Weed Science 2015 63:416–424

Molecular Basis for Resistance to Fenoxaprop in Shortawn Foxtail (*Alopecurus aequalis*) from China

Wenlei Guo, Weitang Liu, Lingxu Li, Guohui Yuan, Long Du, and Jinxin Wang*

Shortawn foxtail is a competitive annual grass weed widely spread in east, south-central, and southwest China and parts of the Yellow River basin. One shortawn foxtail population (JSQT-1) resistant to fenoxaprop was identified in Jiangyan, Jiangsu province. Whole-plant experiments determined that the resistant population conferred high-level resistance to fenoxaprop (93-fold), clodinafop (21-fold), sethoxydim (107-fold), mesosulfuron (41-fold), and pyroxsulam (12-fold); moderate-level resistance to haloxyfop (8-fold), clethodim (9-fold), and pinoxaden (8-fold), and no resistance to isoproturon. Molecular analyses confirmed that the Ile-1781-Leu mutation was present in the resistant population. A dCAPS marker was used to detect the Ile-1781-Leu mutation. All 97 plants of the resistant population analyzed were homozygous mutants at the 1781 position. Our study established the first case of fenoxaprop resistance in shortawn foxtail, determined cross resistance to other herbicides, and elucidated that the molecular basis of resistance resulted from, at least partly, an Ile to Leu mutation at amino acid position 1781 in the plastid ACCase.

Nomenclature: Clethodim; clodinafop; fenoxaprop; haloxyfop; isoproturon; mesosulfuron; pinoxaden; pyroxsulam; sethoxydim; shortawn foxtail, *Alopecurus aequalis* Sobol.; wheat, *Triticum aestivum* L.

Key words: ACCase gene, ACCase resistance, cross resistance, dCAPS, mutation.

Shortawn foxtail is an annual grass weed widespread in Europe, temperate Asia, and North America (Cope 1982; Hashim et al. 2010). It is one of the most common and troublesome weeds in some regions of China (Zhang 2003), especially in wheat (*Triticum aestivum* L.) and oilseed rape (*Brassica napus* L.) fields with a rice rotation (Huang 2004; Wang and Qiang 2007). Shortawn foxtail has a strong tillering capacity that enhances its competitiveness against wheat seedlings, resulting in reduced tillers, decreased spike length, and reduced yield (Liu et al. 1992). Tang et al. (1990) reported 30.8% yield loss in wheat fields infested with shortawn foxtail at 530 to 1,060 plants m^{-2}. Zhu and Tu (1997) reported 24.2% and 51.9% wheat yield loss from shortawn foxtail interference at 540 to 675 and 1,197 to 1,560 plants m^{-2}, respectively. For the management of shortawn foxtail and other grass weeds, POST treatment of aryloxyphenoxypropionate (APP) herbicides has been used for several decades in China (Li et al. 2014; Zhang 2003). However, with repeated and extensive use, sensitivity declines year by year to these herbicides, and it is no surprise that resistant populations of shortawn foxtail to acetyl-coenzyme A carboxylase (ACCase) inhibiting herbicides have been reported in Jiangsu province of China (Huang 2004).

ACCase is a biotin-dependent enzyme that catalyzes the carboxylation of acetyl-CoA to produce malonyl-CoA, which is the first step in the biosynthesis of fatty acids (Délye 2005; Harwood 1988). In additional to serving as the substrate in fatty-acid synthesis, malonyl-CoA also plays an essential role in many reactions, such as the elongation of long-chain fatty acids and the synthesis of secondary metabolites (Harwood 1988). Plants have two different ACCase forms: one in the chloroplast and the other in the cytosol (Konishi et al. 1996). In all plants, the cytosolic ACCase is a homomeric eukaryotic multidomain protein, consisting of four subdomains—biotin carboxyl carrier protein (BCCP), biotin carboxylase (BC), and carboxyl transferase (CT) α and β. However, the chloroplast ACCase is homomeric eukaryotic in the Gramineae, and heteromeric prokaryotic in most other plants. The heteromeric ACCase is insensitive to ACCase-inhibiting herbicides, whereas the homomeric ACCase in the plastid of most grass weeds can be inhibited. Previous research has determined that the CT domain of the homomeric ACCase in

DOI: 10.1614/WS-D-14-00105.1

* First, second, fourth, fifth, and sixth authors: Graduate Student, Graduate Student, Graduate Student, Graduate Student, and Professor, Key Laboratory of Pesticide Toxicology and Application Technique, College of Plant Protection, Shandong Agricultural University, Shandong Tai'an 271018, People's Republic of China; third author: Lecturer, College of Chemistry and Pharmaceutical Science, Qingdao Agricultural University, Shandong Qingdao 266109, People's Republic of China. Corresponding author's E-mail: wangjx@sdau.edu.cn

chloroplast is the target site for ACCase-inhibiting herbicides (Zhang et al. 2003).

ACCase-inhibiting herbicides were introduced in 1978 and have been used repeatedly to control grass weeds (Powles and Yu 2010). Besides APPs, ACCase inhibitors also include the other two families: the cyclohexanediones (CHDs) and phenylpyrazoline (PPZ). However, 46 weed species have been reported to have resistance to ACCase inhibitors worldwide, including seven species in China (Heap 2014). According to previous studies, amino acid substitution in the CT domain of the plastid ACCase is one of the most common mechanisms that confer resistance to ACCase herbicides (Délye et al. 2003). To date, 13 kinds of substitution are documented, existing in seven positions of the CT domain, that result in various patterns of resistance: Ile-1781-Leu (Délye et al. 2002b; Zagnitko et al. 2001), Ile-1781-Val (Collavo et al. 2011), Ile-1781-Thr (Kaundun et al. 2013b), Trp-1999-Cys (Liu et al. 2007), Trp-1999-Leu (Scarabel et al. 2011), Trp-1999-Ser (Kaundun et al. 2013a), Trp-2027-Cys (Yu et al. 2007), Ile-2041-Asn (Cruz-Hipolito et al. 2011; Délye et al. 2003), Ile-2041-Val (Scarabel et al. 2011), Asp-2078-Gly (Kaundun 2010), Cys-2088-Arg (Yu et al. 2007), Gly-2096-Ser (Beckie et al. 2012; Cruz-Hipolito et al. 2012), and Gly-2096-Ala (Petit et al. 2010). In general, the cross resistance pattern endowed by one mutation mentioned above was related to the position in the ACCase gene, homozygosity/heterozygosity of the plants and the herbicide and dose applied for evaluation (Beckie and Tardif 2012; Powles and Yu 2010). It was also recognized that cross resistance was closely bound up with the specific mutations and weed species (Yu and Powles 2014). Therefore one cannot assume the level and spectrum of a target-site mutation in a new weed species that has never been studied.

In addition to target-site resistance (TSR) resulting from amino acid mutation in ACCase, non–target-site resistance (NTSR) is another mechanism conferring various herbicide resistances. Unlike TSR, NTSR can confer multiple resistances to herbicides with different modes of action (Délye et al. 2013; Powles and Yu 2010). NTSRs are usually governed by complex polygenes in grass weeds (Busi et al. 2011), which makes it more complicated than TSR (Délye 2013).

In recent years, farmers have noticed that fenoxaprop failed to control shortawn foxtail at recommended rate in wheat fields of Jiangsu and Anhui province, China. Resistant shortawn foxtail has been threatening wheat production in the above areas. So the objectives of this article were (1) to determine the susceptibility to fenoxaprop of shortawn foxtail populations, (2) to identify the molecular basis of resistance to fenoxaprop in the resistant shortawn foxtail population, and (3) to study the susceptibility of the resistant population to other herbicides with varied modes of action.

Materials and Methods

Plant Material. Seed samples of shortawn foxtail were collected from Jiangsu province of China in June 2013. The suspected resistant (R) population (JSQT-1) was collected from a wheat field (32.67°N, 120.07°E) where fenoxaprop had been used annually at least 8 yr. The susceptible (S) control, JSSY-1 population was collected from a park (34.11°N, 118.76°E) that had never been treated with ACCase-inhibiting herbicides. Seeds were air-dried and stored in paper bags at 4 C until use.

Whole-Plant Dose–Response Experiments. Prior to planting, seeds of both populations were soaked in 0.08% gibberellin solution at 4 C for 5 d and then were rinsed thoroughly with distilled water before being transferred to petri dishes. Eight milliliters of distilled water was added to each petri dish, which contained two layers of Whatman No. 1 filter paper, and then the dishes were incubated in an artificial chamber (20/15 C, 12/12 h day/night). After 7 d 10 germinated seeds were then sown in a 12-cm diameter plastic pot containing loam soils. Pots were placed in a controlled greenhouse (25/15 C, 14/10 h day/night, 75% relative humidity). The pots were watered every other day (80 to 100 ml water per pot) and fertilized with 0.5 g mixed fertilizer at 18 d after sowing.

When plants had reached the three- to four-leaf stage, the herbicides were sprayed with the use of a moving nozzle cabinet sprayer equipped with one TeeJet 9503EVS flat-fan nozzle calibrated to deliver 450 L ha^{-1} at 280 KPa. A total of six ACCase inhibitors, two acetolactate synthase (ALS) inhibitors, and one photosystem II inhibitor were used in this study. The herbicides and the rates applied to each population are listed in Table 1.

The aboveground parts of the plant were cut at 21 d after treatment (DAT) and oven dried for 72 h at 80 C. Subsequently, the dry-weight data were recorded. The experiment was conducted two times and each treatment had three replications.

https://doi.org/10.1614/WS-D-14-00105.1 Published online by Cambridge University Press

Table 1. Herbicides and doses applied to the resistant (JSQT-1) population and the susceptible (JSSY-1) population of *Alopecurus aequalis* in dose–response experiments.

Herbicide[a]	Application rate[b]	
	JSQT-1 (R[c])	JSSY-1 (S)
	g ai ha^{-1}	
ACCase inhibitors		
Fenoxaprop	**62**, 186, 559, 1,677, 5,030 15,090	0.8, 2.3, 6.9, 20.7, **62**, 186
Clodinafop	22.5, **45**, 90, 180, 360, 720	1.4, 2.8, 5.6, 11.2, 22.5, **45**
Haloxyfop	8, 16, **32**, 64, 129, 259, 518	0.5, 1, 2, 4, 8, 16, **32**
Clethodim	3.4, 6.7, 13.5, 27, 54, **108**	0.8, 1.7, 3.4, 6.8, 13.5, 27, 54
Sethoxydim	93.7, **187.5**, 375, 750, 1,500, 3,000	5.9, 11.7, 23.4, 46.8, 93.7, **187.5**
Pinoxaden	5.6, 11.2, 22.5, **45**, 90, 180, 360	0.7, 1.4, 2.8, 5.6, 11.2, 22.5, **45**
ALS inhibitors		
Mesosulfuron	1.1, 2.2, 4.5, **9**, 18, 36, 72	0.02, 0.07, 0.3, 1.1, 4.5, 18
Pyroxsulam	0.6, 1.3, 2.6, 5.3, **10.6**, 21.1, 42.2	0.04, 0.13, 0.4, 1.2, 3.5, **10.6**
Photosystem II inhibitor		
Isoproturon	14, 28, 56, 112, 225, 450, **900**	14, 28, 56, 112, 225, 450

[a] Fenoxaprop (69 g L^{-1} EW, Bayer, Hangzhou, China), clodinafop (15% WP, Syngenta, Shanghai, China), haloxyfop (108 g L^{-1} EC, Dow AgroSciences, Beijing, China), clethodim (240 g L^{-1} EC, Arysta LifeScience, Shanghai, China), sethoxydim (12.5% EC, Soda, Tianjin, China), Pinoxaden (5% EC, Syngenta, Shanghai, China), mesosulfuron (30 g L^{-1} OF, Bayer, Hangzhou, China), pyroxsulam (7.5% WG, Dow AgroSciences, Beijing, China), isoproturon (50% WP, Bianjing, Suzhou, China).
[b] The recommended field rates are shown in bold.
[c] Abbreviations: R, resistant; S, susceptible.

Molecular Basis of Resistance. *DNA Extraction.* Leaves of an individual shortawn foxtail plant at three- to four-leaf stage were harvested, wrapped in plastic bags, and stored at −80 C until use. Genomic DNA was extracted from 100 mg of young shoot tissue of each plant using a modified CTAB (cetyltrimethylammonium bromide) method (Doyle and Doyle 1987).

ACCase Gene Sequencing. Based on the ACCase gene sequence of black-grass (*Alopecurus myosuroids* Huds., GenBank accession AJ310767) and Japanese foxtail (*Alopecurus japonicas* Steud, GenBank accession AQ068820), one pair of primers (AC-F, AC-R, Table 2) was designed to amplify a 1,327-bp fragment of ACCase gene of shortawn foxtail. This fragment encompassed all the mutation sites that had been proved in the CT domain of ACCase. Each polymerase chain reaction (PCR) was performed in a final volume of 25 µl containing 1 µl of gDNA (about 25 ng μl^{-1}), 1 µl of each primer (10 µM), 2.5 µl of 10× EasyTaq Buffer (TransGen Biotech, China), 2 µl of dNTPs (2.5 mM, TransGen Biotech, China), 0.25 µl of EasyTaq DNA Polymerase (5 U μl^{-1}, TransGen Biotech, China), with double-distilled H_2O added to the final volume of 25 µl. PCR reactions were run on a T100 Thermal cycler (Bio-Rad, Hercules, CA) subjected to the following program: 5 min denaturation at 94 C, 34 cycles of 30 s at 94 C, 40 s at 56 C, 90 s at 72 C, and a final step of 72 C 10 min. PCR products were visualized by electrophoresis on 1.0% agarose gel run in 1× TAE buffer. The intended bands were purified with the use of the TIANgel Midi Purification Kit (Tiangen Biotech, China) and the purified products were cloned with pEASY-T1 vector (TransGen Biotech, China). The recombinant plasmids were introduced into

Table 2. Primers used for sequencing and dCAPS[a] of *Alopecurus aequalis* in this study.

Primers	Sequence (5′-3′)[b]	Annealing temperature	Usage	Product size
AC-F	AGTAAAATCTTGCTTCCGTGT	56 C	CT domain sequencing	1,327 bp
AC-R	CAACTGTTTCTTCCGAGCTTC			
d1781F3	GGACTGATGATAGCAGCCCTGAACG	57.4 C	dCAPS for 1781 mutation	209 bp
d1781R2	AGAATAGGCACTGGCAATAGCAGCACTTCCATGCA			

[a] Abbreviation: dCAPS, derived cleaved amplified polymorphic sequence.
[b] Nucleotide underlined is modified from ACCase sequence to create a recognition site T^ACGTA for restriction enzyme *Eco*T22 I.

https://doi.org/10.1614/WS-D-14-00105.1 Published online by Cambridge University Press

competent *Escherichia coli* (Trans1-T1 Phage Resistant Chemically Competent Cell, TransGen Biotech) according to the manufacturer's instructions. Positive clones were sequenced in both forward and reverse directions on ABI PRISM 3730 DNA sequencer by a commercial sequencing company (Sangon Biotech, China). Eight plants per population were tested for mutation detection and five clones of each plant were sequenced. Sequence data were aligned and compared with the use of DNAMAN version 5.2.2 software (Lynnon Biosoft, Quebec, Canada).

Development of 1781 dCAPS Assay for Shortawn Foxtail. A derived cleaved amplified polymorphic sequence (dCAPS) assay was designed for genotyping shortawn foxtail plants at first base of the 1781 ACCase codon position. The dCAPS assay includes three steps: PCR amplification, restriction digestion, and gel electrophoresis. According to the sequence data, a reverse primer d1781R2 and an enzyme *Eco*T22 I were selected using the dCAPS Finder 2.0 software (Neff et al. 2002) by reference to Kaundun and Windass (2006). Subsequently, a forward primer d1781F3 was designed, and the primers are given in Table 2. A mismatch (T to C) was introduced at the third base of codon 1781 in the reverse primer to create a restriction site T^ACGTA for the enzyme *Eco*T22 I, and any mutation at the first base of codon 1781 would disrupt the restriction site. The PCR amplification system, except the primers, was the same as described above. The PCR program was 5 min denaturation at 94 C, 38 cycles of 30 s at 94 C, 30 s at 57.4 C, 20 s at 72 C, and a final step of 72 C for 10 min. A quantity of 5 µl of the PCR products were digested with 1 µl (10 units) of the *Eco*T22 I (TaKaRa Biotechnology, Dalian, China) according to the manufacturer's recommendations. The digestion products were analyzed on a 2.5% agarose gel in 1× TAE buffer.

Statistical Analysis. Data from the two repeats of dose–response experiment were analyzed by ANOVA (Version 17.0, SPSS Inc.), and data were combined as there were no differences between the two experiments. Combined data were used for nonlinear regression analysis fitted to a four- parameter log-logistic curve, Equation 1 (Seefeldt et al. 1995):

$$y = c + (d - c)/\{1 + \exp[b(\log x - \log \mathrm{ED}_{50})]\}, \quad [1]$$

where c is the lower limit, d is the upper limit, and b is the relative slope around the herbicide dose resulting in 50% growth inhibition (ED_{50}). In the regression equation, the herbicide dose was the independent variable (x), and the growth response (percentage of the untreated control) was the dependent variable (y). This equation was used to estimate the dose causing a 50% dry- weight growth reduction (GR_{50}). All regression analyses were conducted with the use of SigmaPlot (Version 12.3; SigmaPlot Software Inc.).

The resistance ratio, which is calculated by the ratio of GR_{50} of the resistant population to the GR_{50} of the susceptible population, was chosen as an index to assess the level of resistance to herbicides used in this research.

Results and Discussion

ACCase Gene Sequencing. A 1,327-bp DNA fragment was obtained and cloned from the R population and the S population, respectively. The sequence alignment results indicated that both populations had 97.66% homologies with the same region of the documented ACCase gene sequence of blackgrass (GenBank accession AJ310767) and Japanese foxtail (GenBank accession JQ068820). A total of 31 single-nucleotide polymorphisms (SNPs) were detected in the amplified fragments of ACCase gene, among which 25 SNPs were synonymous and six resulted in different amino acid substitutions. The six nonsynonymous SNPs, located at 5356, 5368, 5395, 5497, 5633, and 6499 sites, resulted in Arg-1734-Gly, Met-1738-Leu, Gly-1747-Ser, Ile-1781-Leu, Ile-1826-Asn, and Lys-2115-Gln amino acid substitutions, respectively. Most of them were evenly distributed in plants of both populations (5356, 5368, 5395, 5633, and 6499); thus they are random variations not related to herbicide resistance. The transformation from adenine to thymine at position 5497 was only identified in plants from the R population (Figure 1), which resulted in an isoleucine to leucine substitution. The alignment results also proved that no other mutations, which had been documented to confer resistance to ACCase herbicides, were found in the R population. Given that the Ile-1781-Leu mutation has been reported to confer resistance to fenoxaprop in many other grass weeds (Délye 2005; Liu et al. 2007; Zhang and Powles 2006), this mutation in JSQT-1 (R) population is very likely the factor that results in fenoxaprop resistance. All the nucleotide and amino acid sequence positions described above follow the full-length sequence of blackgrass plastid ACCase (GenBank accession AJ310767).

https://doi.org/10.1614/WS-D-14-00105.1 Published online by Cambridge University Press

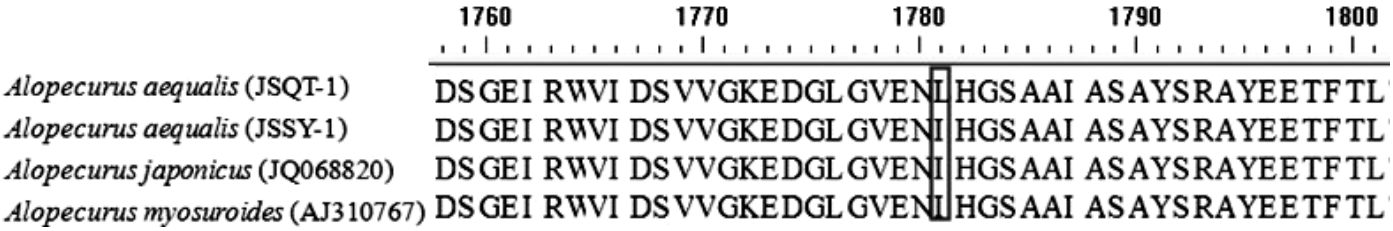

Figure 1. Alignment of partial amino acid sequences of plastid ACCase from resistant (JSQT-1) and susceptible (JSSY-1) *Alopecurus aequalis* population, *Alopecurus japonicus* (GenBank accession JQ068820), and *Alopecurus myosuroides* (GenBank accession AJ310767). The boxed codon indicates the substitution found in codon 1781 of the resistant (JSQT-1) population.

Whole-Plant Dose–Response Experiments. *Fenoxaprop Resistance in Shortawn Foxtail.* Dose–response experiments confirmed that the putative resistant population JSQT-1 (R) was highly resistant to fenoxaprop, whereas the JSSY-1 (S) population was sensitive, with a resistance index of 93 (Table 3). At the recommended field rate (41.4 to 62.1 g ai ha^{-1}), all plants of JSQT-1(R) population survived, which was consistent with the failed control of shortawn foxtail in the wheat field of Jiangyan country, and the plants of the S population were severely injured (data not shown).

A number of weed species have been reported to show resistance to fenoxaprop worldwide since its commercialization in the 1990s (Délye et al. 2002a). In 1998, fenoxaprop (containing mefenpyr-diethyl) was introduced to control annual grass weeds in wheat in China. Tardif and Powles (1994) put forward that resistance to ACCase-inhibiting herbicides may appear after a 3-yr period of selection. In this study, the resistant population JSQT-1 failed to be controlled by fenoxaprop after more than 5 yr of repeated use. These results prove once again that one should rotate with herbicides that have a different site of action as well as other management strategies to control shortawn foxtail in order to avoid or delay the onset of resistance.

Cross-Resistance to other ACCase-Inhibiting Herbicides. Two APP herbicides (clodinafop, haloxyfop),

Table 3. Parameters of the four-parameter log-logistic equation[a] used to calculate the GR_{50}[b] values of the susceptible (JSSY-1) and resistant (JSQT-1) populations of *Alopecurus aequalis* with the use of the whole-plant dose–response experiments. Standard errors (SE) are in parentheses.

		Regression parameters				
Herbicide	Populations	*c*	*d*	*b*	GR_{50}	RI[c]
					g ai ha^{-1}	
Fenoxaprop	JSQT-1(R)	12.9 (2.5)	87.2 (2.1)	−1.0 (0.1)	1,139.6 (100.5)	93
	JSSY-1(S)	11.8 (3.7)	77.7 (3.7)	−1.1 (0.2)	12.2 (1.9)	
Clodinafop	JSQT-1(R)	12.6 (16.90)	101.6 (10.6)	−1.5 (0.7)	170.2 (48.9)	21
	JSSY-1(S)	17.3 (2.0)	84.9 (2.1)	−4.1 (0.6)	8.2 (0.4)	
Haloxyfop	JSQT-1(R)	24.3 (4.9)	91.1 (7.8)	−2.2 (0.9)	32.8 (5.8)	8
	JSSY-1(S)	21.2 (28.0)	87.0 (16.7)	−0.9 (0.8)	4.1 (2.6)	
Clethodim	JSQT-1(R)	13.2 (7.2)	79.4 (8.7)	−1.2 (0.5)	38.6 (10.5)	9
	JSSY-1(S)	18.5 (4.4)	83.5 (7.6)	−1.5 (0.5)	4.4 (0.9)	
Sethoxydim	JSQT-1(R)	13.9 (8.2)	102.7 (7.4)	−1.6 (0.5)	566.8 (89.7)	107
	JSSY-1(S)	16.1 (3.2)	108.1 (11.0)	−1.8 (0.5)	5.3 (1.0)	
Pinoxaden	JSQT-1(R)	8.3 (5.6)	101.3 (8.3)	−1.2 (0.3)	34.0 (3.5)	8
	JSSY-1(S)	18.4 (1.6)	91.2 (2.3)	−1.6 (0.2)	4.2 (0.3)	
Mesosulfuron	JSQT-1(R)	11.8 (1.5)	78.8 (1.7)	−2.0 (0.2)	8.2 (0.4)	41
	JSSY-1(S)	20.5 (3.8)	99.8 (6.8)	−1.5 (0.5)	0.2 (0.05)	
Pyroxsulam	JSQT-1(R)	32.3 (3.2)	95.8 (6.4)	−1.8 (0.5)	2.6 (0.5)	12
	JSSY-1(S)	17.9 (0.2)	92.0 (0.5)	−1.9 (0.04)	0.2 (0.003)	
Isoproturon	JSQT-1(R)	9.5 (2.1)	80.6 (2.1)	−2.4 (0.3)	81.8 (4.5)	2
	JSSY-1(S)	2.7 (1.3)	128.6 (4.6)	−1.5 (0.1)	37.6 (2.0)	

[a] $y = c + (d - c)/\{1 + \exp[b(\log x - \log ED_{50})]\}$, where y is the growth response (percentage of the control), c and d are lower and upper asymptotic limits, b is the slope around the herbicide dose resulting in 50% growth inhibition, x is the concentration of herbicides.

[b] Abbreviations: GR_{50}, dose required to reduce seedling dry weight by 50%; R, resistant; S, susceptible; RI, resistance index.

[c] RI = GR_{50} (resistant)/GR_{50} (susceptible).

https://doi.org/10.1614/WS-D-14-00105.1 Published online by Cambridge University Press

two CHD herbicides (clethodim, sethoxydim), and one PPZ herbicide (pinoxaden) were used in this study. For the sensitive population JSSY-1, the GR50 value of each ACCase-inhibiting herbicide (Table 3) was much lower than the recommended field rate (Table 1), which further proved the susceptibility of this population. For the resistant population JSQT-1, responses to the above herbicides were varied. JSQT-1 population was highly resistant to clodinafop and sethoxydim with resistance ratio of 21 and 107, respectively (Table 3). Compared with the S population, the R population was moderately resistant to haloxyfop, clethodim and pinoxaden (Table 3).

Generally, Ile-1781-Leu mutation can confer high-level resistance to all three classes of ACCase-inhibiting herbicides: APP, CHD, and PPZ (Beckie and Tardif 2012). Liu et al (2007) demonstrated that the Ile-1781-Leu mutation could confer strong resistance to APP and CHD herbicides by using yeast gene-replacement strains. In our study, the R population was highly resistant to clodinafop (Table 3), which indicated to us that clodinafop, introduced into China in 2009, cannot control shortawn foxtail with the Ile-1781-Leu mutation in the ACCase gene. Though haloxyfop was used in the dicot crop fields, it failed to control the R population of shortawn foxtail at the recommended field rate (Table 3). In our study, the R population was more resistant to sethoxydim than clethodim (Table 3). This was consistent with former studies where several weed species displayed a high level of resistance to sethoxydim and a low level of resistance to other CHD herbicides (Heap et al. 1993; Shukla et al. 1997; Volenberg and Stoltenberg 2002). Shukla et al. (1997) reported that green foxtail (*Setaria viridis* L.) from Canada was 423- and 2-fold resistant to sethoxydim and clethodim, respectively. Clethodim was used in the broadleaf but not cereal crops, and was considered the lowest-risk ACCase inhibitor for selecting for target-site resistance (Beckie and Tardif 2012). Jang et al. (2013) proved that the Ile-1781-Leu mutation can confer resistance to pinoxaden in yeast gene-replacement strains. Pinoxaden resistance was observed in black-grass before the introduction of pinoxaden to France (Petit et al. 2010). As a newly commercialized herbicide in China, pinoxaden resistance has been reported in American sloughgrass (*Beckmannia syzigachne* Steud.) in China (Li et al. 2014). In this study, pinoxaden had never been applied to the JSQT-1 (R) population.

Cross-Resistance to ALS and Photosystem II Inhibitors. In recent years ALS inhibitors, such as mesosulfuron and pyroxsulam, were used to control ACCase-inhibitor–resistant grass weeds in wheat fields in China (Li et al. 2014). In this study, susceptibility to the above two ALS herbicides of the R population was apparently lower than that of the S population, with the RI as high as 41 and 12, respectively (Table 3). The plants of the R population survived at 21 DAT of mesosulfuron and pyroxsulam at the rate of 9 g ai ha^{-1} and 10.6 g ai ha^{-1}, respectively (data not shown). Similar to ACCase resistance, ALS-inhibiting herbicides are prone to be selected for resistance in weeds (Yu and Powles 2014). To date, populations of 145 weed species have been confirmed to be resistant to ALS inhibitors (Heap 2014). A 7-yr period of research determined that the control efficacy of sulfonylureas decreased significantly after the fourth treatment in a wheat field from Italy infested with rigis ryegrass (*Lolium rigidum* Gaudin) resistant to ACCase-inhibitors (Collavo et al. 2013). Our unpublished data proved that a shortawn foxtail population, collected from a wheat field, had produced a number of progeny possessing both the ACCase and ALS target-site mutation in a single plant. Hence, to manage the ACCase-resistant weed populations, the single use of ALS inhibitors is not a long-term strategy.

Whole-plant experiments proved that isoproturon, a urea herbicide, could effectively control shortawn foxtail from both the R and S populations (Table 3). It was likely that intrinsic differences between R and S populations resulted in different susceptibilities to isoproturon.

Analysis of the dCAPS Assay. A dCAPS assay was designed to detect the Ile-1781-Leu mutation rapidly. With the use of this dCAPS marker, the plant with wild type 1781-Ile alleles showed one 174-bp digested band on the agarose gel; the homozygous mutant 1781-Leu plant showed one 209-bp undigested band and the heterozygous plant showed both bands (Figure 2). Ninety-seven and 25 plants, from the JSQT-1 (R) and JSSY-1 (S) populations, respectively, were analyzed in this study. As expected, all plants from the S population showed a digested band (174 bp, and a 35-bp band invisible on the agarose gel). It was noteworthy that all 97 plants of the R population were undigested and displayed a 209-bp fragment on the gel (Figure 2). It reflected the fact that a mutation did exist at the first base of codon 1781. To examine the accuracy of this assay, 15 plants from each population were selected randomly and

https://doi.org/10.1614/WS-D-14-00105.1 Published online by Cambridge University Press

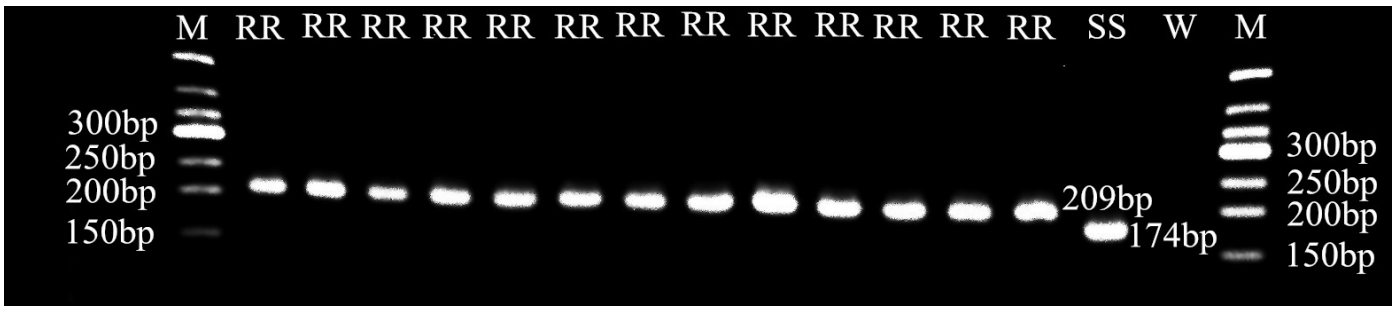

Figure 2. Derived cleaved amplified polymorphic sequence (dCAPS) of individual *Alopecurus aequalis* plants. RR, homozygous mutant 1781-Leu plants from the resistant (JSQT-1) population; SS, wild-type 1781-Ile plants from the susceptible (JSSY-1) population; M, DNA ladder; W, H_2O polymerase chain reaction control (no DNA).

sequenced. The sequencing data proved that all the tested plants from the R and the S population possessed leucine and isoleucine residues at codon 1781, respectively, which was consistent with results of the dCAPS assay.

The accuracy and robustness of this dCAPS assay was proved by the exact matches of the results obtained and the sequencing results. The dCAPS assay used in this research was not a mutation-specific assay in view of several reasons. First, three nucleotide substitutions have been identified at the first base of codon 1781 (Kaundun and Windass 2006; Kaundun et al. 2013b), adenine (A) to thymine (T) or cytosine (C) or guanine (G), and therefore at least three mutation-specific assays are required. Secondly, both A to C and A to T substitution would result in a leucine amino acid substitution, and A to G substitution would result in a valine amino acid substitution. So far, Ile-1781-Val substitution was only identified in a hood canarygrass (*Phalaris paradoxa* L.) population from Italy (Collavo et al. 2011). Specific digestion of wild type fragments implies that nondigestion of fragment is very likely to result from Ile-1781-Leu substitution. Thus, this dCAPS assay together with gene sequencing can help discriminate amino acid substitutions at codon 1781 of ACCase in shortawn foxtail.

Shortawn foxtail is a partly cross-pollinated (40%) weed species (Morishma and Oka 1980). When the mutate allele generated, it was easily spread to the other plants from the resistant plants via pollen. In China, most farmers applied one herbicide repeatedly as the single method for weed control until resistance evolution. Additionally, Chinese farmers were accustomed to increasing herbicide dose arbitrarily to ensure control efficacy. Given these factors, it is no surprise that JSQT-1 population has high level resistance to fenoxaprop and all the tested plants contained homozygous mutant 1781-Leu alleles.

To avoid and manage ACCase-resistant shortawn foxtail, we should adopt an integrated weed management system rather than rely solely on one kind of herbicides. Herbicide rotation or mixture with different action sites is an effective strategy in delaying resistance evolution (Cavan et al. 2000; Beckie and Reboud 2009). In this study, isoproturon is still an alternative herbicide for ACCase resistant shortawn foxtail, though it has been used since 1980s. Crop rotation with dicot crops, such as oilseed rape, faba been (*Vicia faba* L.), or other winter crops, is an effective cropping system for reducing grass weed in some temperate regions of China (Zhang 2003). Many other herbicides with different kinds of action site can be selected to control grass weeds in the dicot crop fields. In the regions with a rice–wheat rotation system, no-tillage cultural technique is usually taken before wheat sowing, which creates a suitable condition for seedling emergence of shortawn foxtail. Huang (2004) reported that shortawn foxtail seedlings were prone to emerge with burial depth of 0 to 3 cm and no seedlings emerged when burial depth was 7 cm or more. Hence, deep plowing in autumn may reduce emergence rate of shortawn foxtail by sinking the seeds 30 cm into the soil.

Target-site resistance and NTSR may occur in a weed population simultaneously (Délye et al. 2013; Kaundun et al. 2013a). In this study, the effect of an NTSR mechanism was not examined and thus we could not exclude the possibility of NTSR. Given that many weed species have NTSR, it is reasonable to deduce NTSR in shortawn foxtail. Nevertheless, this research primarily elucidated that the Ile-1781-Leu mutation was the molecular basis for fenoxaprop resistance in JSQT-1 (R) population of shortawn foxtail. This is the first report of the

https://doi.org/10.1614/WS-D-14-00105.1 Published online by Cambridge University Press

molecular basis and associated cross-resistance from an ACCase resistant shortawn foxtail population. The dCAPS marker developed in this research can detect mutations at codon 1781 of ACCase effectively. Our research will be useful in understanding resistance to ACCase-inhibiting herbicides in grasses.

Acknowledgments

The authors sincerely thank all the workers for assistance in conducting this research. We are also grateful to the farmers for the help in collecting the shortawn foxtail seeds. This work was supported by the National Natural Science Foundation of China (31171866, 31201529), and the Special Fund for Agroscientific Research in the Public Interest (201303031).

Literature Cited

Beckie HJ, Reboud X (2009) Selecting for weed resistance: herbicide rotation and mixture. Weed Technol 23:363–370

Beckie HJ, Tardif FJ (2012) Herbicide cross resistance in weeds. Crop Prot 35:15–28

Beckie HJ, Warwick SI, Sauder CA (2012) Basis for herbicide resistance in Canadian populations of wild oat (*Avena fatua*). Weed Sci 60:10–18

Busi R, Vila-Aiub MM, Powles SB (2011) Genetic control of a cytochrome P450 metabolism-based herbicide resistance mechanism in *Lolium rigidum*. Heredity 106:817–824

Cavan G, Cussans J, Moss SR (2000) Modelling different cultivation and herbicide strategies for their effect on herbicide resistance in *Alopecurus myosuroides*. Weed Res 40:561–568

Collavo A, Panozzo S, Lucchesi G, Scarabel L, Sattin M (2011) Characterisation and management of *Phalaris paradoxa* resistant to ACCase-inhibitors. Crop Prot 30:293–299

Collavo A, Strek H, Beffa R, Sattin M (2013) Management of an ACCase-inhibitor-resistant *Lolium rigidum* population based on the use of ALS inhibitors: weed population evolution observed over a 7 year field-scale investigation. Pest Manag Sci 69:200–208

Cope TA (1982) Poaceae. Page 678 *in* Nasir E, Ali SI, eds. Flora of Pakistan. Fascicle No. 143. Islamabad: National Herbarium, and Karachi, Pakistan: Botany Department, University of Karachi

Cruz-Hipolito H, Dominguez-Valenzuela JA, Osuna MD, de Prado R (2012) Resistance mechanism to acetyl coenzyme A carboxylase inhibiting herbicides in *Phalaris paradoxa* collected in Mexican wheat fields. Plant Soil 355:121–130

Cruz-Hipolito H, Osuna MD, Dominguez-Valenzuela JA, Espinoza N, de Prado R (2011) Mechanism of resistance to ACCase-inhibiting herbicides in wild oat (*Avena fatua*) from Latin America. J Agric Food Chem 59:7261–7267

Délye C (2005) Weed resistance to acetyl coenzyme A carboxylase inhibitors: an update. Weed Sci 53:728–746

Délye C (2013) Unravelling the genetic bases of non-target-site-based resistance (NTSR) to herbicides: a major challenge for weed science in the forthcoming decade. Pest Manag Sci 69:176–187

Délye C, Jasieniuk M, Corre VL (2013) Deciphering the evolution of herbicide resistance in weeds. Trends Genet 29: 649–658

Délye C, Matéjicek A, Gasquez J (2002a) PCR-based detection of resistance to acetyl-CoA carboxylase-inhibiting herbicides in black-grass (*Alopecurus myosuroides* Huds) and ryegrass (*Lolium rigidum* Gaud). Pest Manag Sci 58:474–478

Délye C, Wang T, Darmency H (2002b) An isoleucine–leucine substitution in chloroplastic acetyl-Co A carboxylase from green foxtail (*Setaria viridis* L. Beauv.) is responsible for resistance to the cyclohexanedione herbicide sethoxydim. Planta 214:421–427

Délye C, Zhang XQ, Chalopin C, Michel S, Powles SB (2003) An isoleucine residue within the carboxyl-transferase domain of multidomain acetyl-coenzyme A carboxylase is a major determinant of sensitivity to aryloxyphenoxypropionate but not to cyclohexanedione inhibitors. Plant Physiol 132: 1716–1723

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

Harwood JL (1988) Fatty acid metabolism. Annu Rev Plant Physiol 39:101–138

Hashim S, Hachinohe M, Matsumoto H (2010) Cloning and expression analysis of alpha-tubulin genes in shortawn foxtail (*Alopecurus aequalis*). Weed Sci 58:89–95

Heap IM (2014) International Survey of Herbicide Resistant Weeds. http://weedscience.org/Summary/MOA.aspx. Accessed July 14, 2014

Heap IM, Murray BG, Loeppky HA, Morrision IN (1993) Resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides in wild oat (*Avena fatua*). Weed Sci 41:232–238

Huang SX (2004) Studies on biology and resistance of *Alopecurus aequalis* Sobol. to acetyl-coenzyme A carboxylase inhibitors. M.A. thesis. Nanjing, China: Nanjing Agric Univ. 74 p [In Chinese]

Jang SR, Marjanovic J, Gornicki P (2013) Resistance to herbicides caused by single amino acid mutations in acetyl-CoA carboxylase in resistant populations of grassy weeds. New Phytol 197:1110–1116

Kaundun SS (2010) An aspartate to glycine change in the carboxyl transferase domain of acetyl CoA carboxylase and non-target-site mechanism(s) confer resistance to ACCase inhibitor herbicides in a *Lolium multiflorum* population. Pest Manag Sci 66:1249–1256

Kaundun SS, Bailly GC, Dale RP, Hutchings SJ, McIndoe E (2013a) A novel W1999S mutation and non-target site resistance impact on Acetyl-CoA carboxylase inhibiting herbicides to varying degrees in a UK *Lolium multiflorum* population. PLoS One 8:e58012

Kaundun SS, Hutchings SJ, Dale RP, Mclndoe E (2013b) Role of a novel I1781T mutation and other mechanisms in conferring resistance to Acetyl-CoA carboxylase inhibiting herbicides in a black-grass population. PLoS One 8:e69568

Kaundun SS, Windass JD (2006) Derived cleaved amplified polymorphic sequence, a simple method to detect a key point mutation conferring acetyl CoA carboxylase inhibitor herbicide resistance in grass weeds. Weed Res 46:34–39

Konishi T, Shinohara K, Yamada K, Sasaki Y (1996) Acetyl-CoA carboxylase in higher plants: most plants other than Gramineae have both the prokaryotic and the eukaryotic forms of this enzyme. Plant Cell Physiol 37:117–122

Li LX, Du L, Liu WT, Yuan GH, Wang JX (2014) Target-site mechanism of ACCase-inhibitors resistance in American slough-grass (*Beckmannia syzigachne Steud.*) from China. Pestic Biochem Physiol 110:57–62

Liu DJ, Wang B, Jiang YP (1992) Effects of *Alopecurus aequalis*, *Malachium aquaticum* and rain days on production of wheat of Shanghai. Acta Agric Shanghai 4:73–76 [In Chinese]

Liu W, Harrison DK, Chalupska D, Gornicki P, O'Donnell CC, Adkins SW, Haselkorn R, Williams RR (2007) Single-site mutations in the carboxyltransferase domain of plastid acetyl-CoA carboxylase confer resistance to grass-specific herbicides. Proc Natl Acad Sci USA 104:3627–3632

Morishma H, Oka HI (1980) The impact of copper pollution on water foxtail (*Alopecurus aequalis* Sobol.) populations and winter weed communities in rice fields. Agro-Ecosystem 6:33–49

Neff MM, Turk E, Kalishman M (2002) Web-based primer design for single nucleotide polymorphism analysis. Trends Genet 18:613–615

Petit C, Bay G, Pernin F, Délye C (2010) Prevalence of cross- or multiple resistance to the acetyl-coenzyme A carboxylase inhibitors fenoxaprop, clodinafop and pinoxaden in blackgrass (*Alopecurus myosuroides* Huds.) in France. Pest Manag Sci 66:168–177

Powles SB, Yu Q (2010) Evolution in action: plants resistant to herbicides. Annu Rev Plant Biol 61:317–347

Scarabel L, Panozzo S, Varotto S, Sattin M (2011) Allelic variation of the ACCase gene and response to ACCase-inhibiting herbicides in pinoxaden-resistant *Lolium* spp. Pest Manag Sci 67:932–941

Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-logistic analysis of herbicide dose–response relationships. Weed Technol 9: 218–227

Shukla A, Leach GE, Devine MD (1997) High-level resistance to sethoxydim conferred by an alteration in the target enzyme, acetyl-CoA carboxylase, in *Setaria faberi* and *Setaria viridis*. Plant Physiol Biochem 35:803–807

Tang HY, Wang XE, Shen GH (1990) Study on the yield loss of wheat and barley caused by the infestation of *Alopecurus japonicus* and *A. aequalis*. J Weed Sci 1:8–12 [In Chinese]

Tardif FJ, Powles SB (1994) Herbicide multiple-resistance in a *Lolium rigidum* biotype is endowed by multiple mechanisms: isolation of a subset with resistant acetyl-CoA carboxylase. Physiol Plant 91:488–494

Volenberg D, Stoltenberg D (2002) Altered acetyl-coenzyme A carboxylase confers resistance to clethodim, fluazifop and sethoxydim in *Setaria faberi* and *Digitaria sanguinalis.* Weed Res 42:342–350

Wang KJ, Qiang S (2007) Quantitative analysis of weed communities in wheat fields in Jiangsu. Acta Pratacul Sin 1:118–126 [In Chinese]

Yu Q, Collavo A, Zheng MQ, Owen M, Sattin M, Powles SB (2007) Diversity of acetyl-coenzyme A carboxylase mutations in resistant *Lolium* populations: evaluation using clethodim. Plant Physiol 145:547–558

Yu Q, Powles SB (2014) Resistance to AHAS inhibitor herbicides: current understanding. Pest Manag Sci 70:1340–1350

Zagnitko O, Jelenska J, Tevzadze G, Haselkorn R, Gornicki P (2001) An isoleucine/leucine residue in the carboxyltransferase domain of acetyl-CoA carboxylase is critical for interaction with aryloxyphenoxypropionate and cyclohexanedione inhibitors. Proc Natl Acad Sci USA 98:6617–6622

Zhang HL, Yang ZR, Shen Y, Tong L (2003) Crystal structure of the carboxyltransferase domain of acetyl-coenzyme A carboxylase. Science 299:2063–2067

Zhang XQ, Powles SB (2006) Six amino acid substitutions in the carboxyl-transferase domain of the plastidic acetyl-CoA carboxylase gene are linked with resistance to herbicides in a *Lolium rigidum* population. New Phytol 172:636–645

Zhang ZP (2003) Development of chemical weed control and integrated weed management in China. Weed Biol Manag 3:197–203

Zhu WD, Tu SX (1997) Study on damage from *Alopecurus aequalis* Sobol and its economical threshold in wheat fields of Hubei province. J Huazhong Agric Univ 3:268–271 [In Chinese]

Received July 25, 2014, and approved December 23, 2014.