Cross-resistance to acetolactate synthase (ALS) inhibitors associated with different mutations in Japanese foxtail (*Alopecurus japonicus*)

Ning Zhao[1,2], Yaling Bi[3], Cuixia Wu[4], Dandan Wang[1,2], Ludan You[1,2], Weitang Liu[5,6] and Jinxin Wang[7,8]

[1]Graduate Student, College of Plant Protection, Shandong Agricultural University, Tai'an, China; [2]Graduate Student, Key Laboratory of Pesticide Toxicology and Application Technology, Shandong Agricultural University, Tai'an, China; [3]Associate Professor, College of Agronomy, Anhui Science and Technology University, Fengyang, China; [4]Research Fellow, Tai'an Academy of Agricultural Sciences, Tai'an, China; [5]Associate Professor, College of Plant Protection, Shandong Agricultural University, Tai'an, China; [6]Associate Professor, Key Laboratory of Pesticide Toxicology and Application Technology, Shandong Agricultural University, Tai'an, China; [7]Professor, College of Plant Protection, Shandong Agricultural University, Tai'an, China and [8]Professor, Key Laboratory of Pesticide Toxicology and Application Technology, Shandong Agricultural University, Tai'an, China

Abstract

Japanese foxtail (*Alopecurus japonicus* Steud.) is an invasive grass weed that severely threatens the production of wheat (*Triticum aestivum* L.) and canola (*Brassica napus* L.) crops in eastern Asia. Mesosulfuron-methyl is a highly efficient acetolactate synthase (ALS)-inhibiting herbicide widely used for control of this species in China. However, in recent years, some *A. japonicus* populations have evolved resistance to mesosulfuron-methyl by different amino acid substitutions (AASs) within the *ALS* gene. In the current study, 11 populations of *A. japonicus* were collected from Anhui Province, China, where the wheat fields were severely infested with this weed. Based on single-dose screening, eight of these populations evolved resistance to mesosulfuron-methyl, and gene sequencing revealed three AASs located in codon 197 or 574 of the *ALS* gene in the different resistant populations. Subsequently, three typical populations, AH-1, AH-4, and AH-10 with Trp-574-Leu, Pro-197-Thr, and Pro-197-Ser mutations, respectively, in *ALS* genes were selected to characterize their cross-resistance patterns to ALS inhibitors. Compared with the susceptible population AH-S, AH-1 showed broad-spectrum cross-resistance to sulfonylureas (SUs), imidazolinones (IMIs), triazolopyrimidines (TPs), and sulfonyl-aminocarbonyl-triazolinones (SCTs); whereas AH-4 and AH-10 were resistant to SUs, TPs, and SCTs but sensitive to IMIs. Moreover, all three resistant populations were sensitive to both photosystem II inhibitor isoproturon and 4-hydroxyphenylpyruvate dioxygenase inhibitor QYM201 (1-(2-chloro-3-(3-cyclopropyl-5-hydroxy-1-methyl-1H-pyrazole-4-carbonyl)-6-(trifluoromethyl)phenyl)piperidin-2-one). Based on the current state of knowledge, this study is the first report of *A. japonicus* evolving cross-resistance to ALS-inhibiting herbicides due to a Pro-197-Ser mutation in the *ALS* gene.

Introduction

Japanese foxtail (*Alopecurus japonicus* Steud.), a tetraploid species, is a competitive annual grass weed that heavily infests wheat (*Triticum aestivum* L.) and canola (*Brassica napus* L.) fields in eastern and central China (Bi et al. 2016). The strong tillering capacity of *A. japonicus* enhances its competitiveness against crop seedlings, resulting in considerable reduction in grain yield (Wang et al. 2018). Since the 1990s, this weed has been controlled with POST applications of the acetyl-CoA carboxylase (ACCase; EC 6.4.1.2) inhibitor fenoxaprop-*P*-ethyl. The plastidic ACCase in grass weeds is the common target of three chemically distinct classes: aryloxyphenoxypropionate, cyclohexanedione, and phenylpyraxoline (Herbert et al. 1997). Nevertheless, resistance to fenoxaprop-*P*-ethyl was confirmed in 2007 (Yang et al. 2007), after approximately 15 consecutive years of application.

Thereafter, one of the primary herbicides used to control the ACCase-resistant *A. japonicus* and other grass weeds was mesosulfuron-methyl. Mesosulfuron-methyl, a sulfonylurea (SU) herbicide, inhibits the activity of the enzyme acetolactate synthase (ALS; EC 2.2.1.6) that catalyzes the first step in the biosynthesis of the branched-chain amino acids: valine, leucine, and isoleucine (Zhou et al. 2007). The ALS isozymes are the common target of five different chemical classes: SU, imidazolinone (IMI), triazolopyrimidine (TP), pyrimidinyl-thiobenzoate (PTB), and sulfonyl-aminocarbonyl-triazolinone (SCT) (Duggleby et al. 2008). However, after several years of successful control, many *A. japonicus* populations also evolved resistance to mesosulfuron-methyl, particularly in the province of Anhui (Bi et al. 2016).

Table 1. Geographical locations of the collection sites for the seeds of *Alopecurus japonicus*.

Population	Suspected biotype	Geographic location	GPS coordinates
AH-S	Susceptible	Zhouzhuang Village, Yangcun Town, Tianchang City	118°58′7.7628″E, 32°47′23.3442″N
AH-1	Resistant	Pudong Village, Yongfeng Town, Tianchang City	119°2′43.7784″E, 32°46′17.7096″N
AH-2	Resistant	Guanghua Village, Yangcun Town, Tianchang City	118°56′25.3824″E, 32°48′49.4748″N
AH-3	Resistant	Qiaotian Community, Tongcheng Town, Tianchang City	118°56′15.3888″E, 32°50′14.1576″N
AH-4	Resistant	Lulong Community, Renheji Town, Tianchang City	119°4′11.4816″E, 32°38′37.644″N
AH-5	Resistant	Qimiao Village, Datong Town, Tianchang City	118°53′17.5236″E, 32°51′29.8008″N
AH-6	Resistant	Guanghua Village, Yangcun Town, Tianchang City	118°56′25.3824″E, 32°48′49.482″N
AH-7	Resistant	Longji Community, Yangcun Town, Tianchang City	119°2′8.4336″E, 32°50′53.4156″N
AH-8	Resistant	Chahe Village, Wanshou Town, Tianchang City	119°7′23.3508″E, 32°44′37.968″N
AH-9	Resistant	Matang Village, Jinji Town, Tianchang City	118°53′52.386″E, 32°53′59.3196″N
AH-10	Resistant	Jiqiao Village, Yangcun Town, Tianchang City	118°59′5.9994″E, 32°48′59.0004″N

The ACCase and ALS inhibitors are both susceptible to the evolution of resistance (Beckie and Tardif 2012; Powles and Yu 2010), with 48 and 160 weed species identified as resistant phenotypes, respectively (Heap 2018). As repeatedly documented (Kaundun 2014; Yu and Powles 2014), target-site resistance (TSR) is the most common mechanism resulting in resistance to an ACCase or ALS inhibitor. To date, as many as 15 amino acid substitutions (AASs) have been identified at seven codons of *ACCase*, and 28 AASs have been found at eight codons of *ALS* (Tranel et al. 2018). In *A. japonicus*, mutations at five *ACCase* codons (Ile-1781, Trp-1999, Trp-2027, Ile-2041, and Asn-2078) and at three *ALS* codons (Pro-197, Asp-376, and Trp-574) impact the efficacies of ACCase- and ALS-inhibiting herbicides (Bi et al. 2013, 2016; Chen et al. 2017; Cui et al. 2015; Feng et al. 2016; Tang et al. 2012; Xu et al. 2013, 2014).

Since the first report in 2013 (Bi et al. 2013), resistance of *A. japonicus* to ALS inhibitors has only been identified in two other studies (Bi et al. 2016; Feng et al. 2016). Currently, since its patent expired in October 2014, more than 52 pesticide manufacturers have obtained the registration for mesosulfuron-methyl in China (Institute for the Control of Agrochemicals 2018). Therefore, mesosulfuron-methyl will likely remain in large-scale use, and further herbicide resistance is expected to evolve. In previous studies concerning resistance to mesosulfuron-methyl (Bi et al. 2013, 2016; Feng et al. 2016), the focus was primarily on one or a few specific populations, and thus the extent of resistance in a specific area remains unclear. In addition, although the proline-to-threonine AAS at codon position 197 of *ALS* has been reported (Bi et al. 2013), its cross-resistance pattern has not been investigated in *A. japonicus*. Some of the AASs at the known mutation sites of *ALS* are also not yet identified or characterized in this species. Therefore, in this study, 11 populations were collected from different sites in Tianchang, Anhui Province, China, where the wheat fields are the most severely infested with *A. japonicus*. The aims of this study were the following: (1) determine the sensitivity of different populations of *A. japonicus* to mesosulfuron-methyl; (2) identify the mutations associated with the resistance; (3) and determine the cross-resistance patterns to other selected herbicides.

Materials and Methods

Plant Material and Chemical Herbicides

In May 2017, mature seeds of putative resistant (R) and susceptible (S) *A. japonicus* populations were collected from different sites in Tianchang, Anhui Province, China (Table 1). The wheat fields where the R populations were collected received fenoxaprop-P-ethyl and/or mesosulfuron-methyl application(s) for many years; whereas the S population was collected from uncultivated land (roadside) with no history of herbicide application. The seeds were air-dried and stored in paper bags at 4 C until further use. The information for the herbicides used for the dose–response experiments is given in Table 2.

Table 2. Herbicides and their doses used in whole-plant dose–response experiments for different populations of *Alopecurus japonicus*.[a]

Group	Common name	Trade name	Formulation	Supplier	Populations	Test doses[b]
						––––––––––––g ai ha^{-1}––––––––––––
SU	Mesosulfuron-methyl	Sigma®	30 g L^{-1} OF	Bayer, Hangzhou, China	AH-S, AH-5, AH-9	0, 0.3, 0.6, 1.2, 2.3, 4.5, **9**
					AH-1, AH-2, AH-3, AH-6, AH-7	0, 4.5, **9**, 18, 36, 72, 144
					AH-4, AH-8, AH-10	0, **9**, 18, 36, 72, 144, 288
IMI	Imazethapyr	DOWMAO®	10% AS	Binnong, Binzhou, China	AH-S, AH-4, AH-10	0, 1.57, 3.13, 6.25, 12.50, 25, 50
					AH-1	0, 25, 50, **100**, 200, 400, 800
TP	Pyroxsulam	Powerflex®	7.5% WDG	Dow AgroSciences, Beijing, China	AH-S, AH-4	0, 0.33, 0.66, 1.32, 2.64, 5.28, **10.55**
					AH-1, AH-10	0, 2.64, 5.28, **10.55**, 21.10, 42.20, 84.40
SCT	Flucarbazone-Na	Pre-pare®	70% WDG	Arysta LifeScience, Shanghai, China	AH-S	0, 1.0, 2.0, 4.0, 7.9, 15.8, **31.5**
					AH-1, AH-4, AH-10	0, 7.9, 15.8, **31.5**, 63, 126, 252
Urea	Isoproturon	Alon®	50% WP	Bianjing, Suzhou, China	AH-S, AH-1, AH-4, AH-10	0, 14, 28, 56, 112, 225, 450
HPPD	QYM201[c]	PD20184019[d]	6% OD	King Agroot, Qingdao, China	AH-S, AH-1, AH-4, AH-10	0, 4.3, 8.5, 16.9, 33.8, 67.5, **135**

[a] Abbreviations: AS, aqueous solution; HPPD, 4-hydroxyphenylpyruvate dioxygenase-inhibiting herbicide; IMI, imidazolinone; OD, oil dispersion; OF, oil-miscible flowable; SCT, sulfonyl-aminocarbonyl-triazolinone; SU, sulfonylurea; TP, triazolopyrimidine; WDG, water-dispersible granules; WP, wettable powder.
[b] The field-recommended rates (g ai ha^{-1}) are shown in bold (1×).
[c] QYM201, 1-(2-chloro-3-(3-cyclopropyl-5-hydroxy-1-methyl-1H-pyrazole-4-carbonyl)-6-(trifluoromethyl)phenyl)piperidin-2-one.
[d] PD, pesticide registration number.

Single-Dose Herbicide-Resistance Testing

Single-dose testing was conducted as described previously (Zhao et al. 2019b). Briefly, 50 seeds were randomly selected from each population and pregerminated. The germinated seedlings were then transplanted into 50-cell planting trays (54 cm by 28 cm by 8.5 cm) containing moist loam soil and cultured in a controlled-environment greenhouse (natural light, 25/15 C day/night, and ~75% relative humidity). The trays were watered every other day to maintain an adequate level of soil moisture. At the 3- to 4-leaf stage, the weed seedlings were treated with mesosulfuron-methyl at the field-recommended rate (FRR; 9 g ai ha^{-1}). All herbicides shown in Table 2 were applied using a compressed-air, moving-nozzle cabinet sprayer equipped with a TeeJet® 9503EVS flat-fan nozzle (Spraying Systems Co., Wheaton, IL, USA) following previous methods (Zhao et al. 2019b). After seedlings were sprayed, all plants were returned to the greenhouse and cultured for 3 wk. At 21 d after treatment (DAT), the percentage of dead plants was visually assessed (Kumar and Jha 2017). Resistance was confirmed when ≥50% of the individuals survived the herbicide treatment at the FFR (Deng et al. 2017). After the assessment, genomic DNA was extracted from the young leaves of each surviving individual using the classic cetyltrimethylammonium bromide method (Porebski et al. 1997). For those populations in which all plants died from mesosulfuron-methyl treatment, 30 seeds from each population were randomly selected and cultured to the 3- to 4-leaf stage for DNA extraction.

ALS Gene Amplification

A pair of primers previously reported by Guo et al. (2015) was used to amplify the *A. japonicus ALS* gene fragments containing all the known mutation sites (Ala-122, Pro-197, Ala-205, Asp-376, Arg-377, Trp-574, Ser-653, and Gly-654) (Kumar and Jha 2017). The PCR amplification was performed using 2× Es TaqMasterMix (CWBIO, Beijing, China) according to the manufacturer's instructions. The PCR cycling profile consisted of a "hot-start" at 94 C for 2 min, followed by 35 cycles at 94 C for 30 s, 59 C for 30 s, and 72 C for 1 min, with a final elongation step at 72 C for 2 min. An EasyPure Quick Gel Extraction Kit (TransGen Biotech, Beijing, China) was used to purify all PCR products, which were then directly sequenced from both directions to minimize sequencing errors by Sangon Biotech (Shanghai, China).

DNAMAN v 6.0 software (Lynnon, Quebec, Canada) was used to analyze the sequence data. The DNA fragments and derived amino acid sequence positions refer to the full-length *ALS* gene of *A. japonicus* (GenBank accession: KR534607).

Mesosulfuron-Methyl Dose Response

Whole-plant dose–response experiments were conducted as detailed in Zhao et al. (2019b). Briefly, *A. japonicus* seeds from each population were pregerminated, transplanted into 12-cm-diameter pots containing moist loam soil (15 seeds per pot), and cultured in a controlled-environment greenhouse under the conditions described earlier. When the seedlings reached the 2- to 3-leaf stage, they were thinned to eight uniformly sized seedlings per pot. *Alopecurus japonicus* plants at the 3- to 4-leaf stage were treated with a series of doses of mesosulfuron-methyl (Table 2). At 21 DAT, the plants of the different biotypes were cut at ground level, and their aboveground dry weights were recorded.

Cross-Resistance to Other Herbicides

Based on the *ALS* gene analysis, three R populations, AH-1, AH-4, and AH-10, were selected to determine their cross-resistance to other herbicides. Three ALS inhibitors, a photosystem II (PSII) inhibitor, and a 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor were used in this study (Table 2). A series of doses for the different herbicides were determined based on preliminary tests. At 21 DAT, the aboveground dry weights of the plants were recorded.

Statistical Analyses

All the whole-plant dose–response experiments were performed twice (two runs). The experimental design was a randomized complete block design with three replications for each treatment. The dry weight data were expressed as a percent of the untreated control. No significant differences (P > 0.05) were detected between repeated experiments; therefore, the data from the same trial were pooled across runs and fit to a four-parameter log-logistic curve (Equation 1) in SigmaPlot v. 14.0 (Systat Software, San Jose, CA, USA):

$$y = C + \frac{D - C}{1 + \left(\frac{x}{GR_{50}}\right)^b} \qquad [1]$$

where C is the lower limit of the response, D is the upper limit of the response, x is the herbicide application dose, b is the slope at which the herbicide dose causes a 50% growth reduction (GR_{50}), and y is the response at the herbicide dose x. A resistance index (RI) based on the GR_{50} value was calculated to estimate the level of resistance in the R population relative to that in the S population. The weed populations were categorized as follows: no resistance (RI < 2), low resistance (2 ≤ RI < 5), moderate resistance (5 ≤ RI < 10), and high resistance (RI ≥ 10) (Beckie and Tardif 2012).

Results and Discussion

Resistance Screening and Identification of ALS Gene Mutations

Since 2004, the ALS inhibitor mesosulfuron-methyl has been in popular use for weed management in wheat fields in China, particularly for the control of ACCase-resistant weed species. However, the long-term use of single mode of action (MOA) herbicides has frequently resulted in the evolution of herbicide resistance (Norsworthy et al. 2012). According to Beckie and Tardif (2012), weed resistance can be selected by ALS inhibitors in less than 10 applications. Therefore, predictably, resistance to ALS inhibitors has evolved in 17 weed species in China, including *A. japonicus* (Heap 2018). In this study, the 10 suspected resistant *A. japonicus* populations were all collected from wheat fields with a long-term use history of ACCase and/or ALS inhibitors. In a previous study, these 10 populations were highly resistant to the ACCase inhibitor fenoxaprop-*P*-ethyl, and the Ile-1781-Leu or Trp-2027-Cys mutation in the *ACCase* gene was very likely one of the key reasons resulting in fenoxaprop-*P*-ethyl resistance in the different *A. japonicus* populations (Zhao et al., 2019a). These populations were also treated with mesosulfuron-methyl for many years, and therefore, their sensitivity to different ALS inhibitors and the potential TSR mechanism were investigated further.
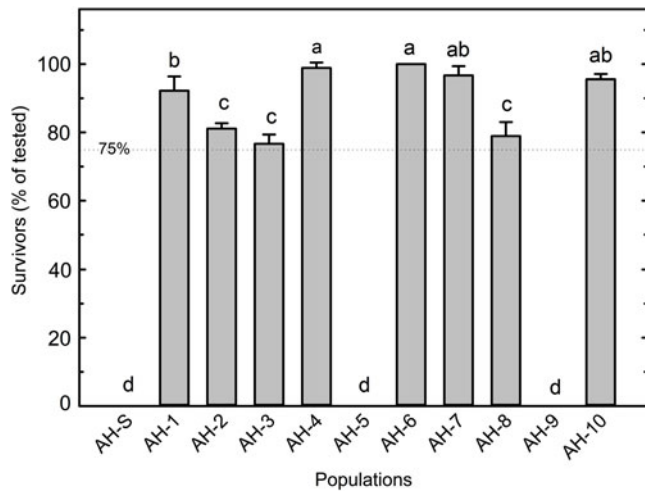
Figure 1. Resistance screening to mesosulfuron-methyl for different populations of *Alopecurus japonicus*. Vertical bars represent the SE of the mean. Bars with the same letters are not significantly different at P ≤ 0.05.

As Figure 1 shows, all tested plants from populations AH-S, AH-5, and AH-9 died from mesosulfuron-methyl treatment at 9 g ha^{-1}, while more than 75% of individuals survived in each of the other eight populations. These results indicated that 8 of the 11 populations tested evolved resistance to mesosulfuron-methyl.

A 1,859-bp fragment spanning all eight known mutation sites of the *A. japonicus ALS* gene was then obtained from each individual ($n \geq 30$) of the different populations. The sequence comparison indicated that the amplified *ALS* genes of all populations had 99.14% sequence similarity with the same region of the documented *ALS* gene of *A. japonicus*. Because they resulted in no AAS, most of the single-nucleotide polymorphisms were synonymous. However, in plants of populations AH-4 and AH-8, a C to A mutation in the *ALS* gene was detected, which resulted in a proline (CCC) to threonine (ACC) substitution at codon position 197 (Figure 2A and B). Similarly, in plants of the AH-1, AH-2, AH-3, AH-6, and AH-7 populations, a G to T mutation in the *ALS* gene was identified, which resulted in a tryptophan (TGG) to leucine (TTG) substitution at codon position 574 (Figure 2D and E). Notably, in plants of the AH-10 population, a C to T mutation was identified in the *ALS* gene, resulting in a proline (CCC) to serine (TCC) substitution at codon position 197 (Figure 2A and C). No known *ALS* gene mutation was detected in plants of populations AH-S, AH-5, and AH-9 (unpublished data).

In the direct sequencing of PCR products, the chromatograms of mutant codon positions always showed double peaks in the surviving plants and sharp, single peaks in the susceptible plants (Figure 2). In addition, no homozygous mutation was detected in sequenced individuals, suggesting multiple alleles of the *ALS* genes (Iwakami et al. 2017). These results are consistent with a previous finding that three or four alleles of *ALS* were present in the different accessions of *A. japonicus* (Feng et al. 2016). Because this study primarily focused on identifying the AASs associated with herbicide resistance, we did not identify the allele(s) holding the specific mutation(s) in each resistant population.
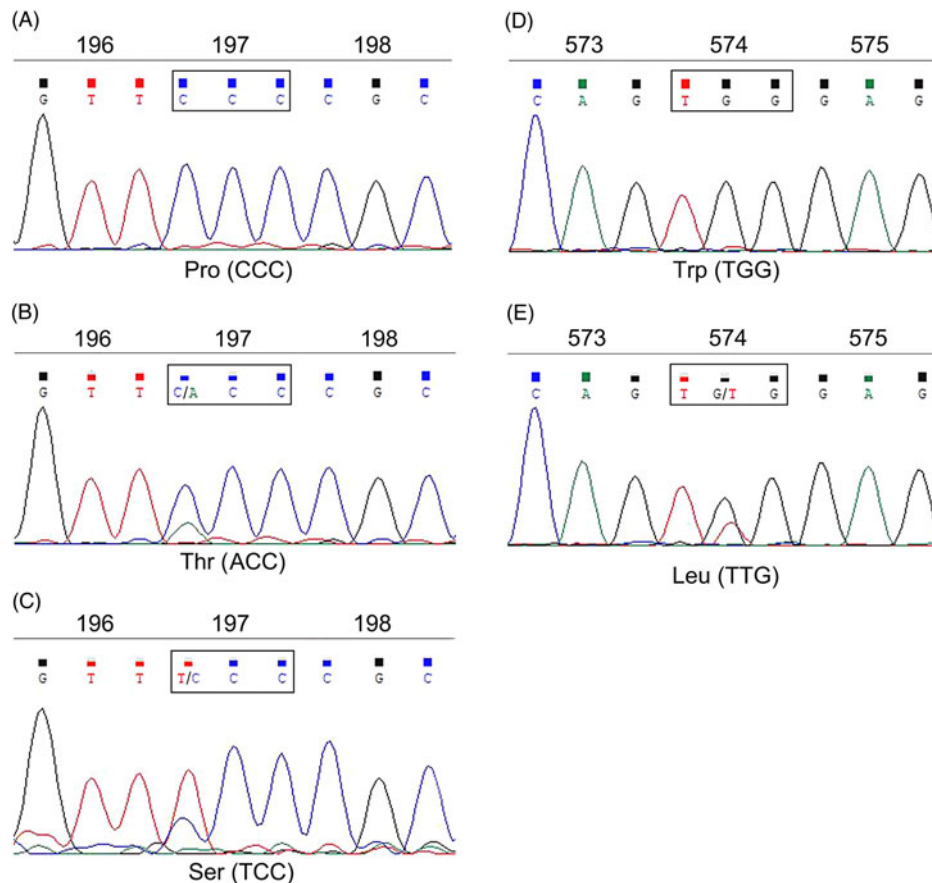

Figure 2. Acetolactate synthase (ALS) gene sequencing showing (A) Pro (CCC), (B) Thr (ACC), or (C) Ser (TCC) at codon position 197 and (D) Trp (TGG) or (E) Leu (TTG) at codon position 574 for *Alopecurus japonicus*.

Table 3. Parameter values of the four-parameter log-logistic equation (Equation 1)[a] used to fit the plant growth response of *Alopecurus japonicus* (as a percent of the untreated control) resulting from the different application doses of mesosulfuron-methyl.

Population	Biotype	Regression parameters[b]				GR_{50}[b,c]	Resistance index
		C	D	B	R^2		
						g ai ha^{-1}	
AH-S	Susceptible	25.17 (1.25)	105.72 (4.33)	2.63 (0.34)	0.9975	0.76 (0.05)	1.0
AH-1	Resistant	26.38 (6.00)	98.62 (2.90)	3.19 (0.87)	0.9923	47.50 (5.15)	62.5
AH-2	Resistant	24.61 (5.00)	100.06 (2.54)	2.36 (0.48)	0.9964	41.44 (3.83)	54.5
AH-3	Resistant	21.83 (5.40)	101.76 (2.94)	1.94 (0.37)	0.9971	37.97 (3.76)	50.0
AH-4	Resistant	24.04 (2.40)	98.55 (2.75)	2.60 (0.61)	0.9951	84.91 (8.39)	111.7
AH-5	Susceptible	26.02 (0.83)	91.72 (1.76)	3.43 (0.28)	0.9992	0.81 (0.03)	1.1
AH-6	Resistant	28.63 (6.84)	97.51 (2.68)	2.70 (0.73)	0.9927	52.70 (6.52)	69.3
AH-7	Resistant	33.42 (3.75)	98.72 (1.77)	2.80 (0.50)	0.9969	46.07 (3.49)	60.6
AH-8	Resistant	28.43 (0.78)	98.64 (0.62)	6.07 (1.20)	0.9997	71.10 (4.70)	93.6
AH-9	Susceptible	25.95 (2.76)	99.15 (2.91)	4.36 (1.07)	0.9956	1.39 (0.09)	1.8
AH-10	Resistant	32.23 (1.43)	98.06 (0.96)	6.25 (2.35)	0.9991	75.14 (1.83)	98.9

[a] Equation 1: $y = C + (D − C)/[1+(x/GR_{50})^b]$, where C is the lower limit of the response, D is the upper limit of the response, x is the herbicide application dose, b is the slope of the curve at GR_{50}, and y is the plant growth response.
[b] Numbers in parentheses represent the SE of the mean.
[c] GR_{50} refers to the herbicide dose resulting in a 50% growth inhibition.

Sensitivity to Mesosulfuron-Methyl

Whole-plant dose–response experiments were performed to reconfirm the sensitivity of each *A. japonicus* population to mesosulfuron-methyl (Table 3). As expected, the susceptible populations AH-S, AH-5, and AH-9 were completely controlled by mesosulfuron-methyl at 9 g ha^{-1}. The GR_{50} values of these three populations were all below 1.50 g ha^{-1}, which are rates much lower than the FFR. By contrast, the GR_{50} values of the eight suspected resistant populations (AH-1 to 4, AH-6 to 8, and AH-10) to mesosulfuron-methyl treatment were between 37.97 and 84.91 g ha^{-1} and were 50.0- to 111.7-fold greater than those of the susceptible population (AH-S). Based on the corresponding RI values, these eight populations were deemed highly resistant to mesosulfuron-methyl (RI ≥ 10).

Cross-Resistance to Other Herbicides

For the susceptible population AH-S, all plants were killed at the FFR of all herbicides evaluated. Compared with AH-S, AH-1 with the Trp-574-Leu mutation was highly resistant to imazethapyr (RI = 16.36) and pyroxsulam (RI = 20.56) and moderately resistant to flucarbazone-Na (RI = 8.84); AH-4 with the Pro-197-Thr mutation was moderately resistant to flucarbazone-Na (RI = 5.69), had low resistance to pyroxsulam (RI = 3.12), and was sensitive to imazethapyr (RI = 1.91); and AH-10 with the Pro-197-Ser mutation was highly resistant to pyroxsulam (RI = 15.33) and flucarbazone-Na (RI = 13.74) but was sensitive to imazethapyr (RI = 1.31) (Table 4; Figure 3). In addition, all three populations were sensitive to both isoproturon and QYM201, with their GR_{50} values much lower than the corresponding FFRs (Table 4; Figure 3).

Pro-197-Thr and Trp-574-Leu are well-known mutations documented in many resistant weed species (Chen et al. 2015; Deng et al. 2017; Han et al. 2012; Xia et al. 2015), including *A. japonicus* (Bi et al. 2013, 2016). However, the cross-resistance to ALS inhibitors in *A. japonicus* due to the Pro-197-Thr point mutation has not been previously tested (Bi et al. 2013). In the current study, the whole-plant dose–response experiments revealed the resistant population AH-4 with the Pro-197-Thr mutation was highly resistant to SUs, moderately resistant to SCTs, minimally resistant to TPs, and sensitive to IMIs (Tables 3 and 4). Thus, this AAS endowed *A. japonicus* plants with broad-spectrum cross-resistance to ALS inhibitors in chemically distinct classes. As previously documented,

Table 4. Sensitivities of susceptible (AH-S) and resistant (AH-1, AH-4, and AH-10) populations of *Alopecurus japonicus* to different herbicides.

Herbicide	Population	GR_{50}[a]	RI[b]
		g ai ha^{-1}	
Imazethapyr	AH-S	7.43 (0.04)	1.00
	AH-1	121.56 (4.55)	16.36
	AH-4	14.19 (0.38)	1.91
	AH-10	9.73 (0.18)	1.31
Pyroxsulam	AH-S	1.60 (0.01)	1.00
	AH-1	32.90 (1.92)	20.56
	AH-4	4.99 (0.12)	3.12
	AH-10	24.52 (0.05)	15.33
Flucarbazone-Na	AH-S	5.79 (0.26)	1.00
	AH-1	51.19 (2.14)	8.84
	AH-4	32.94 (1.88)	5.69
	AH-10	79.54 (3.37)	13.74
Isoproturon	AH-S	106.99 (14.27)	1.00
	AH-1	103.03 (5.50)	0.96
	AH-4	91.88 (3.19)	0.86
	AH-10	124.71 (13.84)	1.17
QYM201[c]	AH-S	29.51 (3.76)	1.00
	AH-1	26.75 (2.61)	0.91
	AH-4	25.80 (2.58)	0.87
	AH-10	30.12 (4.32)	1.02

[a] GR_{50} refers to the herbicide dose resulting in a 50% growth inhibition. Numbers in parentheses represent the SE of the mean.
[b] RI, resistance index.
[c] QYM201, 1-(2-chloro-3-(3-cyclopropyl-5-hydroxy-1-methyl-1H-pyrazole-4-carbonyl)-6-(trifluoromethyl)phenyl)piperidin-2-one.

the *ALS* gene Pro-197-Thr mutant flixweed [*Descurainia sophia* (L.) Webb ex Prantl] showed similar cross-resistance to ALS inhibitors (Deng et al. 2017). This substitution can also confer resistance to SUs, TPs, and IMIs in shortawn foxtail (*Alopecurus aequalis* Sobol.) (Xia et al. 2015). In addition, in the current study, the *A. japonicus* plants with the Trp-574-Leu mutation were highly resistant to SUs, IMIs, and TPs and moderately resistant to SCTs, which are results consistent with previous findings (Bi et al. 2016).

In a comparison with other known *ALS* mutations, the proline to serine mutation is the most common AAS at the 197 codon of the *ALS* gene (Heap 2018). This substitution confers cross-resistance to ALS inhibitors in many other weed species, such as wild radish (*Raphanus raphanistrum* L.) (Yu et al. 2012), corn poppy (*Papaver rhoeas* L.) (Kaloumenos et al. 2011), mayweed chamomile (*Anthemis cotula* L.) (Intanon et al. 2011), water starwort
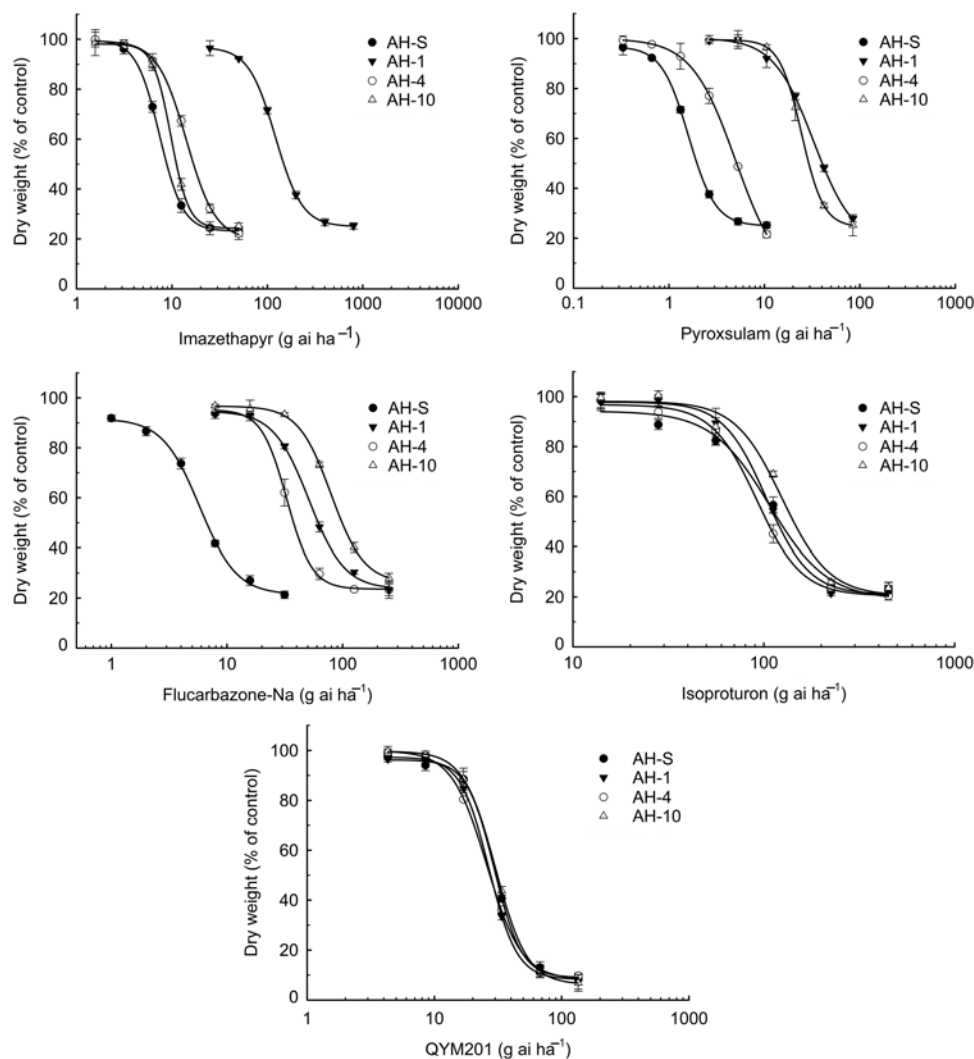
Figure 3. Dose–response curves of the aboveground dry weights of susceptible (AH-S) and resistant (AH-1, AH-4, and AH-10) populations of *Alopecurus japonicus* for a series of application rates of different herbicides. Vertical bars represent the SE of the mean.

[*Myosoton aquaticum* (L.) Moench] (Liu et al. 2013), and eclipta [*Eclipta prostrata* (L.) L.] (Li et al. 2017). In these weeds, the Pro-197-Ser substitution is correlated with broad-spectrum resistance to SUs, TPs, PTBs, or SCTs but low or no resistance to IMIs. However, to our knowledge, this AAS has not been documented in *A. japonicus*. This study is the first to confirm that the resistance to mesosulfuron-methyl in an *A. japonicus* population, AH-10, was due to a Pro-197-Ser mutation in the *ALS* gene. Moreover, subsequent dose–response bioassays indicated the Pro-197-Ser mutant *A. japonicus* plants were highly resistant to SUs, TPs, and SCTs but sensitive to IMIs.

The ultimate aim of investigating the resistance mechanisms in weeds is to develop more effective pest management practices. In addition to a recent study (Zhao et al., 2019a) that demonstrated the 10 populations tested were all highly resistant to fenoxaprop-*P*-ethyl, in this study, eight of these populations also showed the evolution of resistance to mesosulfuron-methyl. This outcome indicates that resistant *A. japonicus* plants have spread over almost all the wheat fields in this specific area. Notably, many Chinese pesticide manufacturers are continuing to register mesosulfuron-methyl for use in wheat fields (Institute for the Control of Agrochemicals 2018). Therefore, if the use of

this chemical continues in this area, the expectation is that the "susceptible" populations will soon develop resistance to mesosulfuron-methyl (Beckie and Tardif 2012). Fortunately, in the current study, the PSII inhibitor isoproturon continued to display good efficacy for the control of resistant plants (Table 4). Therefore, according to Collavo et al. (2013), a biennial rotation between ALS inhibitors and isoproturon or a mixture with diflufenican may efficiently control the ACCase-resistant *A. japonicus* populations and further delay the development of their resistance to mesosulfuron-methyl.

In this study, the efficiency of QYM201 (1-(2-chloro-3-(3-cyclopropyl-5-hydroxy-1-methyl-1H-pyrazole-4-carbonyl)-6-(trifluoromethyl)phenyl)piperidin-2-one) for the control of resistant populations was also evaluated. QYM201 is a novel HPPD inhibitor developed by King Agroot Chemicals (Qingdao, China) in 2011. To our knowledge, such MOA herbicides have not been used for weed control in wheat fields worldwide. In this study, the *A. japonicus* plants with different *ALS* gene mutations all died from QYM201 treatment at the FFR of 135 g ai ha^{-1}. Based on the present data, this chemical has great potential to effectively manage herbicide resistance and thus would be an ideal option for weed control in wheat fields.

In addition, Wang et al. (2018) found that *A. japonicus* seeds buried from 0 to 3 cm in soil have a high germination rate (>90%), whereas no emergence is observed when seeds are buried at a depth of 7 cm. Therefore, deep tillage has the potential to bury the seeds below their maximum depth of emergence, and the development of no-till or minimum-till systems in the following years would be a possible management option for establishing a crop in fields infested with resistant *A. japonicus* (Zhao et al. 2018).

In summary, the first case of an *A. japonicus* population that evolved high-level resistance to mesosulfuron-methyl due to a Pro-197-Ser mutation in the *ALS* gene was identified. Because the proline to serine mutation is considered the most common AAS at the 197 codon of the *ALS* gene, the occurrence of the mutation in the species *A. japonicus* fills a gap in our knowledge. The comprehensive cross-resistance patterns of TSR resulting from a Pro-197-Thr, Pro-197-Ser, or Trp-574-Leu mutation in *A. japonicus* were also characterized. The evolution of herbicide resistance in *A. japonicus* has reached a critical point in Tianchang, Anhui Province, and thus the development of an integrated weed management program, including herbicide use or crop rotation, tillage, or other practices that deplete the soil seedbank, is urgently needed.

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