Research Note • DOI: 10.15212/ijafr-2022-0106 IJAFR • 61(2) • 2022 • 407-411

Irish Journal of Agricultural and Food Research

A note on current pyrethroid susceptibility in the bird cherry-oat aphid *Rhopalosiphum padi* in Ireland

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Abstract

The objective of this study was to observe the response of the bird cherry oat aphid, Rhopalosiphum padi (Linnaeus, 1758) to field rate equivalents of insecticides, by using bioassays of vials coated with the pyrethroid, λ-cyhalothrin. The results from the geographically separated Irish R. padi colonies indicated a susceptible response, which was a similar finding to the UK which showed sensitivity in this species of cereal aphids. Monitoring the susceptibility status of aphids using bioassays gives information regarding developments of any tolerance, which could be a precursor, or resistance against the target chemical insecticide, which is an important integrated pest management tool.

Keywords

Bird cherry-oat aphid • pyrethroid • Rhopalosiphum padi • tolerance

Introduction

Cereal aphids can cause extensive damage to economically important arable crops, making them an important pest in agriculture (Foster & Leybourne, 2021). Aphids inflict injury via direct feeding and, most often, due to virus-vectoring (Quisenberry & Ni, 2007). The status aphids have as a crop pest is based on their ability to cause damage to a crop by direct feeding, which accounts for up to 20% yield loss and by spreading viruses such as the Barley yellow dwarf virus (BYDV) that can cause up to 80% yield loss in crops. The bird cherry-oat aphid *Rhopalosiphum padi* is a serious agricultural pest in temperate regions worldwide (Mehrabi *et al.*, 2014), with a wide geographic distribution (Peng *et al.*, 2017) causing significant damage to cereal crops and pasture grasses on a global level (Rubio-Meléndez *et al.*, 2019). Pyrethroids are the main chemical insecticides used for controlling aphid populations on cereal crops. Pyrethroids act on the voltage-gated sodium channels (VGSCs), by blocking the neurotransmission receptors causing paralysis and, normally, fast-acting, death in the aphid. However, overuse of pyrethroids has led to an increase of tolerance and resistance developing within aphid populations against these insecticides as observed in both *Sitobion avenae* (Foster *et al.*, 2014) and *R. padi* (Wang *et al.*, 2020) populations. In China, it has been observed that *R. padi* has the potential to develop insecticide resistance with a super-knock down resistance (kdr), target-site-based mutation of the M918L region identified on the VGSCs (Wang *et al.*, 2020). In contrast, a recent survey and assessment of UK *R. padi* populations reported that *R. padi* did not show reduced susceptibility to pyrethroids (Foster & Leybourne, 2021).

A recent study investigating resistance in cereal aphids in Ireland, identified an aphid colony, initiated from a single *R. padi* individual, which was considered to express a level of tolerance to the pyrethroid compound λ-cyhalothrin (Walsh *et al.*, 2020), when compared with two field-collected susceptible colonies of *Metopholophium dirodum* (rose-grain aphid), a field-collected susceptible *S. avenae* (grain aphid) clone and a pyrethroid-resistant *S. avenae* clone (Sa3). As this was the first indication of possible pyrethroid tolerance in *R. padi* recorded in Ireland and the British Isles, it was therefore important to carry out a more detailed study on aphid samples to further evaluate the potential susceptibility of this species to pyrethroid insecticides. In our current study, six *R. padi* individuals were collected and established as colonies from four Irish counties (Carlow [Cw1, Cw2], Laois [LS], Kilkenny [Kk] and Cork [Ck1, Ck2]). Dose–response assays, using vials coated with varied concentrations of the

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pyrethroid, λ-cyhalothrin, were done on the collected colonies with several asexually reproduced generations reared for a period of a year. Controls used in this study were the colonies initially collected and tested by Walsh *et al.* (2020): the suspected tolerant *R. padi* clone (Tipp 1 and Tipp 2) and a partially resistant *S. avenae* standard clone (Sa3).

Materials and methods

Pyrethroid sensitivity assay

The objective of this study was to survey the response status of Irish *R. padi* colonies to different rates of the pyrethroid insecticide, λ-cyhalothrin, ranging from 0% (control) up to the equivalent of the recommended full spray field rate, 5.0 g a.i./ha or 50.0 ng a.i./cm^2 (Table 1), using a coated glass vial dose–response assay, following the approach used by Walsh *et al.* (2019) and Foster *et al.* (2014), to measure the LC_{50} value of each distinct aphid colony.

Following the dose–response assays, a 470-bp polymerase chain reaction (PCR) fragment which amplified the domain IIS4–IIS6 region in the VGSC for each of the Irish *R. padi* colonies was sequenced. The molecular protocol for sequencing this region of interest followed a study that searched the VGSC of the *S. avenae* for mutations (Foster *et al.*, 2014), with the adaptation of *R. padi* equivalent primers for this study. This enabled screening for known mutations associated with resistance to pyrethroids found in other insects such as the M918, L925, T929, F932 and L1014F (Foster *et al.*, 2014).

Individual *R. padi* were collected from tillage crops (spring barley and maize) from several counties: Carlow, Laois, Kilkenny and Cork (Table 2). Clonal lineages were initiated, each using individual non-winged asexual parent aphid reared on spring barley, *Hordeum vulgare* var. Planet and Cassia in long-term cage colonies, maintained at 20°C under a 16:8 h (long day) light: dark photoperiod. Dose–response assays, using λ-cyhalothrin (a pyrethroid), as described in the methods from Foster *et al.* (2014) and Walsh *et al.* (2019), were done on the aphid colonies. The colonies tested are summarised in Table 2, plus the pre-existing *R. padi* colonies, collected by Walsh (2019), collected in 2017 in Co. Tipperary (Tipp 1 and Tipp 2) and an *S. avenae* clone, collected in 2018 in Co. Carlow (Sa3).

Table 1: λ-Cyhalothrin field rate conversions in % field rate to g a.i./ha and ng a.i./ha

Equivalent field rate % of λ-cyhalothrin	g a.i./ha	ng a.i./cm^2
100	5	50
75	3.75	37.5
50	2.5	25
20	1	10
4	0.2	2
0.4	0.02	0.2
0	0	0

Following the approach of Foster *et al.* (2014), 15 adult wingless (apterous) aphids were placed into each glass vial. This was repeated with three replicate vials for each combination of λ-cyhalothrin concentration in acetone × aphid lineage (Table 1). Following the 5-h assay end point, aphids were individually inspected and recorded as being mobile, that is, capable of coordinated movement within 1 min of observation, affected, that is, alive, but not capable of coordinated movement within 1 min of observation, or dead, not moving. The data were corrected for observed control mortality using the Abbott's correction formula (Abbott, 1925) ahead of quantifying dose–response curves using Probit analysis in SAS 9.4 (SAS, 2014) to determine the median lethal concentration that affects/kills 50% of the exposed population (LC_{50}).

A Wald Chi-squared (χ^2) test was used to test the association between the independent variables (predictors) and the criterion variable (inhibition), where *P*-values were generated to establish the goodness of fit of the model to the data, and finally, resistance factors were calculated by dividing the LC_{50} of each clonal lineage by the LC_{50} for the susceptible lineage.

VGSC mutation sequencing of R. padi

A method adapted from Foster *et al.* (2014) was used to identify possible mutations in the *R. padi* VGSC. Total RNA was extracted from adult aphids and converted into cDNA using SuperScript III reverse transcriptase (Invitrogen). A PCR fragment of 470 bp was then amplified from the domain IIS4–IIS6 region of the VGSC using *R. padi* primers Rp1 (CTGCTCTCCGCGGGTTACCAA) and Rp3 (CCGTAGGCACCGATAAATTAGA). PCR fragments for each of the Irish *R. padi* colonies were Sanger sequenced and aligned to search for any mutations.

Results

Colonies of *R. padi* collected from major tillage growing counties in Ireland (Table 2) displayed LC_{50} values (Table 3) ranging from 7.5 to 18.2 ng a.i./cm^2 with 95% confidence intervals overlapping within the range of 3.9–20.8 ng a.i./cm^2, which is within the prescribed field rate (5.0 g a.i./ha). The *R. padi* colony displaying the lowest LC_{50} value of 7.5 ng a.i./cm^2 was Carlow 1 with 95% confidence intervals of 4.0–11.6 ng a.i./cm^2. The colony with the highest LC_{50} value of 18.2 ng a.i./cm^2 was Carlow 2 with 95% confidence intervals of 11.32–20.8 ng a.i./cm^2. Carlow 2 indicated the highest decrease in susceptibility or had less mortality when compared to other *R. padi* colonies, but remained within the full field rate of 5.0 g a.i/ha or 50.0 ng a.i./cm^2, and the confidence intervals overlapped with all other populations apart from the

Table 2: Irish aphid colony details

Colony name	Colony code	County of collection	Collection year	Crop source
R. padi pre-existing colony	Tipp 1	Tipperary	2018	Spring barley
(Walsh *et al.*, 2020)	Tipp 2			
S. avenae pre-existing colony	Sa3	Carlow	2017	Spring barley
(Walsh *et al.*, 2019)				
R. padi Carlow 1	Cw1	Carlow	2020	Maize
R. padi Carlow 2	Cw2	Carlow	2020	Maize
R. padi Laois	LS	Laois	2020	Maize
R. padi Kilkenny	Kk	Kilkenny	2020	Maize
R. padi Cork 1	Ck1	Cork	2020	Spring barley
R. padi Cork 2	Ck2	Cork	2020	Spring barley

Table 3: LC_{50} responses to λ-cyhalothrin (ng/cm^2 after 5 h in coated glass vials) of Irish samples of *Rhopalosiphum padi* versus standard aphid references (shown in red font) from a study in the UK by Foster and Leybourne (2021)

Standard baseline clone/Irish sample	Region of collection	County of collection	Aphid colony	*N*[1]	LC_{50}[2]	95% CI[3]	Response ratio[4]	Ratio calculated using Foster baselines
UK susceptible controls (Foster & Leybourne, 2021)								
R. padi[5]	UK	–	Rp (S) (*R. padi*)	251	6.33	2.449–15.98	1	*
S. avenae[6]	UK	–	Sa-kdr-SS (*S. avenae*)	230	1.14	0.660–1.869	1	**
Irish tolerant/resistant controls (Walsh *et al.*, 2020)								
Irish resistant (Sa3) (Walsh *et al.*, 2019)	Ireland	Carlow	Sa3 (Carlow) (*S. avenae*)	945	36.94	31.52–45.25	32.4	**
Irish potential tolerant *R. padi* clone (Walsh *et al.*, 2020)	Ireland	Tipperary	Rp (Tipp) (*R. padi*)	945	8.18	3.39–18.17	1.3	*
Irish county *R. padi* samples collected (2020)								
	Ireland	Carlow	Cw1	315	7.5	4.0–11.60	1.2	*
		Carlow	Cw2	840	18.2	11.32–20.80	2.9	*
		Laois	LS	315	10.1	4.12–10.69	1.6	*
		Kilkenny	Kk	315	10.0	3.90–10.61	1.6	*
		Cork	Ck1	315	12.92	8.04–17.84	2.0	*
		Cork	Ck2	315	12.04	7.52–16.37	1.9	*

[1]Number of aphids tested.
[2]LC_{50} in ng/cm^2.
[3]95% CI = 95% confidence interval.
[4]Within-species response ratio calculated from LC_{50} sample/LC_{50} for standard baseline Sa-kdr-SS or Rp (S) clones.
[5]Response ratios of 6.33 ng/cm^2 for the standard baseline of the susceptible clone of *R. padi* (Rp SS) from Foster & Leybourne (2021) used to determine response ratio of Irish *R. padi* colonies.
[6]Response ratio of 1.14 ng/cm^2 for standard baselines for the susceptible clone of *S. avenae* (Sa-kdr-SS) from Foster & Leybourne (2021) used to determine response ratio of Irish *S. avenae* colony.
*Susceptible *R. padi* (Rp SS) response ratio of 6.33 ng/cm^2 from Foster & Leybourne (2021) used to calculate the response ratio of Irish colonies.
**Susceptible *S. avenae* (Sa-kdr SS) response ratio of 1.14 ng/cm^2 from Foster & Leybourne (2021) used to calculate the response ratio of Irish colonies.

Kilkenny population. The estimated LC_{50} values and confidence intervals for all the tested colonies are listed in Table 3.

The previously identified pyrethroid-tolerant *R. padi* colony (Walsh *et al.*, 2020) was retested again in 2020 with seven concentrations of λ-cyhalothrin, including a field rate equivalent of 50 ng a.i./cm^2. The LC_{50} value was found to be 8.18 ng a.i./cm^2, with a range in confidence intervals from 3.39 to 18.17 ng a.i./cm^2 (Table 3) for a period of 5 h of exposure. This value overlaps with other tested populations, and with a high level of affected/mortality of aphids, at relatively low concentrations, it is evident that this population is actually susceptible to pyrethroids. Similarly, an *S. avenae* (Sa3) colony which was confirmed to have kdr resistance (L1014F mutation detected) was also tested as a positive control. The LC_{50} value for the *S. avenae* (Table 3) was found to be 36.94 ng a.i./cm^2 (60.3% field rate) when exposed to the same concentrations (Table 1). The Sa3 colony in this study displayed 95% confidence intervals ranging from 31.52 to 45.25 ng a.i./cm^2.

The VGSC region of 470 bp for each *R. padi* colony was sequenced to search for any mutations such as single nucleotide polymorphisms (SNPs) present on single aphids (https://doi.org/10.6084/m9.figshare.20368158.v1) and multiple aphids (https://doi.org/10.6084/m9.figshare.20368197.v1) from each colony collected. The alignment of these 19 *R. padi* sequences did not reveal any amino acid substitutions (https://doi.org/10.6084/m9.figshare.20101373.v1). The IIS4–IIS6 region of the VGSC was selected as it is known to have at least five mutations, which are M918, L929, T929, F932 and L1014 (Foster *et al.*, 2014). A total of 19 *R. padi* samples (single and multiple aphid samples) (Table 4) were Sanger sequenced with LGC Germany (Laboratory of the Government Chemist) with forward (Rp1) and reverse (Rp3) primers. The original individual of the sub-colonies Tipp 1 and Tipp 2 was collected in Co. Tipperary in 2018 by Walsh *et al.* (2019) where a dose–response assay indicated potential tolerance to pyrethroid.

Table 4: Sequenced *R. padi* samples (19 samples of single and multiple aphids)

R. padi colony	Single samples	Multiple samples
Carlow 1 (Cw1)	1	25
Carlow 2 (Cw2)	2[1]	25
Laois (Ls)	1	25
Kilkenny (Kk)	1	25
Cork 1 (Ck1)	1	25
Cork 2 (Ck2)	1	25
Tipp 1	1	25
Tipp 2	1	25
R. padi France PAV	1	–
Suction Tower OP 22.10.2020	1	–

[1]Cw2 singles sequenced twice as it was colony with the highest LC_{50}.

Discussion

This study investigates the response of field-collected Irish colonies of *R. padi* to the pyrethroid, λ-cyhalothrin, to measure if reductions in sensitivity were present in the field population. The laboratory colonies of the *S. avenae* (Sa3) and *R. padi* (Tipp 1 and Tipp 2) colonies, which were subjected to λ-cyhalothrin previously (2017–2018), had LC_{50} values of 60.2 ng a.i./cm^2 for *S. avenae* and 37.0 ng a.i./cm^2 for *R. padi* (Walsh *et al.*, 2020). When these colonies were tested again in this current study in 2020 using the same methodology, the LC_{50} values were reduced to 36.94 ng a.i./cm^2 for *S. avenae* and 8.18 ng a.i./cm^2 for *R. padi*. (Table 3). The results suggest that tolerance was reduced or lost in the lab-based rearing time period since they were last tested. Loss of tolerance to insecticides has been observed before in a study which looked at this phenomenon in German cockroaches, *Blatta germanica*. A decline in the use of insecticides and prolonged periods of no insecticide pressure resulted in tolerance levels declining for several pyrethroids (Cochran, 1993). A reduction in tolerance has also been observed in a study on *Anopheles gambiae*, where the rate of resistance decay for an insect displaying a high intensity of resistance, including enhanced metabolic processes to becoming fully susceptible took approximately 15 generations. This supports the theory that at least a 2-yr interval is needed for the rotational use of insecticides with different modes of action (Machani *et al.*, 2020). There is no current literature to suggest this observation within aphids, but this may explain the reduced LC_{50} expressed within these colonies, due to the length of time in laboratory colonies without exposure to insecticides, or may be due to a general deterioration in fitness under lab-based rearing conditions or alteration between sexual and asexual phases of reproduction, if available in that species.

The dose responses of the geographically separate Irish *R. padi* all proved to be pyrethroid-susceptible. Therefore, the results in this study were within the ranges of a study which surveyed cereal aphid resistance status of *S. avenae* and *R. padi* samples collected from cereal crop regions in the UK from November 2019 to February 2021 (Foster & Leybourne, 2021), where it was concluded that there was no evidence showing the evolution or selection of pyrethroid resistance within *R. padi* beyond what was already known within this species. The UK *R. padi* clones tested showed responses similar to pyrethroid-susceptible aphids of this species in Ireland, and found no evidence of any shift in reduced sensitivity seen in recent years (Foster & Leybourne, 2021).

The sequencing of the VGSC showed no amino acid substitutions, which indicated no known insecticide resistance mutations at present. These results were expected as the Irish *R. padi* individuals showed no evidence of increased tolerance or resistance to pyrethroid insecticides based on the dose–response assay results. The genetic sequencing of *R. padi* populations in this study generates the first dataset of this kind for Irish *R. padi* populations, which may be useful to study the evolutionary responses to pesticides over time.

Continued testing and bioassay monitoring is required to ensure the continued full efficacy of pyrethroids against this *R. padi*. This regular screening of aphid populations for any reduced sensitivity will help safeguard the future efficacy of these compounds for controlling cereal aphid populations, and if selection is seen, identifying the regions where sensitivity or resistance may be present (Foster & Leybourne, 2021).

Acknowledgements

This research was carried out within the Crops Environment Land Use Programme (CELUP) based at Teagasc Crop Research Centre, Carlow, Ireland. This research was funded under the South East Technological University, President's Fellowship scheme under Envirocore and Teagasc Internal Funding. Rothamsted Research receives grant support from BBSRC.

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