethroid resistance

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32 1. Introduction

33 The cabbage stem flea beetle, *Psylliodes chrysocephala* (Coleoptera: Chrysomelidae) is an
34 established and key insect pest of winter oilseed rape, particularly in the UK (Graham and
35 Alford, 1981) and Germany (Zimmer *et al.*, 2014), and is a significant pest of other *Brassica*
36 species in several European countries (Bromand, 1990; Bartlet and Williams, 1991; Bartlet,
37 Mithen and Clark, 1996). *P. chrysocephala* inflicts damage at both the larval and adult stage,
38 with the tunnelling of the larvae into the leaf petioles and main stems causing the most
39 damage through weakening of the upper section of the roots and lower parts of the stems
40 (Williams, 2004). When infestation is high, the plant tips distort, the stems wilt and the
41 infested plants become more susceptible to fungal infections such as *Phoma lingam* (Alford,
42 2003), the bacterial disease *Erwinia* sp. and frost damage (Højland *et al.*, 2015; Højland and
43 Kristensen, 2018). Adult *P. chrysocephala* cause damage by feeding on stems, cotyledons
44 and the first true leaves during crop emergence resulting in ‘shot-holing’ symptoms, leading
45 to poor plant vigour or potential seedling death before emergence when fields are heavily
46 infested (Williams, 2010). Prior to 2014, *P. chrysocephala* affected approximately 67% of the
47 area of oil seed rape grown in the UK causing an annual 1% yield loss (Clarke *et al.*, 2009).
48 However, in 2014, serious crop losses due to adult beetles (2.7% of the national crop) were
49 recorded, the most serious losses (5–14%) being in eastern and southern England (Wynn,
50 Ellis and Alves, 2014). In the autumn of 2015, a more extensive survey found that over 65%
51 of crops had some damage, and that the damage was more widely distributed across the
52 country than in 2014, although nationally only 1% of crops were lost (Alves, Wynn and
53 Stopps, 2015). Subsequent surveys have confirmed that the average numbers of larvae per
54 plant have risen substantially in all regions since 2014 (as summarised in Dewar, 2017).

55

56 Prior to December 2013, control of *P. chrysocephala* relied on the protection of oilseed rape
57 seedlings by systemic neonicotinoid seed treatments containing either imidacloprid,
58 thiamethoxam or clothianidin, followed by the application of foliar pyrethroid sprays later in
59 the season if needed (Højland *et al.*, 2015; Højland and Kristensen, 2018). However, in
60 December 2013, the European regulatory authorities (EU commission, 2013) banned the use
61 of neonicotinoid seed treatments on all outdoor flowering crops, thus preventing their use in
62 oilseed rape, leading to the increase in *P. chrysocephala* and the increased use of pyrethroid
63 sprays. Today, pyrethroids (e.g. lambda-cyhalothrin) are the only class of insecticide that
64 remain for chemical control of *P. chrysocephala* in the UK and other parts of mainland
65 Europe.

66 The continuous use of pyrethroids to control *P. chrysocephala*, coupled with the lack of
67 alternative insecticides with different modes of action, has led to a high selection pressure,
68 driving the development and spread of resistance. Resistance to pyrethroids was first
69 reported in 2008, in north-western Mecklenburg, Western Pomerania, a major oilseed rape
70 growing area in Northern Germany (Heimbach and Müller, 2013). Zimmer *et al.*, (2014)
71 reported the presence of the L1014F *kdr* mutation in the voltage-gated sodium channel, with
72 high frequencies of the allele (90-100%) being found in populations collected from across
73 Northern Germany, with the beetles exhibiting a low level resistance against a range of
74 pyrethroids including lambda-cyhalothrin. More recently, studies by Højland *et al.*, (2015)
75 and Højland and Kristensen (2018) have shown that pyrethroid resistance resulting from the
76 *kdr* mutation is also present in populations from both Denmark and the UK, whilst in Germany
77 it has spread further south. Despite the presence of *kdr* in UK populations, Højland *et al.*,
78 (2015) found that the high pyrethroid resistance levels, with control failures being observed at
79 the full field rate, did not completely correlate with the *kdr* genotype suggesting that another
80 mechanism of resistance, such as metabolic resistance, is also present. Given the lack of

81 alternative insecticides with different modes of action, the presence and spread of pyrethroid
82 resistance is concerning for the chemical control of *P. chrysocephala*.

83 The present study has determined the current status, extent and geographical spread of
84 pyrethroid resistance in UK populations of *P. chrysocephala*. Bioassays, based on glass vial
85 exposure of adult beetles to lambda-cyhalothrin, were carried out on samples collected in
86 2018 and 2019 to examine how resistance had changed over this time across the UK. The
87 presence of the *kdr* and super-*kdr* target-site mutations in UK populations was also
88 monitored, and the potential contribution of a metabolic resistance component in the beetles
89 assessed by pre-treatment with the synergist PBO, which is a cytochrome P450 inhibitor.

90 **2. Methods**

91 2.1 Collection of field samples of *Psylliodes chrysocephala*

92 In July/August 2018 and 2019, live *P. chrysocephala* adults were collected from oilseed rape
93 pods freshly harvested from the fields at Rothamsted Research, Harpenden, Hertfordshire,
94 using a hand-held battery-powered pooter. Insects were maintained at $15\pm 1^{\circ}\text{C}$, with 65%
95 relative humidity in a light:dark photoperiod of 12:12h. Adults were kept in a mesh cage and
96 fed continuously on a diet of Chinese cabbage (*Brassica rapa* spp). Further samples were
97 received by post from oilseed rape fields across the UK and were kept in sealed plastic bags
98 or plastic containers containing Chinese cabbage or oilseed rape plant material and moist
99 tissue paper, maintained in the same environmental conditions as the Rothamsted samples.

100 2.2 Bioassays to test the effect of pyrethroids on *Psylliodes chrysocephala*

101 *P. chrysocephala* samples were tested for resistance to the pyrethroid lambda-cyhalothrin
102 using a glass vial bioassay based on IRAC (Insecticide Resistance Action Committee)
103 Method 031 (www.ircac-online.org/methods/weevils-and-flee-beetles/2014). Glass vials
104 (14ml: 7cm tall/ 2cm diameter) (S Murray and Co, Surrey, UK) were prepared by coating the

105 inner surface with different concentrations of the insecticide. Initial stock solutions were
106 prepared by diluting the technical grade insecticide in technical grade acetone. Three doses,
107 equivalent to 4%, 20% and 100% of the recommended field application rate of lambda-
108 cyhalothrin (7.5 g ai/ha) were used. The controls were glass vials treated with acetone only.
109 To coat vials, 500µl of solution was pipetted into the vials which were then placed
110 horizontally without lids on a roller in a fume hood. Vials were rotated at room temperature
111 for at least 2 hours until all the acetone had evaporated. Vials were then left vertically at 4°C
112 overnight before attaching the screw tops the following day.

113 The adult beetles (see 2.1) were used within a few days of collection and only those capable
114 of walking or jumping when released onto a tray inside a three-sided Perspex cage were
115 collected, using a hand-held battery-powered pooter. A minimum of ten beetles were
116 transferred from the inverted pooter through a small funnel into each vial. The vials were then
117 resealed and left at 18±1°C under a 16:8h light:dark photoperiod. After 24 hours, the beetles
118 were transferred to untreated glass vials without lids under upturned 200ml plastic disposable
119 cups (VWR International Ltd, Dublin, Ireland), to allow for a potential recovery which can
120 occur in insects with metabolic resistance. After a further 24 hours, the beetles were released
121 onto a tray and individuals scored using a fine paint brush according to three categories:
122 ‘mobile’ (capable of jumping or walking in a coordinated way), ‘affected’ (incapable of
123 jumping or coordinated movement) or ‘dead’ (no movement). Scoring of the beetles from
124 each vial was done for 10 minutes to avoid adults that were simulating death, a behaviour
125 shown by this species that has probably evolved through predation pressure. Results were
126 expressed as percentage mortalities. Following scoring, beetles in each category were
127 transferred to Eppendorf tubes and snap frozen using liquid nitrogen before being stored in a
128 freezer at -80°C.

129 2.3 TaqMan PCR assay to detect the presence of *kdr/skdr* in *Psylliodes chrysocephala*

130 TaqMan genotyping assays (Livak, 1999) were used to determine the presence of the
 131 mutations responsible for the kdr (L1014F) and super-kdr (L925I) sodium channel
 132 substitutions in individual adult beetles. Primer Express v.2.0 (Life Technologies) was used
 133 to design the primer and probe sequences for the assays (Table 1). In both assays, VIC
 134 reporter dye-labelled probes were used to detect the wild-type susceptible allele and 6-FAM
 135 reporter dye-labelled probes to detect the resistant allele. Each probe contained a 3' non-
 136 fluorescent quencher dye.

137 **Table 1.** Primer and probe sequences used for TaqMan assays to detect the L1014F (kdr) and
 138 L925I (skdr) mutations in *Psylliodes chrysocephala*.

Primer/Probe	Sequence	
Primers	kdr-F	GGACTGTATGCTAGTCGGTGATGT 140
	kdr-R	GCAAAGCCAAGAAGAGATTCAGTA
	skdr-F	GCCAAGTCATGGCCAACTT 141
	skdr-R	TATAATGCACAGCACAAAGGTCA
Probes	kdr-VIC	TTACCACAAGATTACC 142
	kdr-FAM	TTACCACAAAATTACC
	skdr-VIC	TGGGTGCTTTAGGTAA 143
	skdr-FAM	TGGGTGCTATAGGTAA

144

145 PCR reactions (15µl) contained 1.5µl (50ng) genomic DNA, 7.5µl SensiFast probe mix
 146 (Bioline Reagents Ltd, UK), 0.375µl of kdr or skdr primer/probe mix (800nM of each primer
 147 and 200nM of each probe) and sterile water. Reactions were run on an Applied Biosystems
 148 7900HT real-time PCR system, with initial incubations at 50°C for 2 minutes and 95°C for 10
 149 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 45 seconds. The increase
 150 in VIC and 6-FAM reporter dye fluorescence was monitored in real time and an allelic
 151 discrimination analysis performed using the 7900HT Sequence Detection System software.

152 2.4 Use of a synergist to identify the presence of metabolic resistance in *Psylliodes*
 153 *chrysocephala*

154 Pre-treatment with the insecticide synergist Piperonyl butoxide (PBO), obtained from Sigma-
155 Aldrich (Missouri, USA), was used to detect potential metabolic resistance mechanisms *in-*
156 *vivo*. PBO was diluted in technical grade acetone to give an equivalent concentration of
157 0.011mg cm^{-2} (Højland *et al.*, 2015). This dose was chosen because it did not cause control
158 mortality when tested. 500 μl of solution was then used to coat glass vials (see 2.2). Ten
159 beetles per replicate were transferred to the PBO-coated vials for 1 hour before being
160 transferred to either untreated control vials or vials coated with lambda-cyhalothrin at the
161 100% field rate (7.5 g ai ha⁻¹). The beetles were then bio-assayed in parallel to beetles from
162 the same sample not pre-exposed to PBO.

163 3. Results and Discussion

164 3.1 Survey of pyrethroid resistance in *Psylliodes chrysocephala* across the UK

165 To determine the current extent and geographical spread of resistance to pyrethroid
166 insecticides in UK *P. chrysocephala* populations, and how this compares to previous reports
167 (Højland *et al.*, 2015), bioassays with lambda-cyhalothrin were conducted on adult beetle
168 samples from Rothamsted Research's farm in Hertfordshire and oilseed rape fields located
169 across England, Scotland and Wales. The bioassays allowed the samples to be categorised as
170 being either completely susceptible, or to contain beetles that were 0-25%, 25-50%, 50-75%,
171 75-99% and 100% resistant, depending on the percentage of beetles per sample surviving
172 treatment with 7.5 g ai ha⁻¹ lambda-cyhalothrin. Although lambda-cyhalothrin is used as an
173 exemplar in these studies, other pyrethroids also contribute to the selection pressure in *P.*
174 *chrysocephala* populations across Europe. The bioassay is an approved test method (method
175 031) for determining resistance in *P. chrysocephala* (IRAC, 2014) and was used by Zimmer
176 *et al.*, (2014) to monitor the emergence and geographic spread of pyrethroid resistance in *P.*
177 *chrysocephala* in Germany, by Højland *et al.*, (2015) to determine the spread of pyrethroid

178 resistance in Danish, British and German samples and most recently by Højland and
179 Kristensen (2018) when investigating lambda-cyhalothrin resistance in Danish populations.
180 Similar bioassays have also been used to monitor the spread of pyrethroid resistance in
181 European populations of pollen beetle (*Brassicogethes aeneus*), another major pest of oilseed
182 rape (Zimmer and Nauen, 2011; Slater *et al.*, 2011; Nauen *et al.*, 2012).

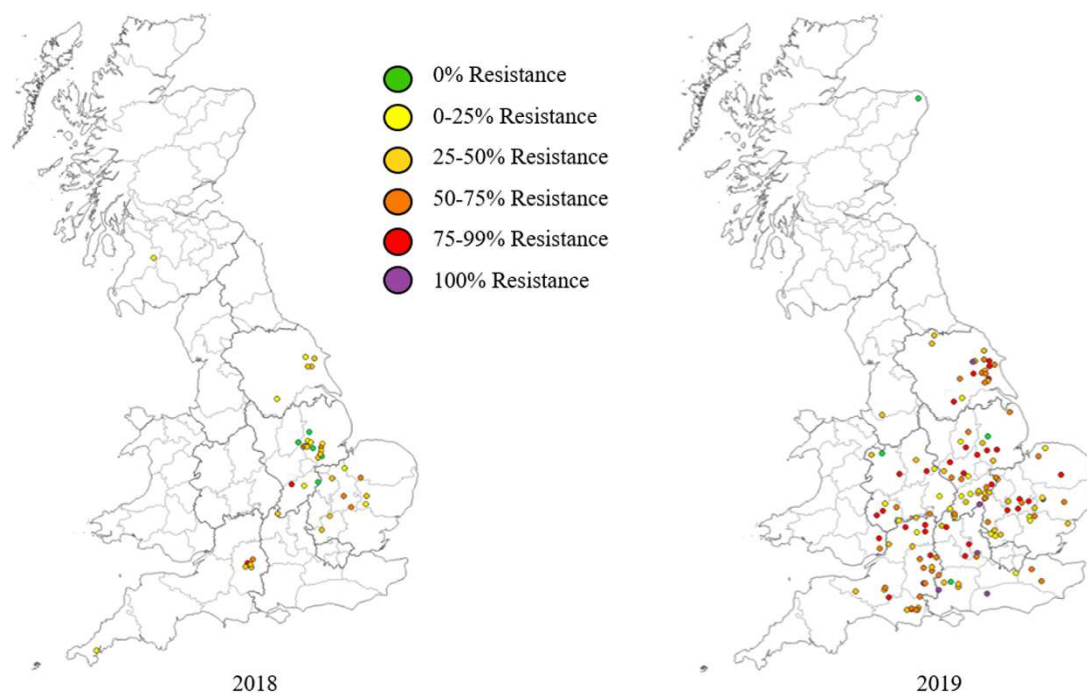
183 In 2018, a total of 41 *P. chrysocephala* samples, obtained from four different regions across
184 England, but primarily from counties in the East (Fig. 1), were tested. Of these only five
185 samples were found to contain no mobile beetles at 100% of the recommended field rate for
186 lambda-cyhalothrin, which would be expected if the sample was susceptible. However, for
187 these five samples mortality was found to be <90% at 20% of the field rate, suggesting
188 resistance is present as judged by the IRACs 'susceptibility rating scheme' (IRAC, 2014).
189 The other 37 samples all showed some level of resistance with the highest resistance, at 89%
190 being the sample from Bishop Cannings (Wiltshire).

191 In 2019 a total of 146 *P. chrysocephala* samples were obtained from across England,
192 representing more of the country (Fig. 1), two samples were received from Wales and one
193 from Scotland. Only the Scottish sample was found to be truly susceptible to lambda-
194 cyhalothrin, displaying 100% mortality at 20% of the recommended field rate. Worryingly,
195 several populations containing 100% resistant beetles were recorded for the first time in the
196 UK. Overall, the distribution maps for pyrethroid resistance in UK populations of *P.*
197 *chrysocephala* (Fig. 1) suggest that higher levels of resistance have spread to the North and
198 West of England and that resistance levels continue to remain high in the South East.

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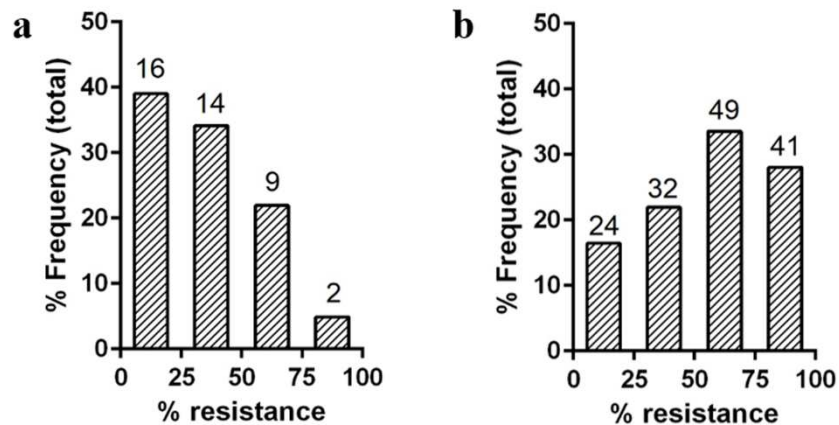
203

204 **Figure 1.** Pyrethroid resistance in *P. chrysocephala* in the UK for 2018 and 2019. The maps
205 were created using QGIS (version 3.0.3) and use a 6-category colour scale to show the level
206 of resistance. The map is divided into counties (light grey borders) and regions (dark grey
207 borders).

208

209 Over the two years of monitoring, the percentage of highly pyrethroid-resistant beetles in the
210 samples increased. The mean resistance level was significantly greater in 2019 (55.64%)
211 compared to 2018 (32.9%) (two-sample t-test, $t_{185} = -5.02$, $p < 0.001$, $SED = 4.529$). Over the
212 two years there was found to be a significant difference in the distribution of measurements
213 across the resistance categories, $X^2(3) = 18.47$, $p < 0.001$ (Fig. 2). In 2018 the percentage of
214 beetles in the 0-25% resistance category was 39%, whereas in 2019 this decreased to 16%. In
215 2018 5% of samples were in the 75-100% resistance category whereas in 2019 this increased
216 to 28%.

217



218

219 **Figure 2.** Histograms showing the shift in the relative % frequency of pyrethroid resistant *P.*
 220 *chrysocephala* in (a) 2018 and (b) 2019. Numbers show the raw count data.
 221

222 To assess whether there was any impact of the spatial variation over which the samples were
 223 collected, analysis of covariance was undertaken based on year, adjusting for the easting and
 224 northing coordinates as covariates. There was no evidence of a linear association between the
 225 covariates and the outcome at the 5% significance level. The analysis was then repeated with
 226 3 geographically extreme data values omitted (two observations in Scotland and one in
 227 Cornwall). Again, there was no evidence of a linear association between the covariates and
 228 the outcome. However, in both cases the analysis showed a significant difference in mean
 229 resistance between the two years ($p < 0.001$). Scatter plots of the easting and northing
 230 coordinates plotted against resistance did not indicate any other non-linear association.

231 Further analysis was undertaken to assess regional and county-level differences for the 2019
 232 data. The mean resistance levels (Table 2a) were higher than the national average in the
 233 South East (60.39%), South West (57.83%), and Yorkshire and the Humber (63.32%). South-
 234 East Wales had the highest mean resistance (72.50%) but only two samples were tested.
 235 Analysis of variance (ANOVA), incorporating a nested treatment structure to reflect counties
 236 nested within regions, showed that mean resistance levels did not differ significantly between

237 regions ($F_{8,113} = 1.28, p=0.262$). The standard errors of the differences between means (SEDs)
 238 at the regional level were also calculated (Table 2b). There was also found to be no
 239 significant difference in mean resistance levels between counties within the same region
 240 ($F_{24,113} = 1.06, p=0.395$). The residual mean square from the ANOVA was 675.3. The absence
 241 of statistically different mean resistance levels suggests there are no resistance ‘hotspots’ and
 242 that resistance is highly localised, almost on a farm-by-farm basis.

243 **Table 2. (a)** Summary of average resistance levels by region and county and **(b)** Standard
 244 error of differences between means at the regional level

a

Region and County	Number of Samples	Average resistance level (%)
East Midlands	29	51.28
Leicestershire	7	60.43
Northamptonshire	12	48.58
Nottinghamshire	3	49.67
Lincolnshire, Parts of Kesteven	6	45.33
Lincolnshire, Parts of Lindsey	1	60
East of England	29	53.72
Bedfordshire	3	57.33
Cambridgeshire	8	72.25
Essex	4	31.5
Hertfordshire	3	26
Huntingdonshire	2	57
Norfolk	3	53.33
Suffolk	6	55
North West	1	40
Lancashire	1	40
Scotland	1	0
Aberdeenshire	1	0
South East	18	60.39
Berkshire	2	80
Hampshire	6	50.67
Kent	3	61.67
Oxfordshire	5	64
Surrey	1	18
Sussex	1	100
South East Wales	2	72.5
Monmouthshire	2	72.5
South West	35	57.83

Dorset	7	53.57
Gloucestershire	8	57.88
Somerset	4	57.5
Wiltshire	16	59.75
West Midlands	12	48.33
Herefordshire	4	60.25
Shropshire	3	46.67
Staffordshire	3	46.33
Warwickshire	1	10
Worcestershire	1	50
Yorkshire and the Humber	19	63.32
East Riding of Yorkshire	14	68.93
North Riding of Yorkshire	3	43.33
West Riding of Yorkshire	2	54
Grand Total	146	55.64

245

b	Region	Standard Error of Differences												
		1	2	3	4	5	6	7	8	9				
	East Midlands	1	*											
	East of England	2	6.82	*										
	North West	3	26.43	26.43	*									
	Scotland	4	26.43	26.43	36.75	*								
	South East	5	7.8	7.8	26.7	26.7	*							
	South East Wales	6	19	19	31.83	31.83	19.37	*						
	South West	7	6.53	6.53	26.36	26.36	7.54	18.89	*					
	West Midlands	8	8.92	8.92	27.05	27.05	9.68	19.85	8.69	*				
	Yorkshire and the Humber	9	7.67	7.67	26.66	26.66	8.55	19.32	7.41	9.58	*			
			1	2	3	4	5	6	7	8	9			

246

247 3.2 Pyrethroid resistance mechanism(s) in *P. chrysocephala*

248 The TaqMan assays (see 2.3) were used to detect the presence of the L1014F (kdr) and L925I
249 (s-kdr -like) substitutions in the 2018 and 2019 *P. chrysocephala* samples (Table 3). In 2018,
250 40 beetles from seven UK samples of *P. chrysocephala* were tested using only individuals
251 that had survived the 100% field rate of lambda-cyhalothrin, enabling the genotype
252 associated with the resistant, mobile phenotype to be determined. The samples were from
253 Great Saxham (Suffolk), Bishop Cannings (Wiltshire), Rothamsted (Hertfordshire), Linton
254 (Cambridgeshire), Feltwell (Norfolk) and Horbling and Grantham (Lincolnshire). The

255 L1014F mutation was present at all sites, with 47.5% of the beetles being homozygous for the
256 resistant allele (RR), 37.5% heterozygous (SR) and the remaining 15% *kdr* SS, although this
257 genotype was not present in the Suffolk or Wiltshire populations. The detection of *kdr* SS
258 genotypes in beetles that displayed the mobile phenotype after treatment with the label rate of
259 lambda-cyhalothrin, strongly suggests the presence of another resistance mechanism in *P.*
260 *chrysocephala*. We also identified *kdr* RR (homozygote) genotypes in beetles that did not
261 survive lambda-cyhalothrin treatment, confirming that the L1014 mutation on its own is not
262 able to confer protection to the field rate dose (results not shown). In contrast to L1014F, the
263 L925I mutation, which is predicted (based on studies in other insects) to be associated with
264 higher resistance levels to pyrethroids than *kdr*, was much less common, with two samples
265 (Norfolk and Hertfordshire) showing only the SS genotype and the overall percentage of
266 beetles showing the homozygous L925I genotype (RR) being only 2.5%. Of the six beetles
267 homozygous for the susceptible *kdr* allele (SS), one also displayed the homozygous resistant
268 *s-kdr* allele (RR) and two displayed the heterozygous resistant *s-kdr* allele (SR) (data not
269 shown). As three beetles were susceptible for both the *kdr* and *s-kdr* allele this suggests the
270 presence of another resistance mechanism. Direct sequencing of sodium channel fragments
271 carrying the mutations showed that L1014F (*kdr*) and L925I (*s-kdr*) are mutually exclusive
272 and have arisen independently in different sodium channel alleles, thus limiting the number
273 of genotypic combinations possible within individual beetles.

274 In 2019, *P. chrysocephala* individuals were screened for *kdr* from sites close to those
275 sampled in 2018 (a sample from Oxfordshire was also included) and again, only beetles that
276 survived the 100% field rate of lambda-cyhalothrin were tested. The L1014F mutation was
277 present at all sites except the one from Scotland. The percentage of beetles homozygous for
278 the *kdr* resistance allele (RR) increased in three of the samples, Suffolk, Norfolk and
279 Hertfordshire but decreased overall from 47.5% to 36%. Given that the percentage of beetles

280 resistant to lambda-cyhalothrin in each sample increased between 2018 and 2019, but there
 281 was an overall decrease in the homozygous and heterozygous L1014F mutation, this further
 282 suggests the presence of another resistance mechanism in *P. chrysocephala*. Whilst the L925I
 283 (s-kdr) mutation was less common than the L1014F (kdr) mutation, it was found to be present
 284 in the Wiltshire and Hertfordshire samples which contained only the wild-type metabolic
 285 genotype (SS) in 2018. In the Oxford sample 15% of the beetles tested for the s-kdr mutation
 286 were homozygous for the resistant allele (RR). Overall the percentage of beetles showing the
 287 homozygous genotype (RR) was 6.6%.

288 **Table 3.** Detection of kdr/skdr alleles in *P. chrysocephala* using TaqMan assay

289
290 **2018**

Region	County	Populations	No°	<i>kdr status</i>			
				SS	SR	RR	%RR
East Midlands	Suffolk	1	4	0	3	1	25%
	Wiltshire	1	8	0	4	4	50%
	Lincolnshire	2	10	2	2	6	60%
East of England	Cambridgeshire	1	8	2	2	4	50%
	Norfolk	1	6	1	3	2	33%
	Hertfordshire	1	4	1	1	2	50%
Total		7	40	6 (15%)	15 (37.5%)	19 (47.5%)	

291

Region	County	Populations	No°	<i>skdr status</i>			
				SS	SR	RR	%RR
East Midlands	Suffolk	1	4	3	1	0	0%
	Wiltshire	1	8	5	3	0	0%
	Lincolnshire	2	10	9	1	0	0%
East of England	Cambridgeshire	1	8	5	2	1	13%
	Norfolk	1	6	6	0	0	0%
	Hertfordshire	1	4	4	0	0	0%
Total		7	40	32 (80%)	7 (17.5%)	1 (2.5%)	

292

293 **2019**

Region	County	Populations	No°	<i>kdr status</i>			
				SS	SR	RR	%RR
East Midlands	Suffolk	1	4	0	1	3	75%

	Wiltshire	1	5	3	1	1	20%
	Lincolnshire	2	16	2	8	6	38%
East of England	Cambridgeshire	1	8	1	6	1	13%
	Norfolk	1	8	2	4	5	63%
	Hertfordshire	1	5	1	1	3	60%
SE England	Oxfordshire	1	19	7	4	8	42%
Scotland	Aberdeenshire	1	10	10	0	0	0%
	Total	9	75	26 (34.7%)	25 (33.3%)	27 (36%)	

294

Region	County	Populations	No°	<i>skdr status</i>			
				SS	SR	RR	%RR
East Midlands	Suffolk	1	5	5	0	0	0%
	Wiltshire	1	3	1	1	1	33%
	Lincolnshire	2	16	11	5	0	0%
East of England	Cambridgeshire	1	8	5	3	0	0%
	Norfolk	1	8	7	1	0	0%
	Hertfordshire	1	6	4	1	1	17%
SE England	Oxfordshire	1	20	13	4	3	15%
Scotland	Aberdeenshire	1	10	10	0	0	0%
	Total	5	76	56 (73.7%)	15 (19.7%)	5 (6.6%)	

295

296 3.3 Bioassays of *P. chrysocephala* using lambda-cyhalothrin and the synergist PBO

297 The insecticide synergist piperonyl butoxide has been shown to inhibit both P450
 298 monooxygenases and esterases, thereby acting as a tool for the identification of metabolic
 299 resistance in insect samples (Young, Gunning and Moores, 2006). To investigate the lack of
 300 correlation between lambda-cyhalothrin resistance and *kdr* frequency, and to determine
 301 whether P450 monooxygenases (and/or esterases) may play a role in mediating pyrethroid
 302 resistance in UK *P. chrysocephala* populations, synergist bioassays with PBO pre-treatments
 303 were conducted on five *P. chrysocephala* samples.

304 When exposed to lambda-cyhalothrin at the recommended field rate, the percentage of
 305 beetles affected was 47% (North Yorkshire), 75% (Wiltshire), 8% (Wiltshire), 40%
 306 (Leicestershire) and 40% (Hertfordshire) (Fig. 3). However, all adults pre-treated with PBO,
 307 prior to exposure to lambda-cyhalothrin at the same field rate were killed. This strongly

326 the exact mechanism of resistance. This resistance to pyrethroids has resulted in widely-
327 reported control problems for this pest in the farming press (e.g. Clark 2014; Casswell, 2014;
328 FarmingUK team, 2015; Hill, 2017; FarmingUK team, 2017; Case, 2018; Allison, 2019;
329 Dyer, 2019; Gillbard, 2019) since the introduction of the neonicotinoid ban

330 Despite the development of resistance in *P. chrysocephala*, pyrethroids continue to be used
331 on UK farms as there remains a lack of insecticides with alternative modes of action that can
332 be deployed for resistance management. However, this continued reliance on pyrethroids is
333 failing as a control strategy in many regions and is not sustainable in areas where resistance
334 levels may appear low or non-existent. Since 2014, there has been a significant year by year
335 decrease in the area of oilseed rape production in the UK, declining from 634,000 hectares in
336 2014 (DEFRA, 2014) to 497,000 hectares in 2019 (DEFRA, 2019). It is therefore particularly
337 important that the extent and geographical spread of pyrethroid resistance in this pest
338 continues to be monitored at a time when synthetic pesticides are becoming less favoured
339 through EU legislation. Clearly there needs to be informed decision making on how to best
340 deploy pesticides effectively in the future. Alternative strategies, such as the potential of the
341 parasitoid *Microctonus brassicae* for biological control (Jordan *et al.*, 2020), trap cropping
342 (Barari *et al.*, 2005) and the use of insect-resistant varieties of oilseed rape also offer options
343 for *P. chrysocephala* control and are being further explored.

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Journal Pre-proof

HIGHLIGHTS

- UK populations of cabbage stem flea beetle exhibit high levels of resistance to pyrethroid insecticides
- Pyrethroid resistance in UK populations is largely the result of increased metabolism
- Resistance has advanced to the North and West of England
- Resistance levels are highest in Yorkshire and the Humber and the South East of the UK

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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