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The First Case of Short-Spiked Canarygrass (*Phalaris brachystachys*) with Cross-Resistance to ACCase-Inhibiting Herbicides in Iran

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Abstract: The weed *Phalaris brachystachys* Link. severely affects winter cereal production. Acetyle-CoA Carboxylase (ACCase)-inhibiting herbicides are commonly used to control this weed in wheat fields. Thirty-six populations with suspected resistance to ACCase-inhibiting herbicides were collected from wheat fields in the Golestan Province in Iran. A rapid test performed in Petri dishes and whole-plant dose–response experiments were conducted to confirm and investigate the resistance level of *P. brachystachys* to ACCase-inhibiting herbicides. The seed bioassay results showed that 0.02 mg ai L^{-1} clodinafop-propargyl (CP) and 1.36 mg ai L^{-1} of the diclofop-methyl (DM) solution were the optimal amounts for reliably screening resistant and susceptible *P. brachystachys* populations. In the whole plant bioassay, all populations were found to be resistant to CP, resistance ratios ranging from 2.7 to 11.6, and all of the CP-resistant populations exhibited resistance to DM. Fourteen populations showed low resistance to cycloxydim, and thirteen of these populations were also 2-fold resistant to pinoxaden. The results showed that DM resistance in some *P. brachystachys* populations is likely due to their enhanced herbicide metabolism, which involves Cytochrome P450 monooxygenases, as demonstrated by the indirect assay. This is the first report confirming the cross-resistance of ACCase-inhibiting herbicides in *P. brachystachys* in Iran.

Keywords: herbicides resistance; seed bioassay; EC_{50}; GR_{50}; cytochrome P450

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1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important crops in Iran. Approximately 52% of arable land in Iran is used for wheat cultivation, with a 23% yield reduction caused by weeds [1]. The annual *Poaceae* species short-spiked Canarygrass (*Phalaris brachystachys* Link) is a common and troublesome weed in winter cereals in Mediterranean countries [2]. This is a vigorous and prolific weed that can significantly reduce wheat and barley yields and has been shown to decrease wheat yield by 16 to 60% [3,4]. Currently, *P. brachystachys* is found in the northern part of the Iran, where it infests crops during autumn and winter [5].

The use of herbicides is the most efficient and economical means of controlling grass weeds, and several ACCase-inhibitors have been registered in Iran in the last three decades [6]. The target site of these herbicides is Acetyl-CoA Carboxylase (ACCase; EC 6.4.1.2), which is a key enzyme that catalyzes the primary step in fatty acid biosynthesis [7]. ACCase-inhibiting herbicides are classified into three major families: aryloxyphenoxypropionates (APP), cyclohexanediones (CHD), and phenylpyrazolines

(PPZ). These herbicides, which inactivate ACCase, block fatty acid biosynthesis and reduce the production of phospholipids are major constituents of cell membranes [8]. APP and CHD herbicides have been used to control weeds since they were introduced in the 1970s and 1980s, respectively [9]. Furthermore, pinoxaden, which belongs to the PPZ group, was introduced in 2006 to control grass weeds during wheat cultivation [10]. Inescapably, the continuous use of ACCase herbicides, sometimes two or three times in a season in a wide range of crops, has led to resistance in various weed species. In recent decades, there has been a rising number of reports of graminicide-resistant weeds. Currently, 48 weed species have been reported to be resistant to ACCase inhibitors—the third highest in terms of the number of resistance cases in the world [11]. In Iran, the first case of resistance to ACCase herbicides was reported in *Phalaris minor* Retz. in winter wheat fields from the Fars, Golestan and Khuzestan provinces in 2004 [11].

In 2014, *P. brachystachys* populations that were resistant to diclofop-methyl (DM), fenoxaprop-p-ethyl and clodinafop-propargyl (CP) were found on farms in Golestan province, Iran [12]. Since then, the number of *P. brachystachys* populations resistant to these herbicides in wheat fields has increased, and these populations have been found in other fields in the Golestan province. Currently, no studies have reported the resistance level of *P. brachystachys*, and there has been no confirmation of resistance in this species. Therefore, the objectives of this study were to confirm the resistance of *P. brachystachys* to ACCase-inhibiting herbicides and quantify the level of resistance and cross-resistance patterns to APP, CHD and PPZ in suspected populations of *P. brachystachys* from wheat fields in Iran.

2. Materials and Methods

2.1. Seed Collection

The thirty-seven seeds of suspected resistant *P. brachystachys* populations were collected from winter wheat fields of the Golestan province in Iran during the spring of 2015, 2016 and 2017 (four, twenty-nine and four populations each year, respectively). Additionally, one susceptible population was collected from the same region that had never been treated with herbicides in 2016. Seeds from at least 15 plants that had reached physiological maturity were randomly collected by hand and bulked. The seeds were air dried and stored in paper bags at room temperature until used in the experiment. A global positioning system unit used to take latitude and longitude measurements for each field, and their locations, were mapped (ArcGIS 9.2) (Figure 1). Information regarding the collection position of each population is shown (Supplementary Materials).

2.2. Seed Bioassay

Does-response experiments were conducted using 9-cm diameter plastic Petri dishes. After breaking seed dormancy (seeds were immersed in sulfuric acid (98%) for 3 min then kept in Petri dishes containing moist filter paper with 5 mL distilled water for 72 h in a refrigerator at 4 °C in the dark), five germinated seeds were placed on two sheets of filter paper. Each Petri dish was considered to be one replicate, and the experiment was conducted with three replications for each herbicide and each population. This experiment was repeated three times. A 5-mL aliquot of an aqueous solution of the commercial formulation of DM was applied at 0, 0.1, 0.5, 1, 2, 4, 8, 16 and 32 mg ai L^{-1}, and CP was applied at concentrations of 0, 0.005, 0.02, 0.04, 0.08, 0.32, 0.64, 1.28, 5.12, 20.46 and 40.96 mg ai L^{-1}. For each population, the control treatment (without herbicide solution) with 5 mL distilled water was also considered. Then, the Petri dishes were placed in an incubator at 25 °C. After seven days, the shoot lengths of the coleoptiles in all of the seedlings were measured and expressed as the percentage of the shoot length of coleoptile compared to the control [13].

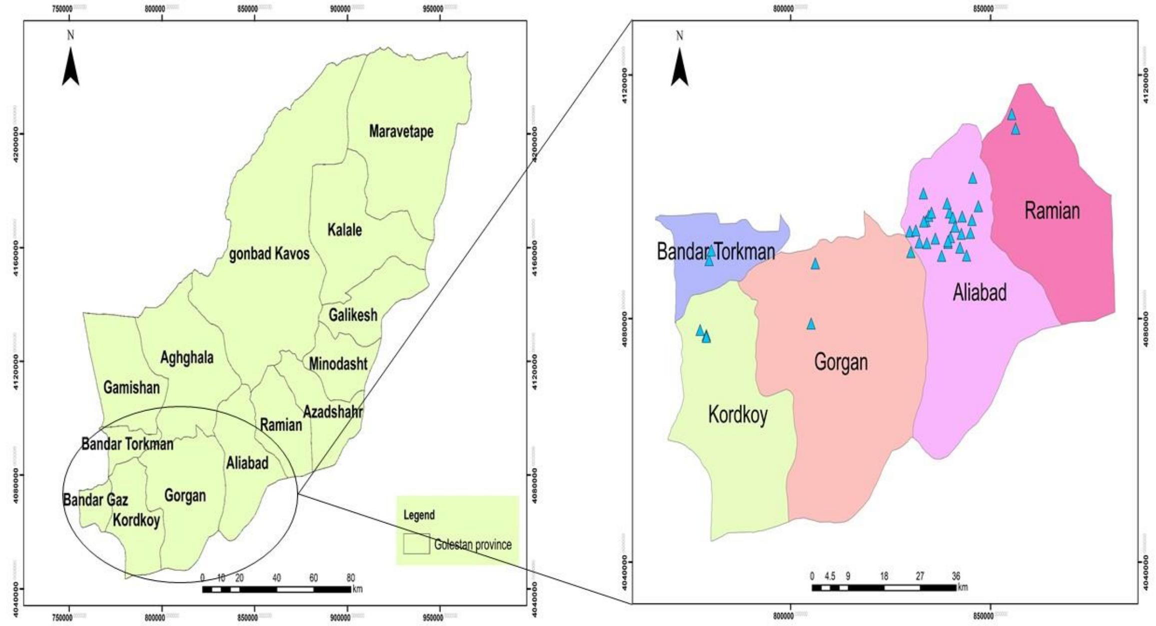

Figure 1. Geographical position of counties in Golestan province and distribution map of surveyed wheat fields in these counties. Circles indicate the field positions of the collected samples.

2.3. Whole Plant Dose–Response Assay

The experiment was repeated three times and arranged in a completely randomized design with three replications for *P. brachyhstachys* populations with different doses of clodinafop-propargyl, diclofop-methyl, cycloxydim and pinoxaden. Seed dormancy was broken as previously described and, then, the germinated seeds were sown in 11-cm plastic pots filled with a mixture (1:2 *v*/*v*) of peat and soil and placed in a greenhouse. The pots were irrigated daily as required. Each replicate pot contained five plants. Four weeks after sowing, at the three-to-four-leaf stage (as BBCH scale in 13–14), herbicides were applied at different rates using a calibrated sprayer with a flat-fan nozzle (TeeJet 8001) to deliver 250 L ha^{-1} of spray solution at 200 kPa. One untreated control (without herbicide application) for each population has been used. The detailed information of the herbicides used for the dose-response assay is presented in Table 1. The plants were harvested and oven dried for 48 h at 70 °C 28 days after herbicide application, and the dry-weight data were recorded. The data were expressed as a percentage of the untreated control. It should be noted that the populations with a high resistance factor based on DM herbicide results were used in the dose-response assay with pinoxaden and cycloxydim herbicides.

Table 1. Herbicide treatments applied for dose-response assays in a *Phalaris brachystachys* population.

Herbicides	Formulation	Trade Name, Manufacturer, City, and Country	Field Recommended Dose (g ai ha^{-1})	Test Doses (g ai ha^{-1})
Clodinafop-propargyl	8% EC [1]	Topik, Kavosh, Kerman, Iran	80	0, 20, 40, 80, 160, 320, 640, 1280
Diclofop methyl	36% EC	Iloxan, Kavosh, Kerman, Iran	900	0, 225, 450, 900, 1800, 3600, 7200, 14,400
Cycloxydim	10% EC	Focus® Ultra, BASF, Germany	100	0, 37.5, 75, 150, 300, 600, 1200
Pinoxaden	4.5% EC	Axial® Syngenta, Basel, Switzerland	45	0, 16.725, 33.75, 67.5, 135, 270, 540

[1] EC = emulsifiable.

2.4. Growth Assay in Combination with CytP450 Inhibitor

Seedlings of different DM-resistant (based on resistance factor) and S populations at the 3–4 leaf stage were treated with DM at the rate of 300 g ai ha^{-1} with and without amitrole (AM) to study whether metabolism was responsible for resistance in the resistant populations. AM was applied at 13.1 g ha^{-1} 24 h prior to application of DM. Twenty-one days after application, the plants were harvested, and the shoot fresh weight was measured. The experiment was repeated three times and it included ten replicates per repetition.

2.5. Statistics Analysis

The data obtained from the Petri dish and pot experiments were fit to a nonlinear log-logistic regression model with four parameters in the "*R*" statistical software with the "drc" package [14].

$$y = c + \frac{d - c}{1 + (exp\{b(\log(x) - \log(e))\}} \tag{1}$$

In the model, y represents the shoot dry weight or shoot length of coleoptile (percentage of the untreated control) at a herbicide dose of x; c and d denote the lower and upper limits, respectively; b is the slope of the response curve at e; and e denotes GR_{50} (or GR_{90}). The effective concentration of herbicide that caused 50% inhibition of the shoot length of coleoptile (EC_{50}) was calculated from the log-logistic regression model, which allowed us to screen resistant and susceptible populations according to the EC_{50} Equation (1). From each model, the effective herbicide doses which inhibited plant growth by 50 and 90% (GR_{50} and GR_{90}) with respect to the untreated control, were determined for each population. The resistance factor (RF), which is the ratio of the EC_{50}, GR_{50} or GR_{90} of the resistant population to the EC_{50}, GR_{50} or GR_{90} of the susceptible population, was considered as an index in order to compare the resistance levels of tested populations [15].

3. Results

3.1. Seed Bioassay

A difference in the shoot length of the populations was visible after 7 days of incubation. The resistance factors and estimated nonlinear regression parameters for the applied herbicides are shown in Table 2. The four-parameter log-logistic model provided a good fit to the data ($p < 0.001$; $R^2 > 0.96$). The results of the Petri dish assays showed that the coleoptile length of the seedlings decreased according to a sigmoidal trend and that the decreasing shoot length of the S population observed at lower concentrations than the other populations. This confirmed that the susceptible population was more sensitive to herbicides than the other populations. Regarding DM, the estimated EC_{50} was 1.36 mg ai L^{-1} for S, while for the other populations it ranged between 1.86 and 6.30 mg ai L^{-1} (Table 2). In the Petri dish assays, with the increasing CP concentration, different responses were consistently observed, and all populations had shorter coleoptiles compared to their untreated controls. While 0.02 mg ai L^{-1} of CP inhibited 50% of the shoot length of the S population, for the other populations, it ranged from 0.07 to 0.29 mg ai L^{-1} of CP and the resistance factors ranged from 2.77 to 10.27 (Table 2).

Table 2. Estimated nonlinear regression parameters and resistance factors (RFs) for the *P. brachystachys* in response to different diclofop-methyl (DM) and clodinafop-propargyl (CP) concentrations.

Population	DM						CP					
	d [1] (SE [2])	b [3] (SE)	R^2 [4]	*p* Values [5]	EC_{50} [6] (SE)	RF [7]	d (SE)	b (SE)	R^2	*p* Values	EC_{50} (SE)	RF_{50}
AL01	97.59 (4.13)	1.10 (0.14)	0.98	<0.001	3.08 (0.47)	2.26 (0.38)	102.17 (3.77)	0.95 (0.10)	0.98	<0.001	0.17 (0.02)	6.07 (1.44)
AL02	99.79 (3.72)	1.39 (0.17)	0.99	<0.001	2.44 (0.29)	1.79 (0.24)	100.24 (3.86)	0.89 (0.01)	0.99	<0.001	0.18 (0.03)	6.40 (1.56)
AL03	98.01 (4.00)	1.23 (0.15)	0.99	<0.001	2.36 (0.32)	1.73 (0.26)	95.16 (3.58)	1.15 (0.19)	0.99	<0.001	0.26 (0.04)	9.19 (2.22)
AL04	96.17 (3.57)	1.29 (0.19)	0.97	<0.001	6.30 (0.85)	4.62 (0.70)	103.04 (4.11)	0.71 (0.07)	0.98	<0.001	0.18 (0.03)	6.53 (1.74)
AL05	98.10 (3.96)	1.13 (0.14)	0.98	<0.001	2.23 (0.47)	2.37 (0.38)	100.29 (3.93)	0.83 (00.9)	0.97	<0.001	0.21 (0.03)	7.41 (1.87)
AL06	98.66 (3.95)	1.18 (0.15)	0.99	<0.001	3.09 (0.43)	2.26 (0.35)	103.20 (3.89)	0.82(0.08)	0.98	<0.001	0.19 (0.03)	6.82 (1.71)
AL07	97.59 (3.97)	1.23 (0.16)	0.97	<0.001	2.23 (0.59)	3.10 (0.48)	101.83 (4.36)	0.73 (0.07)	0.99	<0.001	0.08 (0.01)	3.03 (0.80)
AL08	96.53 (3.98)	1.16 (0.15)	0.98	<0.001	3.25 (0.48)	2.38 (0.38)	101.86 (4.17)	0.80 (0.08)	0.98	<0.001	0.11 (0.02)	3.89 (0.98)
AL09	97.67 (3.66)	1.28 (0.17)	0.99	<0.001	4.09 (0.53)	3.00 (0.44)	100.55 (3.92)	0.95 (0.10)	0.98	<0.001	0.14 (0.02)	4.95 (1.20)
AL10	98.10 (3.95)	1.16 (0.14)	0.98	<0.001	3.07 (0.44)	2.25 (0.35)	100.02 (4.44)	0.66 (0.07)	0.98	<0.001	0.13(0.02)	4.53 (1.28)
AL12	98.23 (3.88)	1.25 (0.16)	0.98	<0.001	3.41 (0.47)	2.49 (0.38)	102.03(3.88)	0.86 (0.09)	0.99	<0.001	0.16 (0.02)	5.63 (1.37)
AL13	98.22 (3.00)	1.17 (0.10)	0.98	<0.001	1.86 (0.19)	1.37 (0.17)	99.24 (4.13)	0.82 (0.10)	0.97	<0.001	0.16 (0.03)	5.79 (1.56)
AL14	93.70 (3.81)	1.29 (0.21)	0.95	<0.001	5.92 (0.88)	4.34 (0.71)	99.74 (4.14)	0.74 (0.09)	0.99	<0.001	0.23 (0.05)	8.33 (2.32)
AL15	99.41 (3.91)	1.13 (0.14)	0.98	<0.001	3.53 (0.50)	2.58 (0.41)	99.53 (4.14)	0.89 (0.11)	0.98	<0.001	0.13 (0.02)	4.55 (1.19)
AL16	98.71 (3.86)	1.36 (0.18)	0.99	<0.001	2.31(0.29)	1.69 (0.24)	100.00 (4.40)	0.62 (0.07)	0.99	<0.001	0.21 (0.05)	7.42 (2.27)
AL17	97.66 (4.24)	1.03 (0.13)	0.97	<0.001	2.62 (0.42)	1.92 (0.33)	100.49 (4.15)	0.80 (0.08)	0.98	<0.001	0.15 (0.03)	5.39 (1.40)
AL18	97.39 (3.34)	1.72 (0.25)	0.99	<0.001	3.66 (0.38)	2.68 (0.33)	100.96 (4.04)	0.86 (0.09)	0.99	<0.001	0.12 (0.02)	4.30 (1.06)
AL19	98.67 (4.13)	1.14 (0.14)	0.99	<0.001	2.44 (0.36)	1.78 (0.29)	101.82 (4.00)	0.92 (0.09)	0.99	<0.001	0.10 (0.01)	3.67 (0.88)
AL20	99.49 (4.19)	1.10 (0.13)	0.99	<0.001	1.93 (0.28)	1.42 (0.23)	96.51 (4.30)	0.91 (0.13)	0.97	<0.001	0.12 (0.02)	4.38 (1.21)
AL21	93.10 (3.71)	1.34 (0.22)	0.97	<0.001	6.07 (0.86)	4.44 (0.70)	100.34 (3.72)	0.97 (0.10)	0.98	<0.001	0.17 (0.02)	6.12 (1.45)
AL22	93.50 (4.24)	1.26 (0.19)	0.99	<0.001	3.13 (0.49)	2.29 (0.39)	9.86 (4.29)	0.82 (0.10)	0.98	<0.001	0.14 (0.03)	5.11 (1.43)
AL23	97.02 (3.55)	1.43(0.20)	0.98	<0.001	4.25 (0.52)	3.11 (0.44)	98.83 (3.44)	1.31 (0.19)	0.99	<0.001	0.20 (0.03)	7.12 (1.63)
AL24	98.16 (3.49)	1.54 (0.21)	0.99	<0.001	3.65 (0.41)	2.67 (0.35)	102.57 (4.19)	0.75 (0.07)	0.99	<0.001	0.12 (0.02)	4.32 (1.12)
AL28	98.14 (3.79)	1.21 (0.15)	0.98	<0.001	3.99 (0.55)	2.92 (0.45)	103.75 (4.14)	0.82 (0.08)	0.97	<0.001	0.08 (0.01)	3.05 (0.75)
AL31	99.23 (3.98)	1.15 (0.14)	0.99	<0.001	2.92 (0.41)	2.14 (0.33)	97.23 (4.06)	0.91 (0.13)	0.98	<0.001	0.19 (0.04)	6.82 (1.81)
AL32	97.38 (4.34)	1.11 (0.14)	0.99	<0.001	1.82 (0.28)	1.33 (0.22)	99.03 (3.66)	1.11 (0.14)	0.99	<0.001	0.15 (0.02)	5.28 (1.26)
AL33	96.56 (4.00)	1.14 (0.15)	0.97	<0.001	4.03 (0.61)	2.95 (0.49)	101.04 (4.05)	0.72 (0.08)	0.97	<0.001	0.25 (0.05)	8.84 (2.37)
AL34	96.92 (3.57)	1.28 (0.17)	0.98	<0.001	5.21 (0.69)	3.82 (0.56)	101.79 (3.83)	0.95 (0.10)	0.98	<0.001	0.13 (0.02)	4.54 (1.07)
B02	96.60 (4.25)	1.10 (0.14)	0.98	<0.001	2.30 (0.36)	1.68 (0.28)	101.90 (4.13)	0.75 (0.08)	0.97	<0.001	0.15 (0.03)	5.38 (1.45)
B03	97.96 (3.98)	1.15 (0.15)	0.98	<0.001	3.77 (0.56)	2.78 (0.45)	101.09 (4.20)	0.71(0.07)	0.98	<0.001	0.23 (0.05)	8.23 (2.27)
Kr14	98.69 (3.95)	1.20 (0.15)	0.99	<0.001	2.49 (0.34)	1.83 (0.27)	100.64 (4.02)	0.75 (0.08)	0.99	<0.001	0.25 (0.05)	8.73 (2.31)
Kr15	95.42 (3.79)	1.22 (0.17)	0.98	<0.001	4.33 (0.62)	3.17 (0.50)	96.82 (4.10)	0.80 (0.10)	0.97	<0.001	0.29 (0.06)	10.27 (2.78)
Kr16	97.35 (3.99)	1.12 (0.14)	0.99	<0.001	3.15 (0.36)	2.31 (0.37)	99.32 (4.08)	0.83 (0.09)	0.98	<0.001	0.17 (0.03)	5.96 (1.53)
G04	96.76 (3.97)	1.29 (0.17)	0.96	<0.001	2.39 (0.32)	1.75 (0.26)	102.98 (4.28)	0.75 (0.07)	0.99	<0.001	0.07 (0.01)	2.77 (0.71)
Rm17	97.11(3.93)	1.52 (0.21)	0.99	<0.001	1.92 (0.23)	1.41 (0.19)	102.01(2.80)	0.84 (0.06)	0.98	<0.001	0.15 (0.01)	5.29 (1.12)
Rm18	97.93 (3.99)	1.19 (0.15)	0.99	<0.001	2.45 (0.34)	1.79 (0.28)	103.15 (4.17)	0.74 (0.07)	0.98	<0.001	0.12 (0.02)	4.36 (1.13)
S	93.67 (3.28)	4.21 (0.86)	0.99	<0.001	1.36 (0.09)	-	101.65 (4.60)	0.90 (0.12)	0.99	<0.001	0.02 (0.004)	-

[1] d = upper limit; [2] SE = standard error; [3] b= slope of curve around the dose giving 50% response; [4] $R^2 = 1 -$ (sum of squares of the regression/corrected total sum of squares) [5] *p*-value = is the probability level of significance of the resistance factor; [6] EC_{50} is the concentration producing a response halfway between the upper limit (fixed at 100) and the lower limit (fixed at 0); [7] RF_{50} = Resistance Factor is calculated as (GR_{50} resistant/GR_{50} sensitive).

3.2. Dose–Response Assay

We assessed representatives of all of the different groups of graminicides, such as clodinafop-propargyl, diclofop-methyl, pinoxaden and cycloxydim. The results showed that the susceptible population was considerably controlled by two APP herbicides. The other populations showed resistance to the APP herbicides, but the level of resistance varied substantially. The S population was inhibited by 50% with only 24.22 g ai ha^{-1} of CP compared with the recommended field amount of 80 g ai ha^{-1}. The other populations were resistant to the CP field dose, with resistance levels ranging from 2.7 to 11.6-fold based on the GR_{50} values (Table 3). Among the 36 populations studied, the Kr15 and Kr16 populations had the largest resistance factor based on their GR_{50} values (Table 3). The estimated GR_{50} values indicated different resistance factors (RF = GR_{50} R/GR_{50} S) to DM for the different populations. The S population was inhibited by 50% with only 279.57 g ai ha^{-1} of DM, while the amount required to reach GR_{50} for the other populations was between 563.14 and 3059.90 g ai ha^{-1}. The estimated GR_{90} for the S population was 866.63 g ai ha^{-1}, whereas the GR_{90} values varied in the other populations from 2934.52 to 22929.67 g ai ha^{-1} (Table 4).

Table 3. Estimated non-linear regression parameters for the *P. brachystachys* populations in response to CP, based on the dry weight.

Population	**Regression Parameters**		$R^{2,4}$	**GR_{50} [5] (SE)**	**RF_{50} [6] (SE)**	**GR_{90} [7] (SE)**	**RF_{90} [8] (SE)**
	d [1] (SE)	**b [2] (SE [3])**					
AL01	95.72 (5.85)	1.31 (0.22)	0.99	122.30 (22.38)	5.04 (1.23)	62.89 (150.62)	5.76 (1.95)
AL02	98.23 (5.06)	1.62 (0.27)	0.98	130.13 (18.19)	5.37 (1.14)	501.66 (99.37)	4.43 (1.41)
AL03	97.37 (5.04)	1.75 (0.31)	0.99	133.79 (18.43)	5.52 (1.17)	466.31 (88.24)	4.12 (1.28)
AL04	97.88 (5.10)	1.17 (0.18)	0.99	229.79 (40.25)	9.48 (2.25)	1479.43 (380.17)	13.07 (4.67)
AL05	96.28 (5.05)	1.27 (0.22)	0.97	239.55 (40.89)	9.88 (2.32)	1348.26 (342.19)	11.91 (4.23)
AL06	98.18 (4.81)	1.43 (0.23)	0.98	189.58 (27.89)	7.82 (1.70)	880.00 (198.11)	7.77 (2.61)
AL07	96.47 (5.00)	1.46 (0.26)	0.98	185.22 (28.45)	7.64 (1.70)	832.73 (189.51)	7.35 (2.48)
AL08	94.5 (5.17)	1.88 (0.35)	0.99	112.80 (15.59)	4.65 (0.98)	361.77 (66.57)	3.19 (0.99)
AL09	98.77 (5.00)	1.27 (0.19)	0.99	189.66 (30.47)	7.82 (1.78)	1061.77 (250.97)	9.37 (3.22)
AL10	100.99 (5.30)	1.12 (0.15)	0.97	130.08 (21.98)	5.36 (1.25)	909.40 (230.47)	8.03 (2.85)
AL12	96.59 (5.87)	1.45 (0.23)	0.98	83.36 (13.71)	3.44 (0.79)	376.26 (78.67)	3.32 (1.08)
AL13	94.14 (5.79)	1.75 (0.35)	0.99	104.64 (16.59)	4.31 (0.97)	366.54 (72.09)	3.23 (1.02)
AL14	99.88 (4.92)	1.27 (0.19)	0.97	182.88 (28.32)	7.54 (1.68)	1025.58 (245.35)	9.06 (3.12)
AL15	95.60 (4.87)	2.06 (0.39)	0.98	114.17 (14.15)	4.71 (9.57)	330.11 (57.54)	2.91 (0.88)
AL16	97.91 (5.08)	1.64 (0.27)	0.97	116.99 (15.97)	4.82 (1.01)	445.65 (90.37)	3.93 (1.26)
AL17	96.08 (4.90)	1.92 (0.36)	0.98	115.41 (14.29)	4.76 (0.96)	361.57 (71.10)	3.19 (1.01)
AL18	96.95 (4.21)	2.76 (0.59)	0.99	108.95 (9.78)	4.49 (0.82)	241.32 (40.78)	2.13 (0.64)
AL19	97.25 (5.24)	1.62 (0.27)	0.99	112.09 (15.87)	4.62 (0.99)	434.81 (90.01)	3.84 (1.24)
AL20	96.77 (5.06)	1.64 (0.28)	0.99	128.84 (18.01)	5.31 (1.13)	491.14 (101.46)	4.33 (1.40)
AL21	97.59 (5.12)	1.30 (0.21)	0.99	172.36 (27.80)	7.11 (1.62)	929.41 (224.66)	8.21 (2.85)
AL22	98.14 (5.62)	1.60 (0.25)	0.98	75.50 (10.81)	3.11 (0.67)	296.29 (59.37)	2.61 (0.83)
AL23	97.55 (4.75)	1.33 (0.21)	0.98	231.83 (35.83)	9.56 (2.13)	1196.40 (287.30)	10.57 (3.65)
AL24	98.23 (4.83)	1.39 (0.22)	0.97	178.55 (26.32)	7.37 (1.60)	860.11 (196.74)	7.59 (2.57)
AL28	102.55 (5.40)	1.56 (0.21)	0.98	66.31 (8.69)	2.73 (0.56)	270.78 (54.68)	2.39 (0.76)
AL31	95.96 (4.49)	1.90 (0.36)	0.98	143.45 (16.78)	5.92 (1.17)	454.96 (95.10)	4.01 (1.30)
AL32	98.05 (5.48)	1.65 (0.26)	0.98	86.30 (12.21)	3.56 (0.76)	325.75 (62.72)	2.87 (0.90)
AL33	97.52 (4.58)	1.52 (0.25)	0.99	199.37 (27.43)	8.22 (1.74)	838.85 (186.43)	7.41 (2.47)
AL34	98.47 (5.07)	1.57 (0.25)	0.99	122.72 (17.17)	5.06 (1.08)	496.65 (101.35)	4.38 (1.41)
B02	99.54 (5.37)	1.30 (0.18)	0.97	114.11 (18.19)	4.71 (1.06)	614.2 (137.25)	5.42 (1.81)
B03	97.12 (4.91)	1.33 (0.22)	0.98	212.14 (33.78)	8.75 (1.98)	1096.80 (137.25)	9.69 (3.33)
Kr14	99.75 (3.80)	1.40 (0.26)	0.99	187.21 (25.16)	7.72 (1.62)	893.36 (190.48)	7.89 (2.58)
Kr15	96.30 (6.51)	1.38 (0.26)	0.99	280.44 (51.95)	11.57 (2.84)	1366.68 (325.43)	12.07 (4.16)
Kr16	95.86 (5.29)	1.16 (0.27)	0.99	281.91 (53.40)	11.63 (2.89)	1846.37 (515.82)	16.31 (6.10)
G04	101.23 (5.49)	1.51 (0.22)	0.99	74.13 (10.30)	3.06 (0.65)	314.78 (64.64)	2.78 (0.89)
Rm17	95.17 (4.52)	2.83 (0.69)	0.98	77.80 (6.76)	3.21 (0.58)	168.93 (32.97)	1.49 (0.47)
Rm18	102.26 (4.77)	1.66 (0.27	0.98	102.05 (11.89)	4.21 (0.83)	382.73 (88.91)	3.38 (1.15)
S	99.57 (5.97)	1.42 (0.26)	0.99	24.22 (3.89)	-	113.18 (28.20)	-

[1] d = upper limit; [2] b = slope of curve around the dose giving 50% response; [3] SE = standard error; [4] R^2 = 1−(sum of squares of the regression/corrected total sum of squares); [5] GR_{50} refers to the herbicides rates required for 50% dry weight reduction compared with the non-treated control; [6] RF_{50} = Resistance Factor is calculated as (GR_{50} resistant/GR_{50} sensitive); [7] GR_{90} = refers to the herbicides rates required for 90% dry weight reduction compared with the non-treated control; [8] RF_{90} = Resistance Factor is calculated as (GR_{90} resistant/GR_{90} sensitive).

Table 4. Estimated non-linear regression parameters for the *P. brachystachys* populations in response to DM, based on the dry weight.

Population	Regression Parameters		R^2 [4]	GR_{50} [5] (SE)	RF_{50} [6]	GR_{90} [7] (SE)	RF_{90} [8]
	d [1] (SE)	b [2] (SE [3])					
AL01	97.06 (4.79)	1.41 (0.17)	0.98	847.02 (113.6)	3.02 (0.50)	3981.52 (671.69)	4.59 (1.04)
AL02	96.65 (4.47)	1.85 (0.27)	0.97	894.90 (97.05)	3.20 (0.47)	2922.03 (442.58)	3.37 (0.72)
AL03	97.74 (4.29)	1.41 (0.17)	0.98	1408.70 (178.44)	5.03 (0.81)	6650.75 (1139.93)	7.67 (1.76)
AL04	101.40 (4.25)	0.82 (0.10)	0.99	2503.10 (595.29)	8.95 (0.47)	7492.64 (3005.80)	8.64 (1.11)
AL05	95.94 (3.87)	1.67 (0.23)	0.98	1737.60 (193.34)	6.21 (0.93)	6431.13 (1037.20)	7.42 (1.65)
AL06	101.34 (4.21)	0.97 (0.11)	0.98	2154.90 (318.57)	7.70 (1.38)	20,293.46 (5034.14)	23.41 (6.83)
AL07	100.11 (3.97)	1.49 (0.17	0.98	1426.16 (159.9)	5.10 (0.76)	6172.88 (971.91)	7.12 (1.55)
AL08	98.06 (4.11)	1.58 (0.20)	0.99	1298.50 (145.88)	4.64 (0.70)	5173.73 (844.38)	5.96 (1.34)
AL09	96.36 (4.17)	1.54 (0.21)	0.97	1601.01 (196.65)	5.72 (0.91)	6630.58 (1089.22)	7.65 (1.72)
AL10	99.89 (4.17)	1.22 (0.14)	0.99	1570.00 (201.86)	5.61 (0.91)	9489.82 (1908.51)	10.95 (2.77)
AL12	97.13 (4.08)	2.05 (0.30)	0.98	1007.7 (96.25)	3.60 (0.50)	2934.52 (423.47)	3.38 (0.71)
AL13	96.58 (4.53)	1.32 (0.17)	0.99	1366.80 (190.36)	4.88 (0.84)	7138.97 (1287.40)	8.23 (1.95)
AL14	102.08 (4.09)	1.09 (0.12)	0.98	1970.00 (262.63)	7.04 (1.17)	14,602.58 (3213.70)	16.85 (4.52)
AL15	100.42 (3.76)	1.77 (0.22)	0.99	1371.00 (131.27)	4.90 (0.68)	4727.74 (728.00)	5.45 (1.18)
AL16	98.06 (4.02)	1.58 (0.20)	0.99	1298.54 (142.66)	4.64 (0.68)	5173.91 (825.77)	5.96 (1.31)
AL17	99.15 (3.50)	2.44 (0.42)	0.97	1218.50 (90.83)	4.35 (0.54)	2996.61 (461.14)	3.45 (0.75)
AL18	98.98 (3.34)	2.54 (0.45)	0.98	1351.50 (96.61)	4.83 (0.59)	3203.18 (494.83)	3.69 (0.80)
AL19	96.68 (4.34)	1.97 (0.30)	0.98	951.34 (98.45)	3.40 (0.45)	2897.83 (425.86)	3.43 (0.65)
AL20	96.52 (4.15)	1.83 (0.27)	0.99	1104.40 (114.35)	3.95 (0.57)	3658.36 (585.56)	4.22 (0.93)
AL21	98.87 (4.02)	1.09 (0.14)	0.99	3059.90 (432.50)	10.94 (1.90)	22,929.67 (5461.21)	26.45 (7.50)
AL22	99.77 (4.12)	2.06 (0.28)	0.99	887.66 (81.41)	3.17 (0.43)	2575.31 (356.38)	2.97 (0.61)
AL23	100.64 (3.79)	1.24 (0.15)	0.97	2483.80 (298.76)	8.88 (1.39)	14,428.64 (3005.80)	16.64 (4.31)
AL24	100.26 (3.57)	1.63 (0.20)	0.98	1882.20 (187.85)	6.73 (0.95)	7227.98 (1171.65)	8.34 (1.86)
AL28	99.08 (3.90)	1.57 (0.21)	0.99	1772.60 (196.10)	6.34 (0.95)	7175.31 (1200.87)	8.27 (1.88)
AL31	97.30 (3.39)	2.76 (0.51)	0.97	1257.90 (89.58)	4.49 (0.55)	2782.23 (399.34)	3.21 (0.67)
AL32	100.81 (4.61)	1.12 (0.13)	0.98	563.14 (75.50)	2.01 (0.33)	3948.68 (889.68)	4.55 (1.24)
AL33	92.23 (3.43)	2.19 (0.39)	0.97	2020.60 (189.94)	7.22 (0.99)	5490.71 (860.01)	6.33 (1.39)
AL34	100.26 (3.57)	1.61 (0.18)	0.98	1472.20 (150.65)	5.26 (0.75)	5749.19 (881.94)	6.63 (1.44)
B02	98.71 (3.48)	2.40 (0.42)	0.99	1211.21 (89.74)	4.33 (0.53)	3015.77 (471.24)	3.47 (0.75)
B03	100.65 (4.06)	1.03 (0.12)	0.97	2483.80 (298.76)	8.71(1.51)	20,263.99 (4871.94)	23.38 (6.67)
Kr14	96.62 (4.02)	1.31 (0.18)	0.98	2513.80 (333.88)	8.99 (1.50)	13,300.41 (2568.43)	15.34 (3.79)
Kr15	96.68 (3.63)	1.85 (0.26)	0.98	1705.00 (167.88)	6.09 (0.86)	5576.45(863.50)	6.43 (1.40)
Kr16	97.02 (4.13)	1.22 (0.16)	0.98	2357.50 (326.37)	8.43 (1.44)	14,215.78 (2923.08)	16.40 (4.21)
G04	100.20 (4.07)	1.49 (0.17)	0.99	1290.20 (144.95)	4.61 (0.69)	5582.56 (910.74)	6.44 (1.44)
Rm17	99.08 (4.00)	1.50 (0.18)	0.97	1429.60 (161.31)	5.11 (0.77)	6166.78 (1030.04)	7.11 (1.61)
Rm18	99.32 (3.91)	1.52 (0.18)	0.98	1583.10 (176.83)	5.66 (0.85)	6678.33 (1076.31)	7.70 (1.71)
S	99.20 (4.81)	1.94 (0.28)	0.99	279.57 (28.27)	-	866.63 (133.60)	-

[1] d = upper limit; [2] b = slope of curve around the dose giving 50% response; [3] SE = standard error; [4] $R^2 = 1 -$ (sum of squares of the regression/corrected total sum of squares); [5] GR_{50} refers to the herbicides rates required for 50% dry weight reduction compared with the non-treated control; [6] RF_{50} = Resistance Factor is calculated as (GR_{50} resistant/GR_{50} sensitive); [7] GR_{90} = refers to the herbicides rates required for 90% dry weight reduction compared with the non-treated control; [8] RF_{90} = Resistance Factor is calculated as (GR_{90} resistant/GR_{90} sensitive).

For cycloxydim (CHD family herbicides), all populations were found to exhibit low resistance levels (Table 5). The concentration of cycloxydim that led to a 50% inhibition of shoot growth in the S population was 46.35 g ai ha^{-1} and the cycloxydim resistance factor for all of the resistant populations was between 2-fold and 3-fold. The lowest GR_{90} value for resistant populations was observed in AL21 (295.57 g ai ha^{-1}), whereas a higher GR_{90} value was recorded in Kr15 (425.97 g ai ha^{-1}) (Table 5). Similarly, the pinoxaden herbicide was found to significantly reduce the growth of all the resistant populations, and low resistance levels were recorded for this ACCase-inhibiting herbicide in 13 populations. The pinoxaden GR_{50} values of the resistant populations were approximately two times higher than for the S population, and a large reduction of shoot dry weight was found in all the resistant populations (Table 6).

Table 5. Estimated non-linear regression parameters for the *P. brachystachys* populations in. response to cycloxydim based on dry weight.

Population	Regression Parameters		R^{2} [4]	*p*-Value [5]	GR$_{50}$ [6] (SE)	RF$_{50}$ [7] (SE)	GR$_{90}$ [8] (SE)	RF$_{90}$ [9] (SE)
	d [1] (SE)	b [2] (SE) [3]						
AL04	95.48 (6.73)	2.18 (0.51)	0.96	<0.0001	127.22 (19.69)	2.74 (0.51)	347.271 (65.35)	3.13 (0.89)
AL05	97.12 (6.42)	2.09 (0.44)	0.97	<0.0001	129.03 (18.77)	2.78 (0.50)	367.80 (70.76)	3.32 (0.95)
AL06	97.63 (6.22)	2.17 (0.45)	0.98	<0.0001	126.98 (17.33)	2.73 (0.47)	348.23 (70.76)	3.14 (0.890
AL07	97.91 (5.98)	2.30 (0.50)	0.98	<0.0001	133.05 (16.99)	2.87 (0.48)	344.82 (64.65)	3.11 (0.88)
AL08	97.89 (6.13)	2.21 (0.45)	0.99	<0.0001	120.96 (15.82)	2.60 (0.44)	326.32 (62.23)	2.94 (0.84)
AL14	97.28 (6.36)	2.17 (0.44)	0.97	<0.0001	115.55 (16.07)	2.49 (0.43)	317.38 (58.97)	2.86 (0.81)
AL15	97.80 (5.87)	2.35 (0.52)	0.99	<0.0001	130.06 (15.82)	2.80 (0.45)	331.27 (64.54)	2.99 (0.86)
AL21	95.90 (6.09)	2.57 (0.61)	0.98	<0.0001	125.92 (16.02)	2.71 (0.45)	295.57 (52.24)	2.66 (0.74)
AL23	96.97 (6.20)	2.39 (0.53)	0.97	<0.0001	124.81 (16.54)	2.69 (0.46)	311.85 (54.95)	2.81 (0.78)
AL24	97.30 (6.22)	2.16 (0.46)	0.98	<0.0001	132.62 (18.32)	2.86 (0.50)	365.37 (70.44)	3.29 (0.95)
AL33	95.51 (6.23)	2.43 (0.62)	0.98	<0.0001	140.27 (19.08)	3.02 (0.52)	345.38 (66.14)	3.11 (0.89)
Kr15	97.59 (5.99)	2.07 (0.44)	0.99	<0.0001	147.89 (20.15)	3.19 (0.55)	425.97 (86.82)	3.84 (1.13)
B03	95.42 (6.33)	2.23 (0.56)	0.98	<0.0001	150.96 (21.81)	3.25 (0.58)	363.57 (74.54)	3.64 (0.08)
Rm17	98.50 (6.67)	1.85 (0.35)	0.97	<0.0001	111.03 (17.00)	2.39 (0.45)	363.57 (74.54)	3.28 (0.97)
S	99.98 (6.62)	2.52 (0.59)	0.99	<0.0001	46.35 (5.03)	-	110.77 (23.73)	-

[1] d = upper limit; [2] b = slope of curve around the dose giving 50% response; [3] SE = standard error; [4] $R^2 = 1 -$ (sum of squares of the regression/corrected total sum of squares); [5] *p*-value = is the probability level of significance of the resistance factor; [6] GR$_{50}$ refers to the herbicides rates required for 50% dry weight reduction compared with the non-treated control; [7] RF$_{50}$ = Resistance Factor is calculated as (GR$_{50}$ resistant/GR$_{50}$ sensitive); [8] GR$_{90}$ = refers to the herbicides rates required for 90% dry weight reduction compared with the non-treated control; [9] RF$_{90}$ = Resistance Factor is calculated as (GR$_{90}$ resistant/GR$_{90}$ sensitive).

Table 6. Estimated non-linear regression parameters for the *P. brachystachys* populations in response to pinoxaden, based on the dry weight.

Population	Regression Parameters		R^{2} [4]	*p*-Value [5]	GR$_{50}$ [6] (SE)	RF$_{50}$ [7] (SE)	GR$_{90}$ [8] (SE)	RF$_{90}$ [9] (SE)
	d [1] (SE)	b [2] (SE) [3]						
AL04	96.73 (6.61)	2.05 (0.42)	0.96	<0.0001	52.36 (7.85)	2.09 (0.43)	152.44 (28.36)	1.78 (0.52)
AL05	96.07 (6.62)	2.06 (0.46)	0.97	<0.0001	58.33 (8.92)	2.33 (0.49)	168.65 (32.51)	1.98 (0.59)
AL06	94.48 (6.49)	2.20 (0.58)	0.98	<0.0001	67.59 (10.14)	2.70 (0.56)	183.03 (37.74)	2.14 (0.66)
AL07	95.80(4.72)	3.18 (0.85)	0.99	<0.0001	73.12 (6.56)	2.92 (0.50)	145.87 (27.79)	1.71 (0.50)
AL08	95.55 (6.90)	1.97 (0.43)	0.96	<0.0001	51.44 (8.30)	2.05 (0.44)	156.43 (30.62)	1.83 (0.55)
AL14	96.25 (6.29)	2.37 (0.53)	0.98	<0.0001	53.25 (7.09)	2.12 (0.42)	134.35 (23.92)	1.57 (0.45)
AL15	98.65 (5.99)	2.23 (0.46)	0.99	<0.0001	54.71 (6.81)	2.18 (0.41)	146.53 (28.08)	1.72 (0.51)
AL21	97.57 (6.79)	1.77 (0.33)	0.98	<0.0001	39.48 (6.15)	1.57 (0.33)	135.85 (28.87)	1.59 (0.49)
AL23	95.71 (6.20)	2.21 (0.53)	0.98	<0.0001	64.61 (8.97)	2.58 (0.52)	174.21 (34.79)	2.04 (0.62)
AL24	97.76 (6.52)	1.90 (0.36)	0.97	<0.0001	52.68 (7.94)	2.10 (0.44)	167.21 (32.82)	1.96 (0.59)
AL33	97.14 (6.07)	2.36 (0.53)	0.98	<0.0001	58.52 (7.53)	2.33 (0.45)	148.03 (26.83)	1.73 (0.50)
Kr15	92.92 (5.31)	2.58 (0.70)	0.98	<0.0001	85.37 (10.21)	3.41 (0.64)	199.83 (40.80)	2.34 (0.71)
B03	93.39 (6.49)	2.26 (0.65)	0.97	<0.0001	71.62 (10.79)	2.86 (0.60)	188.98 (40.70)	2.21 (0.69)
Rm17	98.43 (5.85)	2.24 (0.47)	0.99	<0.0001	61.55 (7.67)	2.46 (0.72)	163.77 (31.23)	1.92 (0.57)
S	99.15 (6.73)	1.79 (0.35)	0.98	<0.0001	25.02 (3.65)	-	85.16 (10.48)	-

[1] d = upper limit; [2] b = slope of curve around the dose giving 50% response; [3] SE = standard error; [4] $R^2 = 1 -$ (sum of squares of the regression/corrected total sum of squares); [5] *p*-value = is the probability level of significance of the resistance factor; [6] GR$_{50}$ refers to the herbicides rates required for 50% dry weight reduction compared with the non-treated control; [7] RF$_{50}$ = Resistance Factor is calculated as (GR$_{50}$ resistant/GR$_{50}$ sensitive); [8] GR$_{90}$ = refers to the herbicides rates required for 90% dry weight reduction compared with the non-treated control; [9] RF$_{90}$ = Resistance Factor is calculated as (GR$_{90}$resistant/GR$_{90}$sensitive).

3.3. Growth Assays in Combination with CytP450 Inhibitor

The responses of *P. brachystachys* populations to DM, with and without amitrol are shown in Table 7. The present study found that the combination of DM with amitrol was slightly more effective in the AL33, G04 and Kr15 populations than DM alone and pretreatment with amitrole significantly inhibited the growth of these populations compared to populations without amitrole. However, the fresh weight of the S population did not vary and was independent of the amitrole treatment.

Table 7. Fresh weight of six populations of *P. brachystachys* with treatments of DM and DM + Amitrol (AM).

Population	DM RF [1] (SE [2])	Control (SE) (g)	DM (SE) (g)	DM + AM (SE) (g)	% Reduction [3]
S	-	5.66 (0.24)	2.84 (0.18)	2.71 (0.17)	4.58
AL01	3.02 (0.50)	3.12 (0.30)	3.19 (0.18)	3.16 (0.27)	0.94
G04	4.61 (0.69)	4.68 (0.18)	5.08 (0.26)	3.65 (0.15)	28.15
Kr15	6.09 (0.86)	3.96 (0.23)	4.55 (0.35)	3.20 (0.25)	29.67
AL33	7.22 (0.99)	4.96 (0.42)	5.13 (0.21)	4.05 (0.40)	21.05
AL04	8.95 (0.47)	4.54 (0.55)	5.69 (0.18)	5.20(0.18)	8.61

[1] DM RF = Resistant Factor obtained from diclofop methyl dose–response assay; [2] SE = standard error; [3] The percentage of fresh weight reduction of DM+AM treatment compared to DM treatment.

4. Discussion

The seed bioassay method for determining resistant and susceptible populations has been previously utilized [16–18]. This method is regarded as the most rapid and simplest way to screen resistant and susceptible populations. In this study, this method was applied for the *P. brachystachys* populations. In preliminary tests, each APP herbicide was tested. It is necessary to detect resistance as early as possible to avoid the costly consequences of a resistance spread. Seed bioassays have been shown to be a useful and accurate tool for screening a large number of suspected resistant populations. The identification of concentrations that are effective at separating resistant and susceptible populations is important not only for the rapid diagnosis of potential resistance but also for the screening of seeds used for experiments. From this research, it was determined that the seed bioassay could be developed to be a feasible method to identify resistant populations of *P. brachystacys*. This method has been used to test resistance to ACCase-inhibitors in barnyardgrass (*Echinochloa crusgalli*) [19] and Johnsongrass (*Sorghum halepense* (L.) *Pers.*) [20]. Other researchers described a seed bioassay to detect grass weeds resistant to ACCase-inhibiting herbicides [21].

The dose–response assays confirmed that the *P. brachystachys* populations were resistant to DM and CP herbicides. The seed assay also confirmed the resistance to APP herbicides. According to the results of both the whole plant and seed bioassay, the resistance factor of most of the populations to CP were considerably higher compared to DM. No precise history of herbicide application in the sampled fields was available. However, Golestan is one of the most important provinces for producing wheat in Iran. The use of these herbicides has been the main approach to control weeds in wheat fields. The high percentage of resistance to CP and DM was expected because these two herbicides have a common basic molecular structure [22], and both have been extensively used to control grassy weeds during wheat cultivation, which is the most frequently grown crop in the area. These results indicate that resistance to these herbicides can be attributed to the use of a wheat monoculture in the sampling areas along with the repeated use of these herbicides for a long period of time [23]. Resistance to APP herbicides has been reported in littleseed canarygrass (*Phalaris minor Retz.*) [1]. Also, the level of cross-resistance to APP herbicides in *Avena* spp. has been reported [24]. Notably, most populations were highly resistant to APP herbicides, while their response to PPZ varied. Resistance to APP herbicides is not necessarily associated with resistance to pinoxaden. The AL21 population, which showed high resistance to APP herbicides, was susceptible to pinoxaden. However, the AL04 and Kr15 populations expressed high resistance to CP, with RF values of 9.4 and 11.5, respectively (Table 6). These populations also expressed high resistance to the same chemical class of APP herbicide, DM, with RF values of 8 and 6, respectively, but low cross-resistance was observed to cycloxydim (RF of 2.74 and 3.19, respectively) and pinoxaden (RF of 2.09 and 3.41, respectively) (Table 5).The reduced control of some *P. brachystachys* populations by pinoxaden indicates cross-resistance to this herbicide, regardless of the fact that this herbicide has been used in Iran for the last few years. The whole-plant dose–response assays showed that the cross-resistance levels of ACCase inhibitors varied among *P. brachystachys* populations. APP presented the highest RF values, while the cross-resistance corresponding to CHD and PPZ herbicides was low.

The differences in the cross-resistance patterns in these resistant populations indicate that resistance evolved independently and that each resistant population has likely been exposed to a different selection pressure. Additionally, the differences indicate that more than one resistance mechanism is likely involved in these *P. brachystachys* resistant populations.

To test the hypothesis that enhanced DM metabolism is conferred by CytP450, a known CytP450-inhibitor, amitrole, was tested. Amitrol has long been used as an indicator of the involvement of P450 in metabolic resistance to ACCase herbicides [25–27]. These results of this experiment suggest that CytP450 monooxygenase-mediated metabolism could be present in these populations and contribute to the resistance phenotype. These results indicate that CytP450 is involved in DM-resistance in G04, Kr15 and AL33 populations of *P. brachystachys* and metabolic resistance could be the mechanism responsible for resistance in these populations (Table 7).

The consecutive use of different herbicides with the same mode of action in wheat fields in the Golestan province led to the selection of resistant *P. brachystachys* individuals, and their numbers have increased within the populations. Today, resistant populations have been established in several parts of the province, and if the current weed/crop management method does not change, increasing selection pressure will result in further infestation of resistant populations. However, crop rotation and, consequently, different weed management methods would be the best way to control resistant *P. brachystachys* populations in this region. The results of this study clearly indicate that pinoxaden and cycloxydim have become ineffective at controlling some of the APP resistant *P. brachystachys* populations and these herbicides should not be considered as alternative herbicides for the effective control of resistant populations. Our results regarding this species are in agreement with the results reported by others regarding the different levels of cross-resistance patterns of different weeds resistant to the three groups of ACCase-inhibiting herbicides [18,24]. The Italian ryegrass (*Lolium multiflorum*) with DM resistance was also cross-resistant to pinoxaden [28]. Additionally, there was a level of cross-resistance to APP, CHD, and PPZ in bristly dogstail grass (*Cynosurus echinatus*) populations [27]. Hood canarygrass (*Phalaris paradoxa*) populations have also been reported to have cross-resistance to the APP, CHD and PPZ herbicides [7]. The insensitivity of the ACCase target site is the most common mechanism of resistance to ACCase-inhibiting herbicides [28]. However, resistance likely did not develop via a single mechanism; rather, multiple mechanisms, including enhanced metabolism, an altered target site, and other uncharacterized mechanisms, may be involved [29].

5. Conclusions

This is the first study confirming the cross-resistance of the aryloxyphenoxypropionates, cyclohexanediones and phenylpyrazolines herbicides in *P. brachystachys* in Iran. The CytP450 monooxygenase data in the present study indicate that a metabolic mechanism is probably involved in conferring cross-resistance among ACCase-inhibiting herbicides in some *P. brachystachys* populations. However, the resistance level cannot only be explained by herbicide metabolism to non-toxic forms, and other additional mechanisms should be studied. ACCase enzyme activity and gene analysis are needed to identify the resistance mechanisms in these populations. A goal for further research is the identification of the resistance mechanisms that are involved in ACCase inhibitor herbicides. We plan to study these mechanisms in the future; meanwhile, due to the results of the present study, resistance to ACCase inhibitors in *P. brachystachys* from Iran has been identified. In further studies, we will elucidate the resistance mechanisms of resistance by biochemical and molecular methods.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/9/7/377/s1, Table S1: Geographical location, collection site of *P. brachystachys* suspected resistant populations.

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