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Japanese Foxtail (*Alopecurus japonicus*) Resistance to Fenoxaprop and Pinoxaden in China

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Japanese foxtail is one of the most common and competitive annual grass weeds of wheat in China. Whole-plant dose-response experiments were conducted with fenoxaprop and pinoxaden to confirm and characterize resistant and susceptible Japanese foxtail populations and to elucidate the basis of resistance to these herbicides. The resistant Japanese foxtail population was 49-fold resistant to fenoxaprop and 16-fold (cross) resistant to pinoxaden relative to the susceptible population, which was susceptible to both fenoxaprop and pinoxaden herbicides. Molecular analysis of resistance confirmed that the Ile_{1781} to Leu mutation in the resistant population conferred resistance to both fenoxaprop and pinoxaden. This is the first report of cross resistance of Japanese foxtail to pinoxaden in the world and of a target site mutation that corresponded to resistance to both fenoxaprop and pinoxaden in Japanese foxtail. Prior selection pressure from fenoxaprop could result in evolution of resistance to fenoxaprop and cross resistance to pinoxaden in Japanese foxtail population.

Nomenclature: Fenoxaprop; pinoxaden; Japanese foxtail, *Alopecurus japonicus* Steud; Wheat, *Triticum aestivum* L.

Key words: ACCase inhibitor, fenoxaprop resistance, herbicide resistance, pinoxaden resistance.

Acetyl coenzyme-A carboxylase (ACCase; EC 6.4.1.2) is a biotinylated enzyme that catalyzes the first committed step of fatty acid biosynthesis, which is ATP-dependent carboxylation of acetyl-CoA to malonyl-CoA in eukaryotes and prokaryotes (Harwood 1988; Incledon and Hall 1997). Plants have two ACCase isoforms: one in plastids for fatty acid biosynthesis and the other in the cytosol for synthesis of very long-chain fatty acids and flavonoids (Yu et al. 2010).

All ACCase isoforms contain three catalytic domains, namely the biotin carboxyl-carrier, the biotin carboxylase, and the carboxyl transferase (CT) domains (Délye 2005; Nikolau et al. 2003). The CT domain of plastidic ACCase is the target of widely used ACCase-inhibitor herbicides belonging to three different chemical groups: aryloxyphenoxypropionate (APP), cyclohexanedione (CHD), and phenylpyrazolin (DEN) (Balgheim 2009; Collavo et al. 2011). Grass species have a eukaryotic type of ACCase in the chloroplast that is sensitive to ACCase-inhibitors, whereas most broadleaf species have a prokaryotic type of ACCase that is not sensitive to ACCase-inhibitors (Burke et al. 2006; Incledon and Hall 1997).

Since their commercial introduction in 1977, APP and CHD herbicides have been widely used in world agriculture to control a broad range of grass weeds in many dicotyledonous and some cereal crops (Maneechote et al. 2005). Pinoxaden is a relatively newly introduced ACCase-inhibitor herbicide, belonging to the novel DEN chemistry, for selective control of grass weeds in wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) (Kuk et al. 2008; Porter et al. 2005).

A repercussion of widespread and continuous intensive use of ACCase-inhibitor herbicides worldwide has been the selection for populations of 38 weed species with resistance to these herbicides (Heap 2011). Eighteen weed species in China have evolved resistance to different herbicides groups, including five grass weed species with confirmed resistance to APP and CHD herbicides.

Cross-resistance between members of APP, CHD, and DEN herbicide groups has been reported in various weed species (Collavo et al. 2011; Petit et al. 2010b). Pinoxaden was only introduced in 2006, but resistance in some weeds has already been identified due to cross-resistance with the other two classes of ACCase-inhibiting herbicides. In most cases, resistance exists in plants before their exposure to pinoxaden (Kaundun 2010; Kuk et al. 2008; Petit et al. 2010b; Yu et al. 2010).

Nontarget-site resistance, such as enhanced herbicide metabolism, has been the resistance mechanism for APP, CHD, and DEN herbicide classes in some of the resistant weed species, such as blackgrass (*Alopecurus myosuroides* Huds.) (Cummins et al. 1997; Letouzé and Gasquez 2003; Petit et al. 2010a), wild oat (*Avena fatua* L.) (Cocker et al. 2000), and large crabgrass [*Digitaria sanguinalis* (L.) Scop.] (Hidayat and Preston 1997). Usually resistance to ACCase-inhibiting herbicides is conferred by target-site modifications, often resulting from a single amino acid change that reduces sensitivity of the ACCase enzyme to these herbicides (Preston and Mallory-Smith 2001).

To date, eight conserved amino acid substitutions in the CT domain of the ACCase gene are known to confer ACCase-inhibitor resistance in various weed species: Gln_{1756} to Glu, Ile_{1781} to Leu or Val, Trp_{1999} to Cys or Leu, Trp_{2027} to Cys, Ile_{2041} to Asn or Val, Asn_{2078} to Gly, Cys_{2088} to Arg, and Gly_{2096} to Ala (Collavo et al. 2011; Délye et al. 2011; Powles and Yu 2010; Zhang and Powles 2006). Amino acid substitution at these eight positions can confer different patterns of resistance among ACCase-inhibitors. In general, amino acid mutations at positions 1999, 2027, 2041, and 2096 endow resistance to one or more APPs but not to CHDs or DEN, while amino acid mutations at 1781, 2078, and 2088 confer resistance to all ACCase-inhibitors (Collavo et al. 2011; Powles and Yu 2010).

Japanese foxtail is one of the most troublesome annual grass weeds of wheat-producing regions of China. ACCase-inhibiting herbicides, e.g., fenoxaprop, have been registered for control of Japanese foxtail and other grass weeds for three decades (Li 2007; Li et al. 2007; Qiang 2001). Japanese

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Table 1. Primers used to amplify the ACCase gene of Japanese foxtail populations.

Primers	Sequence (5′–3′)	Amplicon size, bp	Annealing temperature, C
ACVRG1	AATGGGTCGTGGGGCACTCCTATAATTCC	785	62
ACVRG1R	GCTGAGCCACCTCAATATATIAGAAACACC		
ACcp2	AACATTGGTGGACCTCTTCCTATTAC	748	61
ACVII29R	CTTCCATTTGCTCCCTGGAGTCTTG		

foxtail has evolved resistance to the APP herbicides, haloxyfop, fenoxaprop, and quizalofop, and cross-resistance to the CHD herbicide, sethoxydim, after several years of treatment (Yang 2007). Japanese foxtail is cross-pollinated, so resistance may occur via immigration of seed or pollen containing different ACCase alleles from nearby fields containing resistant plants.

Beginning in 2007, producers in the Anhui province, China, observed that fenoxaprop at recommended rates failed to control Japanese foxtail in wheat fields after several years of successful control. Therefore, the objectives of this research were to: (a) investigate and quantify resistance to fenoxaprop and pinoxaden; and (b) determine the molecular basis of ACCase-inhibitor resistance in Japanese foxtail populations from China.

Materials and Methods

Seed Sources. Seeds of a resistant Japanese foxtail population were collected from wheat fields in Yongfeng county, Anhui province, China where fenoxaprop had been used annually for 5 yr and control failure was evident in the fields. A susceptible Japanese foxtail population was collected from wheat field without herbicide applied in Danyang county, Jiangsu province. The resistant and susceptible populations were from fields separated by about 100 km. Seeds from 50 mature plants were collected randomly by hand and bulked. Seeds were air-dried and stored in paper bags at room temperature until used.

Whole-Plant Dose-Response Experiments. The experiments were conducted in a growth-room at China Agricultural University, Beijing, to confirm and characterize fenoxaprop (Puma Super 69EW, Bayer CropScience, Beijing, China) and pinoxaden (Daneng 50 EC, Syngenta Investment Co., Ltd., Shanghai, China) resistance in Japanese foxtail populations. Seeds of suspected resistant and susceptible Japanese foxtail were sown in separate 9-cm-diam plastic pots containing commercial potting soil and peat moss (2:1, v/v). Plants were maintained in the growth-room at 22 ± 2 C, 12-h photoperiod with light intensity of 50 mol m^{-2} s^{-1} and watered as needed.

Herbicide treatments were applied to Japanese foxtail plants at three- to four-leaf stage using a laboratory sprayer equipped with a flat-fan nozzle to deliver 300 L ha^{-1} at 230 kPa. Fenoxaprop at 0, 31, 62, 124, 248, 497, and 745 g ae ha^{-1} was applied to the putative resistant plants and at 0, 1, 2, 4, 8, 16, 31, and 62 g ha^{-1} to the susceptible plants. Pinoxaden at 0, 4.5, 9, 18, 36, and 54 g ae ha^{-1} was applied to the resistant plants and at 0, 0.28, 0.56, 1.1, 2.3, and 4.5 g ha^{-1} to the susceptible plants. The recommended field rates are fenoxaprop at 62 g ha^{-1} and pinoxaden at 45 g ha^{-1}. Also, a nontreated control for each population was sprayed with water. Different herbicide doses were used for the resistant and susceptible populations due to great differences in sensitivities to fenoxaprop and pinoxaden in the preliminary screening experiment (data not showed). Plants were returned to the growth-room after herbicide treatments.

The experiment was in a completely randomized design with three replications and was repeated. Shoots were harvested 21 d after treatment (DAT), and fresh weight was expressed as a percentage of the nontreated control.

Statistical Analyses. The GR_{50} of fenoxaprop and pinoxaden for resistant and susceptible Japanese foxtail populations was calculated using the Probit model: $Y = a + bx$ of MSTAT-C software program (MSTAT-C, Version 2.10, Michigan State University, East Lansing, MI); where Y = Probit, a = intercept, b = slope, and $x = \log_{10}$ (dose), and standard error was calculated. Resistance (*R/S*) ratio was calculated as the GR_{50} of the resistant population divided by the GR_{50} of the susceptible population to indicate the level of resistance for the resistant population.

Differences between the data of the two runs of the experiment for each herbicide were not detected, so the data were pooled. ANOVA was performed on all data using SPSS software (Version 9.0, SPSS Inc., Chicago, IL).

Molecular Basis of Resistance. Japanese foxtail leaves of individual resistant and susceptible plants were harvested at 20 DAT, wrapped separately in aluminum foil, and stored at −80 C. DNA was extracted from leaves of each plant using a modified CTAB (cetyltrimethylammonium bromide) protocol (Doyle and Doyle 1987).

Two forward and reverse overlapping primers (Invitrogen Biotechnology Co., Ltd., Shanghai, China) were designed to amplify the ACCase gene of Japanese foxtail in two segments (Table 1) (Délye et al. 2002b; Liu et al. 2007; Petit et al. 2010b). Primers ACVRG1 and ACVRG1R for a 785 bp were used to amplify a single fragment encompassing codon 1781 in plants that were resistant to fenoxaprop and pinoxaden. Primers ACcp2 and ACVII29R were used to amplify a 748 bp fragment that includes the region containing codons 1999 to 2096, numbered according to ACCase gene sequence of blackgrass (*A. myosuroides* Gene Bank accession AJ310767).

Each 25-µl polymerase chain reaction (PCR) mix contained 3 µM genomic DNA templates, 0.5 µM of each primer, 12.5 µM 2X Taq PCR Master Mix buffer (Tiangen Biotech Co., Ltd., Beijing, China), and ddH2O. The amplification protocol for primers ACVRG1 and ACVRG1R consisted of 94 C for 4 min; followed by 35 cycles of 94 C for 30 s, 62 C for 30 s, 72 C for 30 s, and a final extension cycle at 72 C for 5 min. The PCR amplification protocol for primers ACcp2 and ACVII29R consisted of 95 C for 30 s; 37 cycles at 94 C for 10 s, 61 C for 15 s, 72 C for 45 s, and a final extension cycle at 72 C for 10 min.

The PCR amplification products were confirmed on a 1.0% agarose gel in Tris-Acetate-EDTA (TAE) buffer and then each PCR product was sequenced in forward and reverse directions to minimized sequencing errors by a commercial sequencing company (Sequencing Service Dept., Invitrogen Biotechnology Co., Ltd., Shanghai, China). The sequence data of resistant and susceptible Japanese foxtail populations

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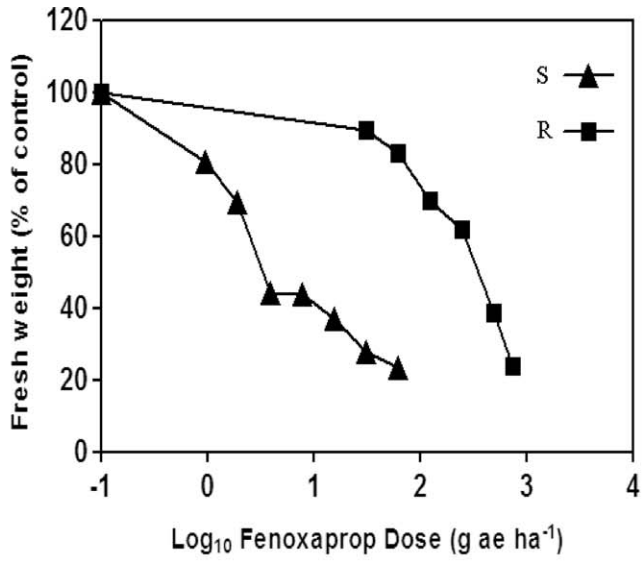

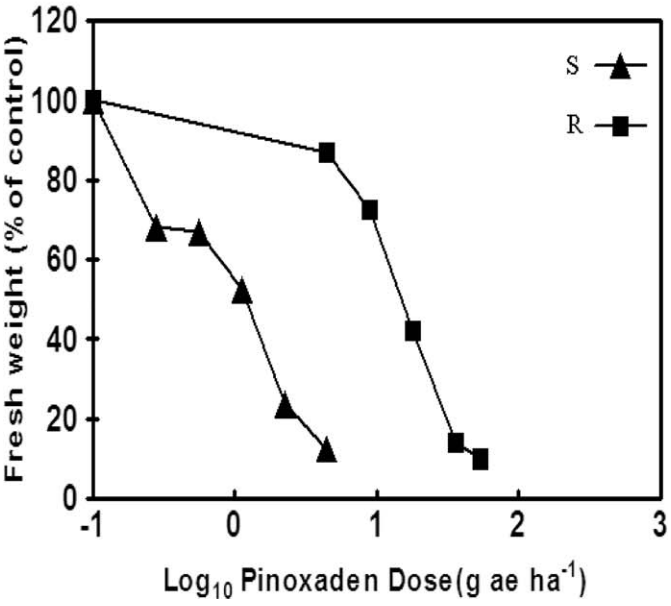

Figure 1. Response of resistant [■] and susceptible [▲] Japanese foxtail populations to different rates of fenoxaprop and pinoxaden. Fresh weight was expressed as a percentage of the nontreated control.

were compared to determine if there was a nucleotide substitution. Sequence data for each Japanese foxtail populations and blackgrass Gene Bank accession AJ310767 were aligned and compared using DNAMAN version 5.2.2 software (Lynnon Biosoft, Quebec, Canada).

Results and Discussion

Whole-Plant Dose-Response Experiments. The resistant Japanese foxtail population was resistant to fenoxaprop and cross-resistant to pinoxaden compared with the susceptible population (Figure 1), but it still was controlled with pinoxaden at the recommended rate. However, the susceptible population was susceptible to both herbicides. Fresh weight reduction of resistant and susceptible populations was affected more by pinoxaden than fenoxaprop, and the two populations displayed decreasing fresh weights with increasing herbicide rates.

Table 2. Estimate of GR_{50} (herbicide rate required to cause 50% reduction in plant fresh weight) and resistance ratio (*R/S*) values of resistant (R) and susceptible (S) Japanese foxtail populations to fenoxaprop and pinoxaden in dose response experiments.

Herbicide	Population		Resistance ratio
	R	S	
	GR_{50}[a] (g ae ha^{-1})		*R/S*[b]
Fenoxaprop	301.2 ± 13	6.1 ± 0.4	49
Pinoxaden	14.6 ± 0.2	0.87 ± 0.2	16

[a] GR_{50} refers to the herbicides rates required for 50% fresh weight reduction compared with the nontreated control. Japanese foxtail populations have practical resistance to pinoxaden but still controlled with the recommended rate of pinoxaden. Each value represents the mean ± standard error.

[b] Resistance ratio (*R/S*) was calculated by dividing GR_{50} value of the resistant population by that of the susceptible population.

Visibly, the resistant population exhibited no mortality with pinoxaden at 4.5 and 9 g ha^{-1}. Most resistant plants were killed by pinoxaden at 36 g ha^{-1} and all plants were killed by pinoxaden at 54 g ha^{-1}. However, the susceptible population was completely controlled with pinoxaden at 4.5 g ha^{-1}. The recommended field rate of pinoxaden was 45 g ha^{-1}.

The GR_{50} values for Japanese foxtail treated with either fenoxaprop or pinoxaden were higher for the resistant population than the susceptible population (Table 2). Moreover, the fenoxaprop GR_{50} value in the resistant and susceptible population was more than those with pinoxaden. The estimated GR_{50} value for the resistant population with fenoxaprop was 301 g ha^{-1} and with pinoxaden was 14.6 g ha^{-1}. However, the GR_{50} value for the susceptible population with fenoxaprop was 6.1 g ha^{-1} and with pinoxaden was 0.87 g ha^{-1}. The *R/S* ratio with fenoxaprop was 49-fold and with pinoxaden was 16-fold, which indicate that the resistant population was more resistant to fenoxaprop than pinoxaden compared with the susceptible population. However, the resistant population was still controlled by pinoxaden at the recommended rate.

Resistance has been defined as the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type (Weed Science Society of America 1998). Traditionally, weed resistance levels are calculated using GR_{50} values estimated from dose–response curves of species to herbicides. Accessions showing significantly higher GR_{50} values than the susceptible standard are classified as resistant.

However, accessions classified as resistance by this process frequently are fully controlled by the recommended herbicide dose, which happens when the species evaluated is naturally highly susceptible to the herbicide. An example is the response of Italian ryegrass (*Lolium multiflorum* Lam.) to clethodim, pinoxaden, and sethoxydim (Kuk et al. 2008). Similarly, the resistant Japanese foxtail population exhibited cross-resistance to pinoxaden relative to the susceptible population (Table 2) and still was killed by pinoxaden at the recommended field rate.

Beckie et al. (2000) reported that resistance is confirmed if the dose-response of the biotypes is statistically different and the putative resistant biotype is not controlled at doses that control susceptible plants. For practical purposes, accessions that can be controlled by the recommended use rate are still susceptible (Kuk et al. 2008). However, the recommended

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Figure 2. Sequence alignment of partial ACCase gene from the resistant and susceptible Japanese foxtail populations and blackgrass (*Alopecurus myosuroides* Gene Bank accession AJ310767). Asterisks (*) identify where amino acids are identical and the free space marks where conservatively substituted residues occur. The (-) denotes the amino acid substitution isoleucine (I) in the susceptible population and blackgrass changed to leucine (L) in resistant population that confer resistance to fenoxaprop and pinoxaden. Abbreviations: S, susceptible; R, resistant; F, fenoxaprop; P, pinoxaden.

herbicide use rate on weeds under greenhouse or growth-room conditions is often much lower than when applied under field conditions. Thus, using the recommended use rate alone is not a sufficient criterion for classification of resistance, and it may indicate that further testing under field conditions is necessary (Heap 2011). From a scientific viewpoint, resistance can be defined as a genetically inherited statistical difference in herbicide response between two weed populations of the same weed species. Clearly, the scientific definition is the lowest hurdle for any weed population to be classified as resistance.

Repeated use of fenoxaprop for 5 yr continuously in monoculture wheat selected for high fenoxaprop-resistant Japanese foxtail plus comparatively low cross resistance to pinoxaden (Table 2). Perhaps this difference occurred because the resistant population had never been exposed to pinoxaden before. Selection with ACCase-inhibiting herbicides for at least 3 yr has selected for resistance, including different patterns of resistance within or among members of APPs, CHDs, and pinoxaden for 38 weed species in the world (Heap 2011). Cross resistance between pinoxaden and APP herbicides, e.g., fenoxaprop, has occurred in wild oat, Italian ryegrass, and blackgrass (Collavo et al. 2011; Kaundun 2010; Kuk et al. 2008; Petit et al. 2010b; Uludag et al. 2008).

The different levels of resistance to fenoxaprop and pinoxaden of Japanese foxtail population may result from more than one mechanism of resistance, with target site mutation and metabolism as the most likely mechanisms (Kuk et al. 2008). Insensitivity of the ACCase target site is the most common mechanism of resistance to ACCase-inhibiting herbicides (Ellis et al. 2010). Therefore, ACCase gene sequencing experiments were done in this study.

Molecular Basis of Resistance. Analysis of the ACCase amino acid sequences of the first fragment, which included codon 1781, showed approximately 98% similarity between the resistant and susceptible populations and the published ACCase sequence of blackgrass Gene Bank accession AJ310767. A single nucleotide substitution from ATA to TTA resulted in an amino acid substitution at position 1781 from isoleucine in susceptible population to leucine in the resistant population (Figure 2). Therefore, the Ile_{1781} to Leu mutation in the resistant Japanese foxtail population conferred resistance to fenoxaprop and cross resistance to pinoxaden.

The sequences of the second fragment, which included the codons 1999 to 2096, in the ACCase gene of the resistant and susceptible Japanese foxtail population showed approximately 99% identity compared to the sequence of blackgrass Gene Bank accession AJ310767, and there were not any substitutions within the sequence of this fragment (Figure 2).

In most plants, target site resistance to ACCase-inhibitors is related to eight allelic point mutations in the CT domain of the ACCase gene, which confer different patterns and levels of resistance to ACCase-inhibitors in many resistant weed species (Collavo et al. 2011; Powles and Yu 2010). The Ile_{1781} to Leu mutation, which is the most common mutation, has been associated with high resistance levels and a wide resistance pattern among APPs, CHDs, and DEN in eight weed species including water foxtail (*Alopecurus aequalis* Sobol.) (Huang 2004), blackgrass (Délye et al. 2002a,b), wild oat (Christoffers et al. 2002), sterile oat (*Avena sterilis* ssp. *ludoviciana* Malzew.) (Liu et al. 2007), rigid ryegrass (*Lolium rigidum* Gaud.) (Zagnitko et al. 2001), Italian ryegrass (White et al. 2005), and green foxtail [*Setaria viridis* (L.) Beauv.] (Délye et al. 2002c). The Ile_{1781} to Leu mutation conferred resistance to pinoxaden at the field rate in ryegrass and blackgrass (Petit et al. 2010b; Scarabel et al. 2011; Yu et al. 2007). The same mutation conferred strong resistance to fenoxaprop in blackgrass (Balgheim 2009; Délye et al. 2008), sterile oat (Liu et al. 2007), and rigid ryegrass (Zhang and Powles 2006). Our results and previous studies by Petit et al. (2010b) and Yu et al. (2010) suggest that APPs, CHDs, and pinoxaden all act by binding roughly at the same site within the ACCase active site cavity.

A population of Japanese foxtail resistant to fenoxaprop and cross resistance to pinoxaden was identified in this study, relative to the susceptible population, but the resistance population still was controlled by pinoxaden at the recommended rate. Prior selection with fenoxaprop resulted in evolution of different levels and patterns of resistance to both fenoxaprop and pinoxaden in Japanese foxtail. This response occurred even though the resistant population was collected from fields that had never been treated with pinoxaden. Moreover, Japanese foxtail resistant to pinoxaden had not been confirmed previously. Thus, this is the first report in the world of cross-resistance of Japanese foxtail to pinoxaden and a target site mutation that correlated to resistance to fenoxaprop and pinoxaden.

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Literature Cited

Balgheim, N. 2009. Investigations on herbicide resistant grass weeds. Ph.D. thesis. Stuttgart, Germany: University of Hohenheim. 80 p.

Beckie, H. J., I. M. Heap, R. J. Smeda, and L. M. Hall. 2000. Screening for herbicide resistance in weeds. Weed Technol. 14:428–445.

Burke, I. C., W. E. Thomas, J. D. Burton, and J. W. Wilcut. 2006. A seedling assay to screen aryloxyphenoxypropionic acid and cyclohexanedione resistance in johnsongrass (*Sorghum halepense*). Weed Technol. 20:950–955.

Christoffers, M., M. L. Berg, and C. G. Messersmith. 2002. An isoleucine to Leucine mutation in acetyl-CoA carboxylase confers herbicide resistance in wild oat. Genome 45:1049–1056.

Cocker, K. M., J.O.D. Coleman, A. M. Blair, J. H. Clarke, and S. R. Moss. 2000. Biochemical mechanisms of cross-resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides in populations of *Avena* spp. Weed Res. 40:323–334.

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

Cummins, I., S. Moss, D. J. Cole, and R. Edwards. 1997. Glutathione transferases in herbicide-resistant and herbicide-susceptible black-grass (*Alopecurus myosuroides*). Pestic. Sci. 51:244–250.
Délye, C. 2005. Weed resistance to acetyl-coenzyme A carboxylase-inhibitors: an update. Weed Sci. 53:728–746.
Délye, C., É. Calmés, and A. Matéjicek. 2002a. SNP markers for blackgrass (*Alopecurus myosuroides* Huds.) genotypes resistant to acetyl CoAcarboxylase inhibiting herbicides. Theor. Appl. Genet. 104:1114–1120.
Délye, C., A. Matéjicek, and J. Gasquez. 2002b. PCR-based detection of resistance to acetyl-CoA carboxylase-inhibiting herbicides in blackgrass (*Alopecurus myosuroides* Huds) and ryegrass (*Lolium rigidum* Gaud). Pest Manag. Sci. 58:474–478.
Délye, C., A. Matéjicek, and S. Michel. 2008. Cross-resistance pattern to ACCase-inhibiting herbicide conferred by mutant ACCase isoforms in *Alopecurus myosuroides* Huds. (black-grass) re-examined at the recommended herbicide field rate. Pest Manag. Sci. 64:1179–1186.
Délye, C., F. Pernin, and S. Michel. 2011. 'Universal' PCR assays detecting mutations in acetyl-coenzyme A carboxylase or acetolactate synthase that endow herbicide resistance in grass weeds. Weed Res. 51:353–362.
Délye, C., T. Wang, and H. Darmency. 2002c. 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.
Doyle, J. J. and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19:11–15.
Ellis, A. T., L. E. Steckel, C. L. Main, M.S.C. de Melo, D. R. West, and T. C. Mueller. 2010. A survey for diclofop-methyl resistance in Italian ryegrass from Tennessee and how to manage resistance in wheat. Weed Technol. 24:303–309.
Harwood, J. L. 1988. Fatty acid metabolism. Annu. Rev. Plant Physiol. 39:101–138.
Heap, I. M. 2011. International Survey of Herbicide Resistant Weeds. www.weedscience.org. Accessed: April 2011.
Hidayat, I. and C. Preston. 1997. Enhanced metabolism of fluazifop acid in a biotype of *Digitaria sanguinalis* resistant to the herbicide fluazifop-*p*-butyl. Pestic. Biochem. Physiol. 57:137–146.
Huang, S. X. 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].
Incledon, B. J. and J. C. Hall. 1997. Acetyl-coenzyme A carboxylase: quaternary structure and inhibition by graminicidal herbicides. Pestic. Biochem. Physiol. 57:255–271.
Kaundun, S. S. 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.
Kuk, Y., N. R. Burgos, and R. C. Scott. 2008. Resistance profile of diclofop-resistant Italian ryegrass (*Lolium multiflorum*) to ACCase- and ALS-inhibiting herbicides in Arkansas, USA. Weed Sci. 56:614–623.
Letouzé, A. and J. Gasquez. 2003. Enhanced activity of several herbicide-degrading enzymes: a suggested mechanism responsible for multiple resistance in blackgrass (*Alopecurus myosurides* Hud.). Agronomie. 23:601–608.
Li, R. 2007. Studies on herbicide-resistance and genetic diversity of wild oat *Avena fatua* L. populations in China. Ph.D. dissertation. Beijing: Beijing Agricultural University. 53 p. [In Chinese].
Li, R., S. Wang, L. Duan, Z. Li, M. J. Christoffers, and L. Mengistu. 2007. Genetic diversity of wild oat (*Avena fatua*) populations from China and the United States. Weed Sci. 55:95–101.
Liu, W., D. K. Harrison, D. Chalupska, P. Gornicki, C. C. O'Donnell, S. W. Adkins, R. Haselkorn, and R. R. Williams. 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.
Maneechote, C., S. Samanwong, X. Zhang, and S. B. Powles. 2005. Resistance to ACCase-inhibiting herbicides in sprangletop (*Leptochloa chinensis*). Weed Sci. 53:290–295.
Nikolau, B. J., J. B. Ohlrogge, and E. S. Wurtele. 2003. Plant biotin-containing carboxylases. Arch. Biochem. Biophys. 414:211–222.
Petit, C., G. Bay, F. Pernin, and C. Délye. 2010b. Prevalence of cross- or multiple resistance to the acetyl-coenzyme A carboxylase inhibitors fenoxaprop, clodinafop and pinoxaden in black-grass (*Alopecurus myosuroides* Huds.) in France. Pest Manag. Sci. 66:168–177.
Petit, C., B. Duhieu, K. Boucansaud, and C. Délye. 2010a. Complex genetic control of non-target-site-based resistance to herbicides inhibiting acetyl-coenzyme A carboxylase and acetolactate-synthase in *Alopecurus myosuroides* Huds. Plant Sci. 178:501–509.
Porter, D. J., M. Kopec, and U. Hofer. 2005. Pinoxaden: a new selective postemergence graminicide for wheat and barley. Proc. Weed Sci. Soc. Am. 45:95.
Powles, S. B. and Q. Yu. 2010. Evolution in action: plants resistant to herbicides. Ann. Rev. Plant Biol. 61:317–347.
Preston, C. and C. A. Mallory-Smith. 2001. Biochemical mechanism, inheritance, and molecular genetics of herbicide resistance in weeds. Pages 23–60 *in* S. B. Powles and D. L. Shaner, eds. Herbicide Resistance and World Grains. Boca Raton, FL: CRC.
Qiang, S. 2001. Weed Science. Beijing: Chinese Agricultural Publishing House. 2471 p.
Scarabel, L., S. Panozzo, S. Varotto, and M. Sattin. 2011. Allelic variation of the ACCase gene and response to ACCase-inhibiting herbicides in pinoxaden-resistant *Lolium* spp. Pest Manag. Sci. 67:932–941.
Uludag, A., K. W. Park, J. Cannon, and C. A. Mallory-Smith. 2008. Cross resistance of acetyl-CoA carboxylase (ACCase) inhibitor resistant wild oat (*Avena fatua*) biotypes in the Pacific Northwest. Weed Technol. 22:142–145.
White, G. M., S. R. Moss, and A. Karp. 2005. Differences in the molecular basis of resistance to the cyclohexanedione herbicide sethoxydim in *Lolium multiflorum*. Weed Res. 45:440–448.
Weed Science Society of America. 1998. "Herbicide resistance" and "herbicide tolerance" defined. Weed Technol. 12:789.
Yang, C. 2007. Study on resistance of Japanese foxtail (*Alopecurus japonicus*) to haloxyfop-R-methyl in oilseed rape field. M.S. thesis. Nanjing: Nanjing Agric. Univ. 77 p. [In Chinese].
Yu, L.P.C., Y. S. Kim, and L. Tong. 2010. Mechanism for the inhibition of the carboxyltransferase domain of acetyl-coenzyme A carboxylase by pinoxaden. Proc. Natl. Acad. Sci. USA. 107:22072–22077.
Yu, Q., A. Collavo, M-Q. Zheng, M. Owen, M. Sattin, and S. B. Powles. 2007. Diversity of acetyl-coenzyme A carboxylase mutations in resistant *Lolium* populations: evaluation using clethodim. Plant Physiol. 145:547–558.
Zagnitko, O., J. Jelenska, G. Tevzadze, R. Haselkorn, and P. Gornicki. 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, X. Q. and S. B. Powles. 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 Phytologist 172:636–645.

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