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First Report on Assessing the Severity of Herbicide Resistance to ACCase Inhibitors Pinoxaden, Propaquizafop and Cycloxydim in Six *Avena fatua* Populations in Ireland

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Abstract: In response to growers reports of poor weed control, resistance to ACCase inhibitors pinoxaden, propaquizafop and cycloxydim was investigated in populations of six wild oats, *Avena fatua*, collected from cereal-dominated crop rotations in Ireland. Glasshouse assays confirmed reduced sensitivity to all three ACCase inhibitors in four of the six populations, R2 to R5. R1 was cross-resistant to pinoxaden and propaquizafop and R6 was resistant to propaquizafop only. Dose-response studies confirmed significant differences in the severity of resistance amongst these populations ($p < 0.05$). For pinoxaden, the ED_{50} or GR_{50} resistance factor (RF) of R1, R3 and R5 were between 11.6 and 13.1 times or 25.1 and 30.2 times more resistant, respectively, compared with the susceptible populations. For propaquizafop, the ED_{50} and GR_{50} RF of R1, R2, R3, R5 and R6 were between >7.8 and >32 or 16.6 and 59 times more resistant, respectively. For cycloxydim, only R5 had both high ED_{50} and GR_{50} RF values of >43.2 and 98.4 respectively. In R2, although the ED_{50} values to both pinoxaden and cycloxydim and additionally, R3 to cycloxydim, were above recommended field rates, their GR_{50} values remained below, suggesting a shift towards cross-resistance. While R4 was the only population, where both ED_{50} and GR_{50} for all ACCase inhibitors remained below recommended field rates, they would not give effective control at these rates, strongly indicating evolving resistance. This is the first study reporting variable cross-resistance types and levels to ACCase inhibitors in *A. fatua* from Ireland.

Keywords: ACCase inhibitors; herbicide resistance; ED_{50}; GR_{50}; cross-resistance; *Avena fatua*

1. Introduction

An Atlantic-influenced Irish climate results in high yield potential for cereal crops but also facilitates rapid development of weeds and fungal diseases [1,2]. Until recently, pesticide efficacy and evolving resistance research in Ireland has been focused primarily on fungal pathogens and to some extent, insect pests and transmitted diseases, with less emphasis on weeds. One weed occurring frequently, in Irish arable fields is wild oats, *Avena fatua* L., which is a largely self-pollinated, predominantly spring-germinating, allohexaploid (Poaceae; $2n = 6x = 42$) grass. *A. fatua* substantially reduce crop yields due to its competitiveness [3], while it also proliferates across all crop establishment

systems, due to seed dormancy and high seed survival in soil, coupled with sporadic seed germination and high seed bank populations [4–6].

Two post-emergent herbicide modes of action, highly active on *A. fatua*, are either acetyl-Coenzyme A carboxylase (ACCase) inhibitors or acetolactate synthase (ALS) inhibitors [7,8]. The most commonly used ACCase inhibitors are divided into three chemically different classes namely, phenylpyrazoline (DEN), aryloxyphenoxypropionates (FOPs) and cyclohexanediones (DIMs) [8,9]. Currently, one DEN (pinoxaden), five FOPs (fenoxaprop-p-ethyl, clodinafop-propargyl, propaquizafop, fluazifop-p-butyl and quizalofop-p-ethyl) and one DIM (cycloxydim) are commercially available in Ireland for controlling *A. fatua* [10]. Of these, pinoxaden and fenoxaprop-p-ethyl give selective control in wheat and barley, and clodinafop-propargyl only in wheat. The other FOPs and DIM are used in break crops including oilseed rape and beans. The ACCase-inhibiting herbicides share similar modes of action by inhibiting the sensitive homomeric plastidic ACCase enzyme activity in monocot species, consequently causing plant death [7]. Although key cereal crops such as wheat and barley contain sensitive homomeric plastidic ACCase, they exhibit tolerance to DEN and certain FOPs, because of their ability to quickly metabolise the herbicides [7]. Additionally, tolerance in cereal crops is enhanced through the inclusion of safeners into the herbicide formulations [7,8]. While the heteromeric plastidic ACCase in dicot species explains the lack of action or target-site insensitivity to ACCase inhibitors [7].

More than 132,000 ha of cereals, oilseed rape and beans crops were treated with ACCase-inhibiting herbicides in 2016, with pinoxaden serving as a key active in the control of *A. fatua*, being applied to >110,000 ha, followed by propaquizafop or fenoxaprop-p-ethyl (between 6200 to 8300 ha) and cycloxydim (2027 ha) [11]. While ALS-inhibiting herbicides that will control *A. fatua* only in winter wheat are less widely used, with mesosulfuron + iodosulfuron applied to 13,664 ha and pyroxsulam + florasulam applied to 3367 ha. Unfortunately, both ACCase/ALS herbicide groups are highly vulnerable to selection for herbicide resistance [12,13], and hundreds of ACCase/ALS resistant *A. fatua* cases have been reported in at least 16 countries [14].

In Ireland, the first incidence of herbicide resistance in *A. fatua* to ACCase inhibitors was reported by Byrne et al. [1] from different populations collected across south-east arable counties in 2017. This initial finding suggests that herbicide resistance in *A. fatua* is widespread in some if not all populations across the country. Unlike the UK, where ACCase resistant field populations of *Alopecurus myosuroides* (black-grass), *Lolium multiflorum* (Italian ryegrass) and *Avena* spp. are well studied [15], research in Ireland has focused on identifying the incidence of emerging weed control issues with grass weeds [1]. Consequently, there has been no study to date which quantifies the levels of resistance in *A. fatua* to important herbicides, which would allow the design of effective weed resistance management strategies, which is suitable for Ireland's mild Atlantic climate and cropping systems, including significant proportions of spring cereals. Therefore, this study aims to characterize the severity of herbicide resistance and cross-resistance in six *A. fatua* populations in Ireland, where growers had reported poor control or difficult to control with commonly used ACCase-inhibiting herbicides pinoxaden, propaquizafop or cycloxydim at recommended field rates.

2. Materials and Methods

2.1. Seed Materials and Growing Conditions

Following reports, from growers participating in the Enable Conservation Tillage (ECT) technology transfer programme, of weed control difficulties where ACCase-inhibiting herbicides were used, seeds from six field populations were collected from the main arable regions in the south-east in June 2019 for resistance testing (Table 1). While most were from one county (Co.), the problem fields within Co. Wexford were located *c.* 7 to 23 km apart. Additionally, *A. fatua* seeds were collected from populations that had not previously received herbicides from Laois and Kildare counties *c.* 30 km apart to use as a susceptible control. The populations were different from those tested by Byrne et al. [1]. Samples were stored in sealed paper bag and kept in a cold room at 8 °C until used.

Table 1. Field history of the six *A. fatua* test populations collected from cereal-dominated crop rotations prior to 2019-harvest for resistance testing.

Population	Field Position	County	Year	Crop	Herbicides Applied
R1	52°49′ N–6°67′ W	Wexford	2019	Spring barley	Pinoxaden
			2018	Spring barley	Pinoxaden
			2017	Spring barley	Pinoxaden
			2016	Spring wheat	Pinoxaden
			2015	Spring oilseed rape	Propaquizafop
R2	52°51′ N–6°62′ W	Wexford	2019	Winter wheat	Pyroxsulam + florasulam
			2018	Winter oilseed rape	Propaquizafop
			2017	Winter wheat	Pyroxsulam + florasulam
			2016	Spring barley	Pinoxaden
			2015	Spring barley	Pinoxaden
R3	52°50′ N–6°40′ W	Wexford	2019	Spring barley	Pinoxaden
			2018	Spring barley	Pinoxaden
			2017	Spring barley	Pinoxaden
			2016	Spring barley	Pinoxaden
			2015	Spring barley	Pinoxaden
R4	52°53′ N–6°41′ W	Wexford	2019	Winter barley	Pinoxaden
			2018	Winter barley	Pinoxaden
			2017	Winter barley	Pinoxaden
			2016	Spring barley	Pinoxaden
			2015	Spring barley	Pinoxaden
R5	52°63′ N–7°24′ W	Kilkenny	2019	Winter barley	No herbicides used to control *A. fatua*
			2018	Winter barley	Pinoxaden
			2017	Winter oilseed rape	Propaquizafop
			2016	Winter barley	Pinoxaden
			2015	Winter barley	Pinoxaden
R6	51°81′ N–8°11′ W	Cork	2019	Spring barley	Pinoxaden
			2018	Faba beans	Propaquizafop
			2017	Spring wheat	Pinoxaden
			2016	Spring oats	Pinoxaden
			2015	Spring barley	Pinoxaden

During winter 2019/20, seeds were pricked in the endosperm using a hypodermic needle and subsequently, placed in a Petri-dish with dense filter paper (VWR® Grade 413), dark-imbibed overnight in a combination of gibberellic acid (GA_3) and potassium nitrate (KNO_3) solution (prepared by dissolving 700 mg of GA_3 + 4 g of KNO_3 in 0.5 L deionised H_20 along with 5 mL of 99.8% aqueous ethanol) to assist seed germination. Seeds were then planted in 96-cell quick pot® propagation trays containing Kettering loam and lime-free grit mix in a 4:1 ratio, with the addition of Osmacote Mini™ (1 kg t^{-1}). Four populations were planted per tray at 24 seeds per population for each replicate. Seedlings were watered as needed to avoid moisture stress. The plants were grown in a glasshouse with 18/12 °C (day/night) temperature regime at a photoperiod of 16 h supplemented with artificial lighting to maintain a minimum light intensity of 250 μ mol quanta m^{-2} s^{-1} at the Teagasc Crops Research Centre, Oak Park, Carlow.

2.2. Single-Rate Herbicide Resistance Testing

Recommended field rates of commercial formulations of ACCase inhibitors pinoxaden (Axial® EC, 100 g a.i. L^{-1} includes cloquintocet-mexyl, Syngenta) at 30 g ha^{-1} plus 1% *v/v* aqueous adjuvant (Adigor® EC, 47% *w/w* methylated rapeseed oil, Syngenta); propaquizafop (Falcon® EC, 100 g a.i. L^{-1}, Adama) at 100 g ha^{-1} and cycloxydim (Stratos Ultra®, EC, 100 g a.i. L^{-1}, BASF) at 150 g ha^{-1}, were applied. Herbicides were applied to the plants at growth stages (GS) 13–14 (BBCH) using a Generation III Research Track Sprayer (DeVries Manufacturing, Hollandale, MN, USA) with a

teejet 8002-EVS flat fan nozzle, at a pressure of 2.5 bar and a water volume equivalent of 200 L ha^{-1}. The experiment was a randomized design with two replicates per herbicide treatment and was repeated twice. In addition to untreated controls, two susceptible populations (S1 and S2) were used as susceptible standards for each treatment.

Visual assessment for survival was conducted 28 days after spraying. Plants which displayed strong growth after treatment were recorded as resistant, and plants with severe symptoms of leaf chlorosis, desiccation or no new active growth and ultimately total plant death as susceptible [16]. Above-ground shoots were harvested, dried at 70 °C for 72 h and weighed. Plant survival was expressed as the percentage of surviving seedlings to the total number of treated seedlings. The above-ground shoot dry weight for each replicate was expressed as a percentage of the mean dry weight of the untreated control replicates of the same population.

2.3. Dose-Response to ACCase-Inhibiting Herbicides

Seeds from the six test populations were tested further for their response in comparison to two susceptible populations, to each of the three ACCase inhibitors pinoxaden, propaquizafop and cycloxydim. Plants (3–4 leaf stage) were sprayed with pinoxaden at 0, 7.5, 15, 30, 45, 60, 120 and 240 g ha^{-1} with each dose mixed with 1% *v/v* adjuvant solutions, with propaquizafop at 0, 25, 50, 100, 150, 200, 400 and 800 g ha^{-1} and with cycloxydim at 0, 37.5, 75, 150, 225, 300, 600 and 1200 g ha^{-1}. The selected dose rates represent 0.25× to 8× recommended field rates. Each dose-response experiment was randomized with two replicates per dose. Plant survival and above-ground shoot dry weights were harvested and measured 28 days after spraying using the methodology above.

2.4. Statistical Analysis

Data analyses were performed using R, version 3.6.3 [17]. For ACCase single-rate resistance testing, ANOVA revealed no significant treatment x experiment interactions (two replicates per experiment) for percent survival (F-value = 0.15; $p = 0.87$) or percent shoot dry weight (F-value = 0.03; $p = 0.97$). Therefore, the data were averaged over the four replicates.

For dose-response to ACCase inhibitors, survival data were regressed over herbicide doses with binomial endpoints and shoot dry weight data by four-parameter models using the *drc* package in R [18]. Lack-of-fit F-tests were performed to assess model fit ($p > 0.05$).

A two-parameter binomial log-logistic model with a constrained slope was selected to model survival data of pinoxaden (ANOVA comparing the models, likelihood ratio [LR] value = 0.22; $p = 0.99$) and cycloxydim (LR value = 2.83; $p = 0.90$). While propaquizafop survival data was fitted by a two-parameter binomial Weibull-1 model with a constrained slope (LR value = 3.30; $p = 0.65$).

A four-parameter Weibull-1 model with a common upper limit was selected to fit shoot dry weight data of pinoxaden (ANOVA comparing the models, F-value = 0.19; $p = 0.99$). For cycloxydim shoot dry weight data, a log-logistic four-parameter model was initially used. The fitted model, however, compromised the data of the population R5. Therefore, R5 was fitted separately by a three-parameter Weibull-2 model (model fit, $p = 0.05$) and the remaining populations by a log-logistic four-parameter model with a common upper limit (F-value = 0.15; $p = 0.98$). For propaquizafop shoot dry weight data, saturated models ($p > 0.05$) did not represent the data with sufficient parsimony to enable comparison between populations. Therefore, despite poor model fitting (model fit, $p < 0.001$), a three-parameter Weibull-2 model with a common upper limit (F-value = 0.46; $p = 0.86$) is presented. As the residuals were not normally distributed, the models and residuals were transformed using Box–Cox procedure [18].

Fitted models estimated the survival ED_{50} (i.e., effective dose causing 50% mortality of the treated plants) or the growth rate GR_{50} (i.e., the effective dose required to obtain a growth reduction of 50% relative to untreated plants). The resistance factor (RF) was then calculated as a ratio of ED_{50} or GR_{50} of a test population to the ED_{50} or GR_{50} of a susceptible standard [16]. For propaquizafop survival data, most resistant populations recorded high survival at the highest dose used, while the control of

two susceptible populations S1 and S2 at the lowest dose was virtually complete, and consequently, a model could not be fitted with the susceptible populations. Therefore, the ED_{50} values of S1 and S2 are reported as less than the lowest dose used (i.e., <0.25× recommended field rate). For some resistant populations, ED_{50} or GR_{50} were larger than the highest dose used (i.e., >8× recommended field rate) and could not be estimated.

3. Results

3.1. Single-Rate Herbicide Resistance Testing

The two susceptible populations S1 and S2 were totally controlled by all three ACCase-inhibiting herbicides at recommended field rates (Figure 1). Compared with the S1 and S2, the populations R2, R3, R4 and R5 were poorly controlled by pinoxaden, propaquizafop and cycloxydim. A significant number of plants of R1 survived pinoxaden and propaquizafop treatments, however, cycloxydim was effective, with only a few survivors. Around 80% of plants of R6 survived propaquizafop, while both pinoxaden and cycloxydim were found to be highly effective. The results from shoot dry weight data were similar to that of plant survival (Figure 1).

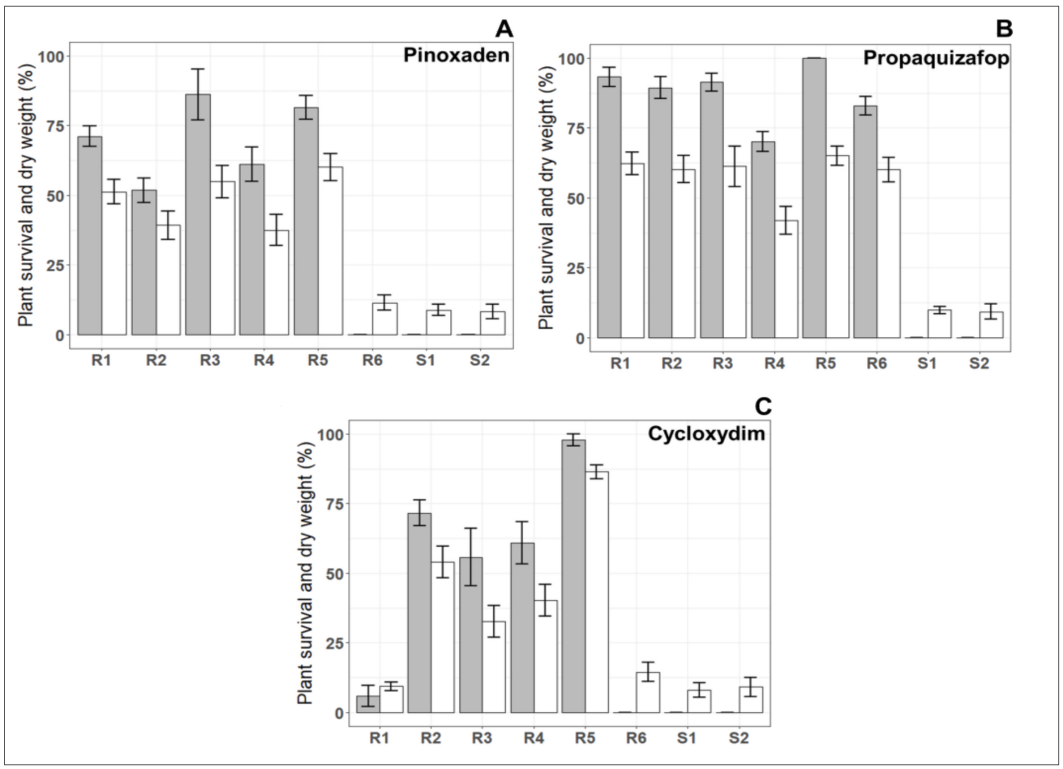

Figure 1. Survival (grey bars) and shoot dry weight (white bars), expressed as a percentage of untreated controls of susceptible (S1 and S2) and test (R1 to R6) populations of *A. fatua* to ACCase inhibitors pinoxaden (**A**), propaquizafop (**B**) and cycloxydim (**C**) applied at recommended field rate, corresponding to 30, 100 and 150 g ha^{-1}, respectively. Vertical bars indicate standard errors.

3.2. Dose-Response to ACCase-Inhibiting Herbicides

Dose-response curves for survival data and shoot dry weight data across the three ACCase-inhibiting herbicides are presented in Figures 2–4. The *t*-statistics determining significant differences between estimated ED_{50} or GR_{50} values, to identify significant differences in parameter estimates between populations, are given in Supplementary Tables S1 and S2. Symptomology of some test populations at specific dose rates across the three ACCase inhibitors are presented in Supplementary Figures S1–S3.

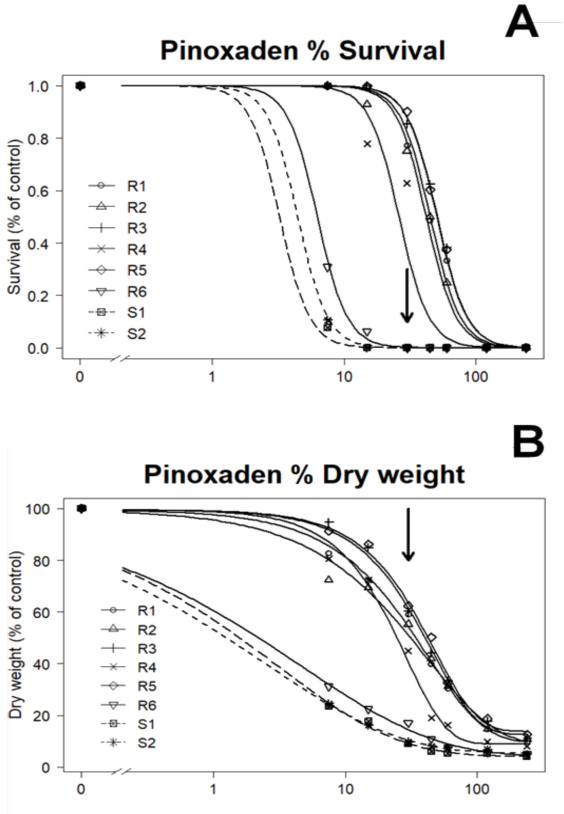

Figure 2. Dose-response curves for survival (**A**) and shoot dry weight (**B**) of susceptible (S) and test populations (R1 to R6) of *A. fatua* treated with a range of dose rates covering from 0.25× to 8× recommended field rate of pinoxaden. Arrows indicate the recommended field rate of 30 g pinoxaden ha^{-1} for *A. fatua* control.

There were significant differences in response amongst the *A. fatua* populations when treated with pinoxaden (ANOVA comparing the models, LR value = 56.37; $p < 0.05$ for ED_{50} and *F*-value = 9.02; $p < 0.001$ for GR_{50}), propaquizafop (LR value = 15.01; $p = 0.01$ for ED_{50} and *F*-value = 73.08; $p < 0.001$ for GR_{50}) and cycloxydim (LR value = 57.51; $p < 0.05$ for ED_{50} and *F*-value = 29.61; $p < 0.001$ for GR_{50}). Shoot dry weight dose-response models confirmed that there were significant differences in response between populations for slope to pinoxaden (*F*-value = 9.21; $p < 0.001$), propaquizafop (*F*-value = 9.20; $p < 0.001$) and cycloxydim (*F*-value = 6.99; $p < 0.001$).

The two susceptible populations S1 and S2 were totally controlled by all three ACCase inhibitors at half their recommended field rates and corresponding shoot dry weight reduction was >80% (Figures 2–4). Although herbicide efficacy in controlled conditions may be higher than the field applications [19], the S1 and S2 proved to be highly sensitive. Consequently, S1 and S2 in the fitted models resulted in very low ED_{50} or GR_{50} values. The *t*-statistics showed that both S1 and S2 were significantly different for ED_{50} or GR_{50}, compared with the test populations ($p < 0.05$) across ACCase inhibitors. However, differences between S1 and S2 were not significant for ED_{50} or GR_{50} ($p > 0.05$), indicating that their response were similar. Therefore mean values of S1 and S2 were reported (Tables 2 and 3).

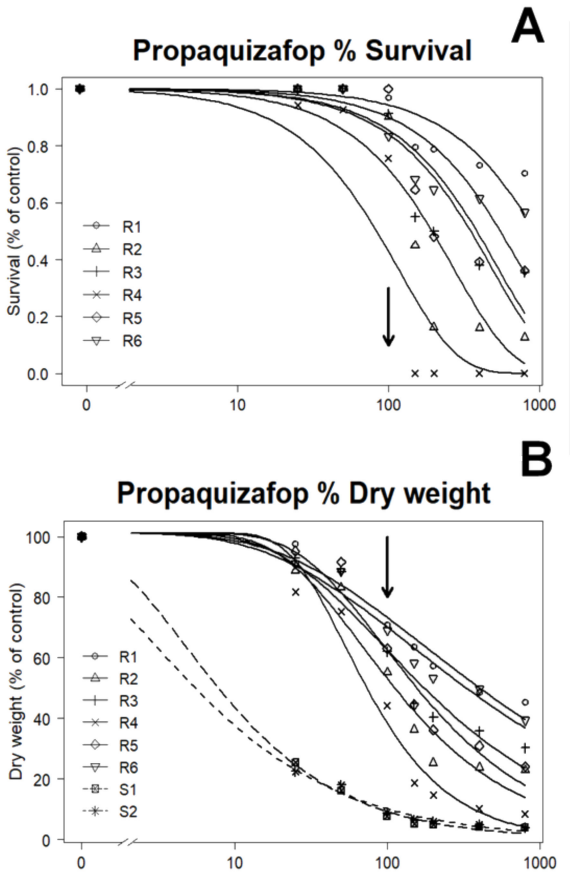

Figure 3. Dose-response curves for survival (**A**) and shoot dry weight (**B**) of susceptible (S) and test populations (R1 to R6) of *A. fatua* treated with a range of dose rates covering from 0.25× to 8× recommended field rate of propaquizafop. Arrows indicate the recommended field rate of 100 g propaquizafop ha^{-1} for *A. fatua* control. Note: most resistant populations recorded high survival at the highest dose used, while the control of two susceptible populations at the lowest dose was virtually complete, therefore, a model (**A**) could not be fitted with the S1 and S2.

Table 2. Survival ED_{50} (standard errors in parentheses) of susceptible (S) (mean values of S1 and S2) and test populations (R1 to R6) of *A. fatua* treated with a range of ±recommended field rate of pinoxaden 30 g ha^{-1}; ±recommended field rate of propaquizafop 100 g ha^{-1} and ±recommended field rate of cycloxydim 150 g ha^{-1}. Resistance factor (RF) was calculated as the ratio of ED_{50} values of test and susceptible populations.

	Pinoxaden (g ha^{-1})		**Propaquizafop (g ha^{-1})**		**Cycloxydim (g ha^{-1})**	
	ED_{50}	**RF of ED_{50}**	**ED_{50}**	**RF of ED_{50}**	**ED_{50}**	**RF of ED_{50}**
S	3.9 (2.69)	-	<25.0	-	27.8 (9.62)	-
R1	45.2 (10.56)	11.6	>800	>32.0	55.9 (14.41)	2.0
R2	42.1 (9.90)	10.8	194.8 (79.64)	>7.8	164.6 (29.92)	5.9
R3	50.6 (11.92)	13.0	350.4 (153.73)	>14.0	155.2 (28.77)	5.6
R4	26.1 (7.04)	6.7	83.4 (35.44)	>3.3	147.2 (28.14)	5.3
R5	51.0 (12.03)	13.1	385.1 (175.39)	>15.4	>1200	>43.2
R6	6.3 (2.33)	1.6	551.6 (281.24)	>22.1	27.6 (9.72)	1.0

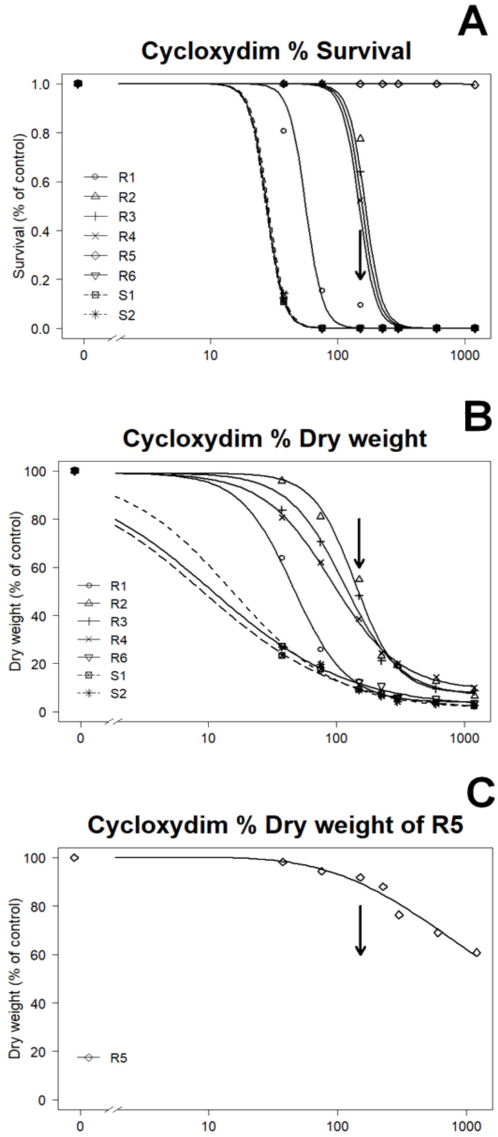

Figure 4. Dose-response curves for survival (**A**) and shoot dry weight (**B**,**C**) of susceptible (S) and test populations (R1 to R6) of *A. fatua* treated with a range of dose rates covering from 0.25× to 8× recommended field rate of cycloxydim. Arrows indicate the recommended field rate of 150 g cycloxydim ha^{-1} for *A. fatua* control.

Table 3. Shoot dry weight GR_{50} (standard errors in parentheses) of susceptible (S) (mean values of S1 and S2) and test populations (R1 to R6) of *A. fatua* treated with a range of ±recommended field rate of pinoxaden 30 g ha^{-1}; ±recommended field rate of propaquizafop 100 g ha^{-1} and ±recommended field rate of cycloxydim 150 g ha^{-1}. Resistance factor (RF) was calculated as the ratio of GR_{50} values of test and susceptible populations.

	Pinoxaden (g ha^{-1})		**Propaquizafop (g ha^{-1})**		**Cycloxydim (g ha^{-1})**	
	GR_{50}	**RF of GR_{50}**	**GR_{50}**	**RF of GR_{50}**	**GR_{50}**	**RF of GR_{50}**
S	1.2 (0.70)	-	6.6 (2.63)	-	12.2 (3.17)	-
R1	30.1 (1.96)	25.1	389.5 (60.29)	59.0	46.0 (2.49)	3.8
R2	28.1 (2.90)	23.4	109.8 (8.82)	16.6	140.1 (6.52)	11.5
R3	34.5 (1.65)	28.8	170.3 (16.34)	25.8	115.2 (6.57)	9.4
R4	22.5 (1.10)	18.8	72.6 (5.26)	11.0	91.7 (6.17)	7.5
R5	36.2 (1.77)	30.2	153.2 (12.34)	23.2	>1200	>98.4
R6	1.8 (0.64)	1.5	318.5 (45.38)	48.3	10.6 (3.45)	0.9

The response of the test population R4, in particular, was different, compared with the remaining populations across ACCase inhibitors, therefore, R4 results will be described later. For pinoxaden, complete control of the populations (R1, R2, R3 and R5) was recorded only from 4× recommended field rate of 30 g pinoxaden ha^{-1} (Figure 2). Estimated ED_{50} values ranged from 42.1 to 51.0 g pinoxaden ha^{-1} and the estimated GR_{50} values ranged from 28.1 to 36.2 g ha^{-1} (Tables 2 and 3). ED_{50} of the four populations were between 1.4 and 1.7 times the recommended field rate. While their GR_{50} was between 1.0 and 1.2 times the recommended field rate, except for R2 which remained slightly below recommended field rate (0.9 times). In other words, the ED_{50} resistance factor (RF) of R1, R2, R3 and R5 was between 10.8 and 13.1 times and the GR_{50} RF was between 23.4 and 30.2 times more resistance, compared with the susceptible populations (Tables 2 and 3).

For propaquizafop, the survival of R1 did not fall below 60 % at the highest dose of 800 g ha^{-1} (8× recommended field rate) used and corresponding shoot dry weight reduction was about 60% (Figure 3). As a result, their estimated ED_{50} exceeded 8 times and the estimated GR_{50} was about 3.9 times the recommended field rate of 100 g propaquizafop ha^{-1} (Tables 2 and 3). In the other populations (R2, R3, R5 and R6), the ED_{50} ranged from 194.8 to 551.6 g ha^{-1}, which was between 2.0 and 5.5 times the recommended field rate, and the GR_{50} ranged from 109.8 to 318.5 g ha^{-1}, which was between 1.1 and 3.2 times the recommended field rate. The ED_{50} RF of these five populations was between >7.8 and >32 times and the GR_{50} RF was between 16.6 and 59 times more resistance than the susceptible populations, respectively (Tables 2 and 3).

For cycloxydim, the survival of R5 was 100% at the highest dose of 1200 g ha^{-1} (8× recommended field rate) used and corresponding shoot dry weight reduction was below 40% (Figure 4). As a result, both their ED_{50} and GR_{50} exceeded by more than 8 times the recommended field rate of 150 g cycloxydim ha^{-1} (Tables 2 and 3). In the populations R2 and R3, the ED_{50} was slightly above the recommended field rate (1.0 or 1.1 times). However, their GR_{50} remained below the recommended field rate (0.8 or 0.9 times), but strongly suggesting reduced sensitivity. The cycloxydim ED_{50} RF of R2, R3 and R5 was 5.6, 5.9 and >43.2 times and the cycloxydim GR_{50} RF was 9.4, 11.5 and >98.4 times more resistance than the susceptible populations, respectively (Tables 2 and 3).

In the population R4, despite ED_{50} or GR_{50} RF for pinoxaden was 6.7 or 18.8 times; propaquizafop RF was >3.3 or 11 times and cycloxydim RF was 5.3 or 7.5 times more resistance, both the ED_{50} and GR_{50} values to each of the three ACCase inhibitors remained well below the recommended field rates (Tables 2 and 3). This strongly suggests that recommended field rates will no longer be effective, and R4 is in the process of evolving greater resistance across ACCase inhibitors. Conversely, although the population R1 cannot be considered as cycloxydim-resistant, its sensitivity to cycloxydim was statistically different to R6 (t-statistics, $p < 0.05$) and to the S1 and S2 (t-statistics, $p < 0.05$). This difference notably in GR_{50} values points to an early indication of possible shifts in cycloxydim susceptibility or tolerance. The sensitivity to both pinoxaden and cycloxydim in the population R6 was consistent with those of S1 and S2 (t-statistics, $p > 0.05$), confirming that R6 was equally susceptible.

4. Discussion

Overall, the dose-response experiments confirmed that the *A. fatua* populations varied significantly in their response when treated with ACCase inhibitors pinoxaden, propaquizafop and cycloxydim in both ED_{50} ($p < 0.05$) and GR_{50} ($p < 0.001$). Model fitting suggested a degree of resistance in all six *A. fatua* populations identified in the ACCase single-rate resistance testing.

The two susceptible populations S1 and S2 that had not previously received herbicides were confirmed to be highly sensitive with very low ED_{50} or GR_{50} values and consequently, elevated the calculated resistance factors for the resistant populations. Population R5 was the only test population found to be cross-resistant to all three ACCase inhibitors. Populations R1 and R3 were found to be cross-resistant to both pinoxaden and propaquizafop, and populations R2 and R6 were found to be resistant to propaquizafop only. In the population R2, although the ED_{50} values to both pinoxaden and cycloxydim and additionally, the ED_{50} of R3 to cycloxydim were higher than the recommended

field rates, even though their GR_{50} values were lower than the recommended field rates, this strongly suggests decreased sensitivity and a shift towards a cross-resistance situation. R4 was the only test population that had both ED_{50} and GR_{50} values well below the recommended field rates across all ACCase inhibitors, while still having a large RF, suggesting that it is in the process of evolving cross-resistance. On the other hand, the pinoxaden- and propaquizafop-resistant population R1 showed early signs of possible shifts in cycloxydim susceptibility or tolerance. The propaquizafop-resistant population R6 was as susceptible as the S1 and S2 to both pinoxaden and cycloxydim.

Our results are in agreement with several studies that have already reported different levels of cross-resistance types to ACCase inhibitors in *Avena* spp. e.g., [16,20–24]. Resistance evolution, especially in *A. fatua*, occurs quite slowly, compared to obligate outcrossing weed species, as it is basically a highly selfing hexaploid, with little standing genetic variation within populations, high levels of seed dormancy and irregular germination in the field making appropriate herbicide timing difficult [20,23]. In that respect, the observed variations in the cross-resistance levels and types in these populations suggest that the resistance has evolved independently and that each population has likely been affected by different selective pressure [16,23]. Additionally, the variations could be due to more than one resistance mechanisms being involved in these populations. Resistance to ACCase inhibitors is usually conferred by changes in amino acid residues at the herbicide-binding site of the ACCase enzyme [25]. However, a non-target-site mechanism of resistance consisting of enhanced degradation of DEN, FOPs or DIM, or uncharacterised non-target-site resistance mechanisms could also exist in these populations. It is already reported that target-site and enhanced metabolism resistance mechanisms can occur concurrently in field populations of *Avena* spp. [26–28] and in obligate outcrossing species such as *A. myosuroides* and *Lolium* spp. [29,30].

In general, the study highlighted that narrow crop rotation and repeated use of herbicides with similar modes of action led to the selection of resistant *A. fatua* individuals, and their numbers had increased within the field populations. Different cross-resistance within a single field (especially for populations R1 to R5) limits the options of changing or alternating active ingredients belonging to the ACCase group. For R6, adequate control may initially be achieved by the use of pinoxaden or cycloxydim. It is evident from this study, evolved resistance to one herbicide can cause cross-resistance to other herbicides within the same group or sometimes even to other herbicide modes of action [8,12], as most tested populations had no history of DIM use and additionally, populations R3 and R4 had no history of FOPs use in the previous 5 years and were unlikely to have been applied previously. Nevertheless, sulfonylurea chemical class of ALS-inhibiting herbicide mesosulfuron + iodosulfuron (Pacifica® Plus®) at recommended field rate was found to be highly effective (0% survival and shoot dry weight reduction of >80%) on all six ACCase resistant populations (Vijaya Bhaskar et al. Unpublished results). This result indicates that ALS modes of action can be used to control these *A. fatua* populations in diverse crop rotations, however, growers must note that ALS resistance in *A. fatua* in other regions has already been reported [12,14].

In these problem fields, to sustain the viability of crop production and to protect alternative modes of action, an integrated weed management (cultural/non-chemical and herbicide control practices) approach is strongly suggested [31,32]. Recommendations for maximising non-chemical integrated weed management to reduce seed return and to deplete the soil weed seed bank including hand roguing for small infestations; whole cropping (cutting, baling and removing the affected straw) if resistant populations are found widespread across the field; delaying post-harvest cultivations as long as possible to encourage natural predation, and machine-hygiene to prevent secondary weed seed dispersal within-field or field-to-field [31].

In general, herbicides are the most effective and convenient means of reducing weed seed production, but at the same time, they exert selection pressure for resistance evolution [32,33]. UK experience shows that non-chemical control practices are often adopted by growers as compensating measures for reduced herbicide efficacy, which could be when resistance is already in the field [32]. In Ireland, complaints of poor *A. fatua* control by key herbicides have been increasing recently

across all major regions, and if current crop/weed management does not change, increasing selection pressure will further increase resistant field populations, causing serious yield depression in cereal crops and reduced profit margins for growers. Therefore, Irish growers need to proactively adopt a complete integrated approach, which includes the use of multiple cultural/non-chemical control tactics such as crop rotations, planting of more competitive crops, use of higher than normal seeding rate, crop establishment techniques, stale seedbeds, hand roguing, and machine hygiene to prevent weed seed accumulation in the soil seed bank and to lessen the selection pressure, while retaining effective herbicides for less frequent but critical use [33,34].

5. Conclusions

This is the first study reporting the severity of herbicide resistance and cross-resistance to ACCase inhibitors in *A. fatua* in Ireland. Our main goal was to characterize the levels of resistance in six *A. fatua* populations following growers' reports of poor control with commonly used ACCase inhibitors pinoxaden, propaquizafop and cycloxydim. Glasshouse assays confirmed a degree of resistance in all six populations with varying resistance levels to ACCase inhibitors. A goal for future research is to explore the resistance mechanisms that are involved in ACCase inhibitors. Meanwhile, our results from this study have practical implications for growers' current weed/crop management, as the level and variety of cross-resistance types recorded, and difficulty in predicting cross-resistance among ACCase inhibitors stresses the need to minimize the selection pressure for resistance by adopting integrated weed management on Irish farms.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/9/1362/s1. Table S1: The *t*-statistics determining significant differences between estimated survival ED_{50} values, to identify significant differences in parameter estimates between *A. fatua* populations to ACCase inhibitors pinoxaden, propaquizafop and cycloxydim, Table S2: The *t*-statistics determining significant differences between estimated shoot dry weight GR_{50} values, to identify significant differences in parameter estimates between *A. fatua* populations to ACCase inhibitors pinoxaden, propaquizafop and cycloxydim, Figure S1: Symptomology of some *A. fatua* test populations at specific dose rates of pinoxaden, recommended field rate 30 g ha^{-1}, Figure S2: Symptomology of some *A. fatua* test populations at specific dose rates of propaquizafop, recommended field rate 100 g ha^{-1}, Figure S3: Symptomology of some *A. fatua* test populations at specific dose rates of cycloxydim, recommended field rate 150 g ha^{-1}.

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