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Short title: Herbicide-resistant sprangletop

Target site mechanism confers resistance pattern of ACCase-inhibitors in bearded sprangletop (*Leptochloa fusca* ssp. *fascicularis*) from California[1]

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Abstract

Bearded sprangletop is a problematic native grass weed in California's rice fields. The widespread and extensive use of Acetyl-CoA carboxylase (ACCase) inhibiting herbicides, such as cyhalofop-P-butyl (cyhalofop), has led to speculation that biotypes of bearded sprangletop have developed herbicide resistance to ACCase. The aim of this study was to evaluate suspected resistant bearded sprangletop biotypes, R1, R2, R3, and the susceptible biotype, S1, in terms of their levels of resistance to three ACCase-inhibiting herbicides and to characterize the molecular mechanisms of resistance. Dose-response experiments suggested that the biotype R1, R2, and R3 had high-level resistance to cyhalofop, and quizalofop-P-ethyl (quizalofop), but not clethodim. It was identified that the resistance to ACCase inhibitors was a target site mechanism resulting from nucleotide substitution. The carboxyl transferase (CT) domain of the ACCase gene's sequence analysis revealed the substitutions Trp-2027-Cys for R1 and R2 biotypes and Ile-2041-Asn for R3 biotype. This study revealed that presence of target-site resistance to cyhalofop and quizalofop in at least two mutation points in representative biotypes of bearded sprangletop in California. This research highlights the significance of careful herbicide selection due to weed species responding quite rapidly to selection pressure to help manage bearded sprangletop in rice field.

Nomenclature: Clethodim; cyhalofop-P-butyl; quizalofop-P-ethyl; bearded sprangletop, *Leptochloa fusca* ssp. *Fascicularis*; rice, *Oryza sativa* L.

Keywords: Dose-response, gene mutation, target site, rice, bearded sprangletop.

Introduction

Bearded sprangletop is one of the most common and competitive annual semiaquatic grasses, and it is widespread in California rice fields (Brim-DeForest et al. 2017; Driver et al. 2020a). Bearded sprangletop is an annual grass native to North America (Bryson and DeFelice 2009) and relies on seed production to complete its life cycle. Bearded sprangletop produces many seeds, and generally, seedlings emerge later than other weedy grasses (McCarty et al. 1995, Driver et al. 2020a). Bearded sprangletop can reduce rice grain yield by up to 36% if not controlled (Smith 1983). As a result of its prolificity and competitiveness, growers must manage this weed using cultural and chemical tools.

While flooding rice fields with deep water is a common practice to suppress bearded sprangletop (Driver et al. 2020a), herbicides are a major component of California's weed-control strategy to achieve adequate bearded sprangletop control and high rice yields (Yasuor et al. 2008). Despite using integrated weed management methods, bearded sprangletop biotypes in California have been suspected to be resistant to herbicides such as cyhalofop (group 1, acetyl-CoA carboxylase (ACCase) inhibitor), thiobencarb (group 15, inhibitor of very-long-chain fatty acids (VLCFA) synthesis, clomazone (group 13, inhibitor of 1-deoxy-d-xylulose 5-phosphate (DXP) synthase), benzobicyclon + halosulfuron-methyl (group 27, 7, 4-hydroxyphenylpyruvate dioxygenase (HPPD) + group 2, acetolactate synthase (ALS) inhibitor) (Brim-DeForest et al. 2015; Driver et al., 2020b; Becerra-Alvarez et al., 2023). Only clomazone resistance has been confirmed in bearded sprangletop biotypes (Driver et al. 2020b). Preliminary studies by Brim-DeForest et al. (2015) suggested target site resistance to cyhalofop in bearded sprangletop. Therefore, identification of the resistance mechanisms might be useful in developing quick molecular diagnostic tests that advisors can use to confirm resistance development in bearded sprangletop.

ACCase inhibitors prevent plants from synthesizing fatty acids (Devine 1997). Fatty acid synthesis inhibition likely prevents the creation of phospholipids needed to construct new membranes for cell development (Gronwald 1991). Three catalytic domains make up ACCase: biotin carboxyl transferase (CT), biotin carboxylase (BC), and biotin carboxyl carrier (BCC) (Nikolskaya et al. 1999). These domains are all involved in the two reversible processes of carboxylation of acetyl-CoA. Initially, a biotin group covalently linked to the BCC domain is carboxylated in an ATP-dependent manner by the BC domain. Subsequently, the carboxyl group is transferred from biotin to acetyl-CoA by the CT domain. The biosynthesis of secondary metabolites and fatty acids depends on the produced malonyl-CoA

(Harwood 1988). There are two distinct ACCase isoforms found in the plants. The homomeric enzyme cytosolic ACCase, present in all eukaryotes, combines the three domains into a single polypeptide. Most plants contain chloroplastic (plastidic) ACCase, a heterodimeric enzyme with three domains distributed among four subunits. Because their ACCase plastidic isoform is homomeric, plants belonging to the *Poaceae* family are unique (Konishi et al. 1996). The selective binding of the CT domain of grasses' plastidic isoform by aryloxyphenoxypropionates (FOPs), the cyclohexanediones (DIMs), and the phenylpyrazolin (DEN) herbicides confers herbicidal effects; other isoforms remain unaffected and insensitive (Nikolskaya et al. 1999; Zhang et al. 2004, Kaundun et al. 2013). Long-term usage of FOPs herbicides, particularly in rice production, has exerted selection pressure on weeds and led to resistant bearded sprangletop biotypes (Rahman et al. 2011; Phongphitak et al. 2014). Cyhalofop, quizalofop, and clethodim are typically used to control grass as a FOPs and DIMs herbicides. Cyhalofop is widely used in conventional rice farming systems and quizalofop is utilized in Provisia® rice farming system to control bearded sprangletop, other weed grasses and weedy rice (Lancester et al. 2018).

The fundamental cause of the resistant weeds is attributed to the development of target and/or non-target site resistance mechanisms to herbicides (Délye 2013). Target site resistance and non-target site resistance mechanisms can contribute to weed survival, depending on the selections made to its genetic changes (Délye 2013). Target site and non-target site resistance mechanisms can also coexist in a single individual or population, raising their resistance to one herbicide or giving them multiple resistance to various herbicides (Vazquez-Garcia et al. 2019). Target site resistance constitutes the most common resistance mechanism to ACCase (Powles and Yu 2010). Herbicide target-site amino acid substitution in the CT domain of ACCase has caused herbicide resistance in various weed species (Laforest et al. 2017). Several amino acid substitutions have been reported in the ACCase gene region in resistant *Leptochloa* spp. such as Ile-1781-Leu, Ile-1781-Trp, Trp-1999-Cys, Trp-2027-Ser, Trp-2027-Leu, Trp-2027-Cys, Ile-2041-Asn, Asp-2078-Gly, Cys-2088-Arg, Gly-2096-Ala (Deng et al. 2019; Peng et al. 2020; Wu et al. 2019; Yu et al. 2007; Yu et al. 2017; Yuan et al. 2019; Zhang et al. 2020; Zhao et al. 2022).

Recently, suspected herbicide-resistant bearded sprangletop biotypes have become a common problem in California rice fields. In annual survey studies conducted by Becerra-Alvarez et al. (2023) in California, an increase in suspected cyhalofop-resistant bearded sprangletop biotypes was observed. This research examines the resistance of three bearded sprangletop to

ACCase-inhibiting herbicides. Specifically, this study aimed to confirm resistance and determine the resistance level of the three suspected bearded sprangletop biotypes through the development of dose-response curves to cyhalofop, quizalofop, and clethodim. The second objective was to establish whether a mutation in the target site gene was responsible for resistance.

Materials and methods

Plant material

A total of four bearded sprangletop biotypes, one known susceptible (S1) and three suspected resistant (R1, R2, and R3) to cyhalofop, were studied. Bearded sprangletop biotype S1 (ST-HR-2015) was collected from California Rice Experiment Station (RES) in Biggs CA (39°27'03.6"N; 121°43'13.5"W) in 2015, and its new generation was produced in the greenhouse in 2019. Suspected resistant bearded sprangletop biotypes were collected from rice fields with history of cyhalofop use and where bearded sprangletop survived the herbicide treatment. R1 (ST-19-10) was collected from Butte County, CA (39°22'46.7"N; 121°44'38.5"W) in 2019, R2 (ST-20-02) was collected from Glenn County, CA (39°37'34.7"N; 122°02'14.0"W) in 2020, and R3 (ST-21.07) was collected from Colusa County, CA (39°19'07.2"N; 122°07'18.2"W) in 2021. The seeds were stored at 4 C until utilized in the experiments. To break bearded sprangletop seed dormancy seeds were placed in a freezer at -20 C for three months before being placed in a refrigerated test tube at 4 C and soaked in deionized water. The water in the tubes was changed daily for two weeks (Driver et al. 2020b). The seeds were then placed on wet filter paper and incubated for 16 hours at 40 C. Germinated seeds were transplanted in 8-cm by 8-cm by 6-cm pots in the greenhouse on Orchard Park Drive at UC Davis. Three seedlings were placed in each pot. Greenhouse temperature was 23 to 34 C, relative humidity was 65-70%, and 14/10-h day/night photoperiod. The supplemental light was 400 µmol m^{-2} s^{-1}. Sterilized media soil was used, comprised of one-part compost (redwood shavings and turkey manure), one-part coarse sand, one-part peat moss, and 1.23 kg m^{-3} dolomite. The soil pH was 6.6, and the soil nutrient was 125 mg kg^{-1} available N, P_2O_5 46 mg kg^{-1} Olsen P, 759 mg kg^{-1} extractable K, 1041 mg kg^{-1} Calcium, 578 mg kg^{-1} magnesium, 7.6 mg kg^{-1} copper, 13 mg kg^{-1} zinc, 58 mg kg^{-1} manganese, 57 mg kg^{-1} iron, and 0.51 mg kg^{-1} boron. Plants were irrigated as needed.

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Dose-response experiment

Herbicides was applied at three- to four-leaf stage. Cyhalofop, quizalofop, and clethodim doses were selected 16-fold below and above the label dose of herbicide with the control application, such as 0×, 1/16×, ⅛×, ¼×, ½×, 1×, 2×, 4×, 8×, and 16× (Table 1). Cyhalofop-P-butyl ((2*R*)-2-[4-(4-cyano-2-fluorophenoxy) phenoxy] propanoate) formulation was Clincher CA (Corteva Agriscience, Indianapolis, IN) which contained 29.6% of active ingredient (ai). Cyhalofop was applied with 2.5% crop oil concentrate (COC). Quizalofop-P-ethyl (2-[4-(6-chloroquinoxalin-2-yl) oxyphenoxy] propanoate) formulation was Targa (Nissan Chemical Corporation, Japan) with contained 10.3% ai. quizalofop was applied with 1% COC. Clethodim (2-[(*E*)-*N*-[(*E*)-3-chloroprop-2-enoxy]-*C*-ethylcarbonimidoyl]-5-(2-ethylsulfanylpropyl)-3-hydroxycyclohex-2-en-1-one) herbicide formulation was Select Max (Valent U.S.A. LLC, San Ramon, CA) which contained 12.6% of the active ingredient (ai). Clethodim was applied with 0.25% nonionic surfactant (NIS). Adjuvants were selected according to product labels, and their application was based on percent volume per volume (%v/v) concentration. Herbicides were applied in a spray chamber (Technical Machinery Incorporated, CA, USA) with one Teejet XR8002VS flat fan nozzle (TeeJet Tech., Illinois, USA) calibrated to deliver 187 L ha^{-1} at 275 kPa pressure, application height of nozzle was 72 cm and, speed was 1.34 m s^{-1}. After herbicide application, pots were placed back inside the greenhouse, the pots irrigated 48 hours after application, they were maintained until harvest, and the aboveground plants were harvested 28 days after treatment (DAT). Plants were dried at 70 C for three days. The herbicide dose required to control 50% of the test biotypes (ED50) was calculated from dry plant weight and converted to dry biomass percentage compared to the non-treated control for presentation (Seefeldt et al. 1995). The experiment was conducted twice as a randomized block design with three replications.

Nucleotide substitution experiment

Fresh leaf tissue from five plants of each bearded sprangletop biotype were collected at 28 DAT. Cetyltrimethylammonium bromide (CTAB) DNA extraction method was used (Doyle and Doyle 1987). Two primer pairs were designed based on sequences of *Leptochloa chinensis* (L.) Nees (GenBank: QWJ75145.1) from National Center for Biotechnology Information, NCBI, (Table 2). The expected coverage of the two pairs was 98.9% and, the 643 number of nucleotides they overlap. Polymerase chain reaction (PCR) amplification was performed using the Qiagen Taq PCR master mix (Qiagen, N.V., Netherlands) which contained 25 μL TAq Master mix, 1 μL each primer (10 μM), 1 μL genomic DNA mixed in

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ddH_2O in 50 μL. Thermal was included initially as denaturing step at 95 C for 5 min, followed by 35 cycles of 45 s denaturation at 95 C, 45 s annealing at 60 C, 60 s elongation at 72 C, and a final extension of 5 min at 72 C. Electrophoresis was performed at 120 V for an hour. Plant DNA was purified by QIAquick PCR Purification Kit (Qiagen, N.V. Nederland). Sequencing was performed at UC Davis Genomic Center. The sequencing data were analyzed by using MEGA 11: Molecular Evolutionary Genetics Analysis version 11 (Tamura et al. 2021).

Statistical analyses

A four-parameter log-logistic model (equation 1) was used to establish the dose of each herbicide that result in 50% dry weight reduction (ED_{50}) (Seefeldt et al. 1995). The ED_{50} estimations was computed using the R DRC package (v4.3-1; Ritz et al. 2015) for statistical analysis.

$$Y = C + \frac{D - C}{1 + exp(b(\log(x) - \log(ED_{50})))} \qquad [1]$$

Where Y is biomass, b is the slope at the inflexion point (ED_{50}), C and D are the lower and higher boundaries of the asymptote, respectively, and × is the herbicide dose. ANOVA was used to examine the p-value that indicated a significant difference between the S and R biotypes. The resistance index (RI) was computed by dividing the ED_{50} of the resistant biotype by that of the susceptible biotype (Guo et al. 2016).

Result and Discussion

Dose response study

All three bearded sprangletop biotypes were found to exhibit high-level resistance to cyhalofop. The ED_{50} value for susceptible bearded sprangletop (S1) treated with cyhalofop was 27.4 g ai ha^{-1}; however, the R1, R2, and R3 resistant biotypes had ED_{50} values higher than 4480 g ai ha^{-1} cyhalofop (16×), resulting in resistance index (RI) values more than 164-fold (Table 3). The exact ED_{50} values could not be calculated for R1, R2, and R3, resistant to cyhalofop (Table 3, Figure 1), since bearded sprangletop survived at all applied cyhalofop doses with no 50% reduction in dry weight.

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It was determined that all three bearded sprangletop biotypes showed high levels of resistance to quizalofop. While all resistant biotypes showed dry weight reduction between 30 and 70 % even at the highest dose of 1792 g ai ha^{-1} (16×) quizalofop, no S1 plants could not maintain their dry weight at 112 g ai ha^{-1} (1×) quizalofop (Table 3, Figure 2). The S1 biotypes had ED_{50} of 12.0 g ai ha^{-1}quizalofop, but the R2 resistant biotype had ED_{50} values higher than 1792 g ai ha^{-1} (16×), resulting in resistance index (RI) values more than 150-fold (Table 3). The exact ED_{50} values could not calculated for resistant R2 biotype due to high resistance to quizalofop and no 50% reduction in dry weight (Figure 2). Bearded sprangletop biotype R1 and R3 exhibited ED_{50} value of 1107.8 and 602.1 g ai ha^{-1} to quizalofop, respectively.

It is evident that the cyhalofop and quizalofop doses used in this study were not high enough to significantly reduce the dry weight of the resistant biotypes. However, the doses used in this study were similar to those used in previous research that examined herbicide resistance in *Leptochloa* spp. (Brim-Deforest et al. 2015; Tehranchian et al. 2016; Deng et al. 2019; Peng et al. 2020; Yuan et al. 2021; Zhang et al. 2022).

For clethodim, all bearded sprangletop biotypes were killed at 1× dose. S1, R1, R2, and R3 biotypes had ED_{50} values of 20.9, 24.3, 23.6, and 20.7 g ai ha^{-1} clethodim, respectively (Figure 3). Therefore, the R biotypes were susceptible to clethodim despite being resistant to the two APP (FOP) herbicides.

One of the main herbicides used since 2003 in California rice fields to control bearded sprangletop has been cyhalofop. ACCase inhibitor-resistant biotypes may emerge after 6 to 10 years of selection pressure, especially in cropping systems where the ongoing use of these herbicides is the sole method of controlling grass weeds (Devine 1997). With the frequent and intense use of cyhalofop in California rice fields, the continuous rice cultivation year after year, and a limited number of available herbicides, it is not surprising that bearded sprangletop has developed resistance to cyhalofop (Becerra-Alvarez et al. 2023; Brim-DeForest et al. 2015). This study revealed that suspected resistant bearded sprangletop biotypes, R1, R2, and R3, had a high-level resistance to cyhalofop, and quizalofop but not to clethodim in California rice fields. This study also suggested that quizalofop, used in the newly developed Provisia rice technology, will have problems combating resistant bearded sprangletop in California. The Provisia system features a non-GMO herbicide-tolerant rice, allowing growers to safely apply quizalofop (Mankin et al. 2021). Although clethodim is not registered in rice, it is understood to be used successfully to control bearded sprangletop and

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other economically important weeds with spot spray applications (Unan et al. 2023). In addition, this study may indicate that clethodim could be a useful tool to control bearded sprangletop if clethodim-resistant rice is developed. However, one must consider the possibility that other biotypes might have resistance to clethodim after selection with cyhalofop and that clethodim if used the same way, could be a strong selection pressure.

Target-site resistance: ACCase mutation detection

Each biotype of bearded sprangletop provided partial ACCase gene sequences. The results of a sequence alignment revealed a 98.8% similarity between the ACCase gene sequences from bearded sprangletop and *Leptochloa chinensis* (L.) Nees (GenBank:QWJ75145.1). All tested plants of the R1 and R2 biotypes displayed a substitution of Ile (ATT) to Asn (AAT) at position Ile-2041-Asn whereas R3 biotype displayed a substitution of Trp (TGG) to Cys (TGC) at position Trp-2027-Cys compared with the S1 biotype and *Leptochloa chinensis* (L.) Nees. The plants from the S1 biotype, meanwhile, showed no signs of any known mutation.

Target-site-based resistance mechanisms frequently make for cross-resistance to herbicides that have the same mode of action (Beckie and Tardif 2012). Several ACCase mutation points in bearded sprangletop have been reported so far. The present study identified two distinct ACCase mutations, Trp-2027-Cys and Ile-2041-Asn, for bearded sprangletop (Figure 4). The Ile-2041-Asn substitution was found in R1 and R2, while the Trp-2027-Cys substitution was found in R3, but these substitutions have been reported before as conferring plants with resistance to FOPs and susceptibility to clethodim (Yu et al. 2007). While the Trp-2027-Cys substitution has been documented before in bearded sprangletop from California (DeForest et al. 2015), this is the first occurrence of the Ile-2041-Asn substitution. Tetranchian et al. (2016) previously identified mutations in the Amazon sprangletop in Trp-2027-Cys in Arkansas. Zhao et al. (2022) identified Trp-2027-Cys substitution *Leptochloa chinensis* sprangletop and noted that it was resistant to cyhalofop. In addition, Peng et al. (2020) reported Trp-2027-Ser and Ile-2041-Asn mutations in which it was resistant to cyhalofop in *Leptochloa chinensis*. Moreover, Yuan et al. (2019) detected Gly-2096-Ala substitution in *Diplachne fusca*, but this substitution was not detected in our study.

Cyhalofop is the only post-emergence herbicide available in California to control bearded sprangletop; however, there are pre-emergence herbicides to control this weed, including clomazone, thiobencarb and benzobicyclon (Becerra-Alvarez et al. 2023). If cyhalofop resistance is known in the field, then using preemergence herbicides becomes essential.

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Practical Implications

In conclusion, bearded sprangletop resistance to selected ACCase inhibitors is present in California rice fields. The bearded sprangletop biotypes under study were resistant to cyhalofop and quizalofop, but not to clethodim. Target site resistance was identified as the primary factor contributing to the resistance to cyhalofop and quizalofop for bearded sprangletop. The Trp-2027-Cys and Ile-2041-Asn target-site substitutions play a crucial role in the resistance to cyhalofop and quizalofop for bearded sprangletop in California. The results can aid in creating scientific approaches for the integrated management of resistant biotypes to ACCase inhibitors in bearded sprangletop. This study also revealed that all tested biotypes were susceptible to clethodim. The possibility of resistant bearded sprangletop genotypes becoming widespread in the coming years may cause greater problems. It might be suggested to rice farmers that integrated weed management such as crop rotation, certified clean seeds, deep flooding (Driver et al. 2020a), till the soil no more than 20 cm deep, and spot spray application (Unan et al. 2023) to control of resistant bearded sprangletop.

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Table 1. Clethodim, cyhalofop-P-butyl and quizalofop-P-ethyl application doses used in this study on the suspected herbicide-resistant bearded sprangletop biotypes.

Active ingredients and trade names	Dose (g ai ha^{-1})	Fraction of used dose
Clethodim (Select Max, Valent U.S.A. LLC, San Ramon, CA)	0, 9.4, 18.8, 37.5, 75, 150, 300, 600, 1200, and 2400	0×, 1/16×, ⅛×, ¼×, ½×, 1×, 2×, 4×, 8×, and 16×
Cyhalofop (Clincher CA, Corteva Agriscience, Indianapolis, IN)	0, 17.5, 35, 70, 140, 280, 560, 1120, 2240, and 4480	0×, 1/16×, ⅛×, ¼×, ½×, 1×, 2×, 4×, 8×, and 16×
Quizalofop (Targa, Nissan Chemical Corporation, Japan)	0, 7, 14, 28, 56, 112, 224, 448, 896, and 1792	0×, 1/16×, ⅛×, ¼×, ½×, 1×, 2×, 4×, 8×, and 16×

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Table 2. Primers of the ACCase gene fragment of bearded sprangletop.

Primers	Sequence (5'-3')	Product Size (bp)	Annealing Temperature (°C)
Primer 1F	TCATTTGGCCCAAGGGAAG	1392	58
Primer 1R	CGAGCTTCTATGCTCTTCTGAA		
Primer 2F	ACATTCGTGACTGGACGGAC	973	58
Primer 2R	TCAACTCTGGGTCAAGCCTA		

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Table 3. Average cyhalofop, quizalofop, and clethodim dose that cause 50% dry weigh reduction (ED_{50}) and resistance index (RI) of bearded sprangletop biotypes.

Active Ingredient	Biotype	ED_{50} (SE)	RI
		g ai ha^{-1}	
Cyhalofop	S1	27.4 (1.8)	-
	R1	>4480	>164
	R2	>4480	>164
	R3	>4480	>164
Quizalofop	S1	12.0 (0.5)	-
	R1	1107.8 (171.0)	92.3
	R2	>1792	>150
	R3	602.2 (66.5)	50.2
Clethodim	S1	21.0 (0.8)	-
	R1	24.3 (0.9)	1.2
	R2	23.6 (0.9)	1.1
	R3	20.7 (0.7)	1

SE, standard error which average of two runs; RI, resistance index ED_{50} value of resistant bearded sprangletop biotype divided by that of susceptible biotype; >4480 and >1792, the data did not allow for the estimation of ED_{50} values since all doses of cyhalofop and quizalofop were not sufficient to no 50% reduction in dry weight.

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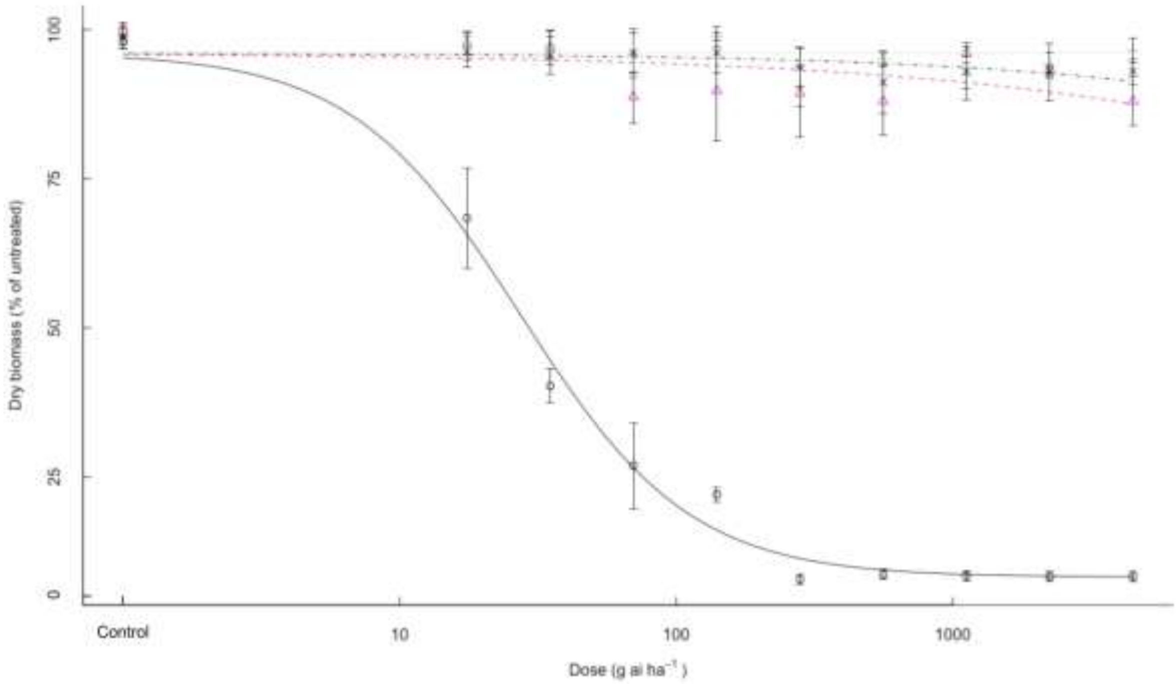

Figure 1. Effect of cyhalofop on the growth biomass of R1 (Δ), R2 (+), R3 (×) and S (O) biotypes of bearded sprangletop. S was the susceptible biotype, R1, R2, and R3 were resistant biotypes. Each point represents the average of six measurements (two runs and three replications) with standard error of the mean. Dose–response curves were generated by non-linear regression using a log-logistic model. Vertical error bars represent the 95% confidence intervals at ED_{50}.

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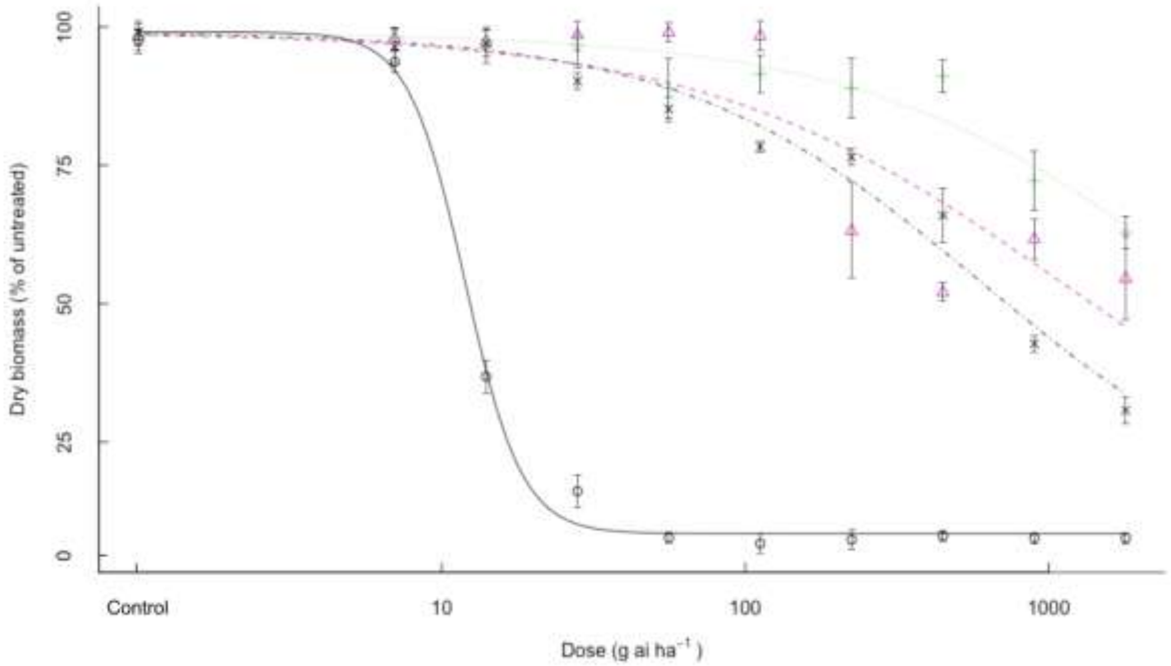

Figure 2. Effect of quizalofop on the growth biomass of R1 (Δ), R2 (+), R3 (×) and S (O) biotypes of bearded sprangletop. S was the susceptible biotype, R1, R2, and R3 were resistant biotypes. Each point represents the average of six measurements (two runs and three replications) with standard error of the mean. Dose–response curves were generated by non-linear regression using a log-logistic model. Vertical error bars represent the 95% confidence intervals at ED_{50}.

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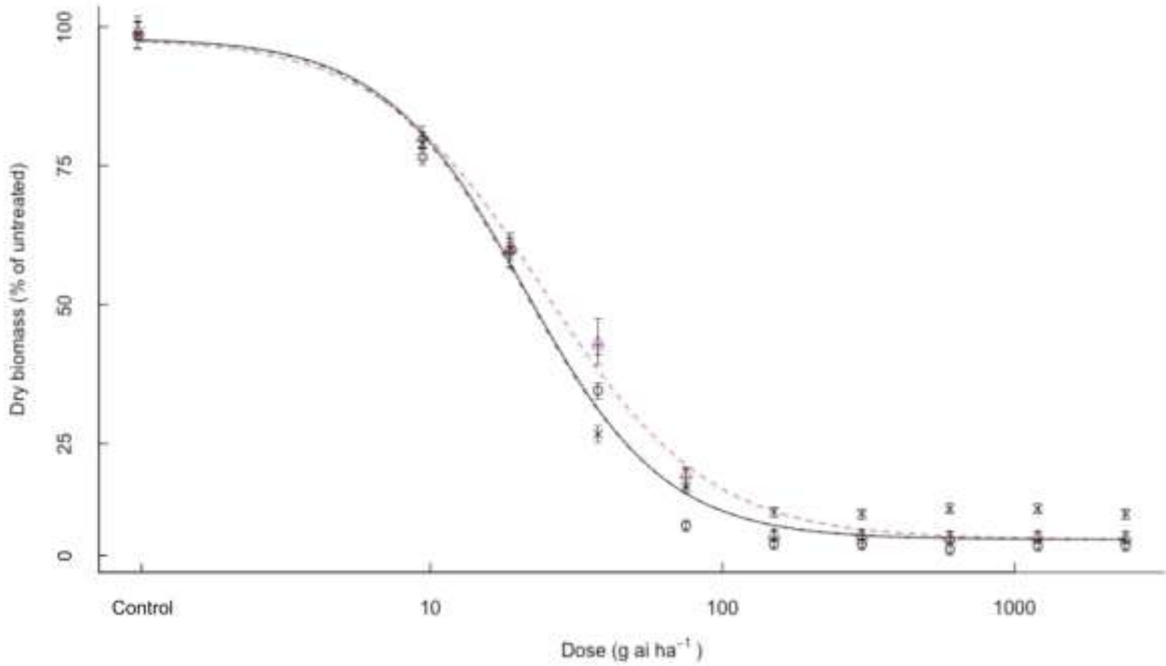

Figure 3. Effect of clethodim on the growth biomass of R1 (Δ), R2 (+), R3 (×) and S (O) biotypes of bearded sprangletop. S1, R1, R2, and R3 were all susceptible to clethodim. Each point represents the average of six measurements (two runs and three replications) with standard error of the mean. Dose–response curves were generated by non-linear regression using a log-logistic model. Vertical error bars represent the 95% confidence intervals at ED_{50}.

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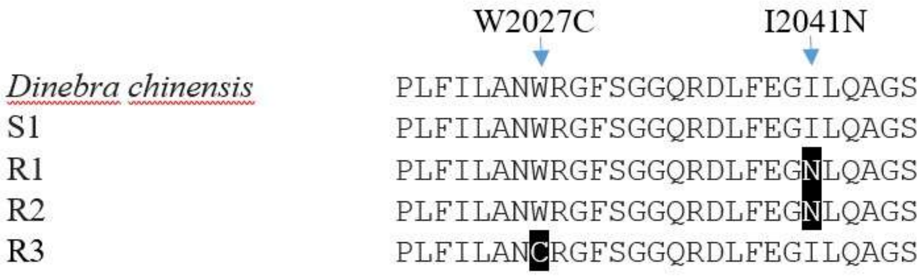

Figure 4: ACCase amino acid sequences of the amplified fragment of *Leptochloa chinensis* (L.) Nees, the susceptible (S1) and resistant (R1, R2, and R3) biotypes of bearded sprangletop. The black boxes illustrated that amino acid substation from tryptophan (W) 2027 to cytosine (C) in R1 and R2, and from isoleucine (I) 2041 to leucine (N) in R3. The *Leptochloa chinensis* (L.) Nees (GenBank:QWJ75145.1) and susceptible bearded sprangletop (S1) ACCase sequence were used as reference.