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Resistance to ACCase-inhibiting herbicides in an Asia minor bluegrass (*Polypogon fugax*) population in China

Wei Tang [a,1], Fengyan Zhou [b,1], Jie Chen [a,*], Xiaogang Zhou [c]

[a] Zhejiang Branch of National Pesticide R&D South Center, Zhejiang Chemical Industry Research Institute, Hangzhou 310023, China
[b] Institute of Plant Protection and Agro-Products Safety, Anhui Academy of Agricultural Sciences, Hefei 230031, China
[c] Institute of Plant Protection, Sichuan Academy of Agricultural Sciences, Chengdu 610066, China

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ABSTRACT

Asia minor bluegrass (*Polypogon fugax*) is a common annual grass weed of winter crops distributed across China. We conducted a study on the resistance level and the mechanism of resistance to ACCase-inhibiting herbicides in a *P. fugax* population from China. Whole-plant dose–response experiments in greenhouse showed that the resistant *P. fugax* population was 1991, 364, 269, 157, and 8-fold resistant to clodinafop-propargyl, fluazifop-*p*-butyl, haloxyfop-R-methyl, quizalofop-*p*-ethyl and fenoxaprop-*p*-ethyl relative to the reference susceptible population, which was susceptible to all the five AOPP herbicides. Much lower *R*/*S* values of 3.5, 2.4 and 3.5, respectively, were detected for clethodim, sethoxydim and pinoxaden. Molecular analysis of resistance confirmed that the Ile_{2041} to Asn mutation in the resistant population conferred resistance to AOPP herbicides, but not to CHD and DEN herbicides. This is the first report of a target site mutation that corresponded to resistance to AOPP herbicides in *P. fugax*. Proper resistance management practices are necessary to prevent ACCase-inhibiting herbicides from becoming ineffective over wide areas.

1. Introduction

Acetyl co-enzyme A carboxylase inhibitors include three chemical groups, aryloxyphenoxypropionates (AOPP), cyclohexanediones (CHD) and phenylpyrazolins (DEN). These herbicides inhibit acetyl-CoA carboxylase (ACCase; EC 6.4.1.2), an essential enzyme in fatty acid biosynthesis in eukaryotes and prokaryotes [1]. In plants, two forms of ACCase have been identified: the first is located in the chloroplast, which is essential in the biosynthesis of primary fatty acids, and the second is located in the cytosol, which is involved in biosynthesis of long chain fatty acids [2–4]. In most plants, chloroplastic ACCase is a "prokaryotic-type", multi-subunit enzyme. Subunits of the prokaryotic ACCase are encoded in the nuclear DNA, except the β-subunit of carboxyltransferase (CT), which is encoded by a chloroplastic gene [2]. However, the Poaceae (Gramineae) family and several members of the Geraniaceae are exceptional by having only the relatively sensitive eukaryotic ACCase in both the chloroplasts and the cytosol and are killed by ACCase-inhibiting herbicides [5,6].

All isoforms of ACCase have three catalytic domains: the biotin carboxyl-carrier (BCC), the biotin carboxylase (BC) and carboxyl transferase (CT) domains [7–9]. The CT domain of the plastid-localised multi-domain ACCase is the target of action for ACCase-inhibiting herbicides [4,10].

Since their commercial introduction of AOPPs, the ACCase-inhibiting herbicides have been widely used in world agriculture to control a broad range of grass weeds in many dicotyledonous and some cereal crops [11,12]. The continued and widespread use of these graminicides has been responsible for selecting populations of 42 grass weed species worldwide and 6 grass weed species in China with resistant to these herbicides [13].

There are three known resistance mechanisms to ACCase-inhibiting herbicides in grasses: enhanced herbicide metabolism, over expression of ACCase, or the presence of an altered target-site [14]. Often resistance to ACCase inhibiting herbicides is conferred by target-site modifications, typically resulting from a single amino acid change that reduces sensitivity of the ACCase enzyme to these herbicides. 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 Ser, Trp_{2027} to Cys, Ile_{2041} to Asn or Val, Asn_{2078} to Gly, Cys_{2088} to Arg, and Gly_{2096} to Ala [8,15–19]. 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 [17,20].

* Corresponding author. Fax: +86 571 85224293.
E-mail address: chenjie01@sinochem.com (J. Chen).
[1] These authors contributed equally to this work.

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Table 1
The recommended field dose and treated dose of the eight ACCase-inhibiting herbicides.

Herbicide	Group	RFD*(g ai ha^{-1})	Treated dose	
Clodinafop-propargyl	AOPP	48	*S*	0, 1/2048×, 1/1024×,1/512×,1/256×, 1/128×, 1/64×,1/32×,1/16×,1/8×,1/4×
			R	0, 1/128×, 1/64×,1/32×,1/16×,1/8×,1/4×,1/2×,1×, 2×, 4×, 8×, 16×, 32×
Fluazifop-*p*-butyl	AOPP	75	*S*	0, 1/1024×,1/512×,1/256×, 1/128×, 1/64×,1/32×,1/16×,1/8×,1/4×,1/2×
			R	0, 1/64×,1/32×,1/16×,1/8×,1/4×,1/2×, 1×, 2×, 4×, 8×, 16×, 32×
Haloxyfop-R-methyl	AOPP	32	*S*	0, 1/1024×,1/512×,1/256×,1/128×, 1/64×,1/32×,1/16×,1/8×,1/4×,1/2×,1×
			R	0, 1/64×,1/32×,1/16×,1/8×,1/4×,1/2×,1×, 2×, 4×, 8×, 16×, 32×
Quizalofop-*p*-ethyl	AOPP	60	*S*	0, 1/256×,1/128×, 1/64×,1/32×,1/16×,1/8×,1/4×
			R	0, 1/64×,1/32×,1/16×,1/8×,1/4×,1/2×, 1×, 2×, 4×, 8×, 16×, 32×, 64×
Fenoxaprop-*p*-ethyl	AOPP	57	*S*	0, 1/1024×,1/512×,1/256×, 1/128×, 1/64×,1/32×,1/16×,1/8×,1/4×,1/2×
			R	0, 1/128×, 1/64×,1/32×,1/16×,1/8×,1/4×,1/2×,1×, 2×, 4×, 8×, 16×
Clethodim	CHD	105	*S* and *R*	0, 1/1024×,1/512×,1/256×,1/128×,1/64×,1/32×,1/16×,1/8×,1/4×
Sethoxydim	DEN	156	*S* and *R*	0, 1/2048×, 1/1024×,1/512×,1/256×,1/128×,1/64×,1/32×,1/16×,1/8×,1/4×,1/2×,1×
Pinoxaden	CHD	45	*S* and *R*	0, 1/2048×, 1/1024×,1/512×,1/256×,1/128×,1/64×,1/32×,1/16×,1/8×,1/4×,1/2×,1×

* RFD = recommended field dose.

Table 2
Primers used to amplify the ACCase gene of *P. fugax* populations.

Primers	Sequence(5′–3′)	Amplicon size(bp)
F1027	CAGTTAGATAGTGGCGAAATCAGGTGGG	
R2029	CATAGCACTCGATGCGATCTGGGTTTAT	1003
F719	CTCCTGAATTTCCCAGCGGCAGACAGAT	
R2070	CCCTTGAGGTTCGAGAACATTACCCTTT	1262

Asia minor bluegrass (*Polypogon fugax* Nees ex Steud.) is a common annual grass weed distributed across China. Its life cycle is closely related to several winter crops, including wheat (*Triticum aestivum* L.) and oilseed rape (*Brassica napus* L.) and greatly reduces their yield and quality [21].

Since 2010, farmers in Sichuan Province, China, observed that clodinafop and haloxyfop at recommended doses failed to control *P. fugax* in wheat fields and oilseed rape fields after several years of successful control. To our knowledge, resistance of *P. fugax* to ACCase-inhibiting herbicides has not been reported. Therefore, the objectives of the present study were to: (1) investigate and quantify resistance of *P. fugax* to clodinafop-propargyl and other ACCase inhibitor herbicides; and (2) determine the molecular basis of such resistance in Chinese populations.

2. Materials and methods

2.1. Plant material

Seeds of a putative resistant *P. fugax* were collected from Qingsheng country (29°54′1″N, 103°48′57″E), Sichuan province, China where clodinafop and haloxyfop had been used annually for over 5 years and control failure was evident in wheat and oilseed rape fields. A susceptible *P. fugax* population was also collected from a non-cultivated area where herbicides had not been applied in Xichang city, Sichuan province (27°50′56″N, 102°15′53″E). Seeds from 50 mature plants were collected randomly by hand and bulked. The collected seeds were air dried and stored in paper bags at room temperature until used.

2.2. Whole-plant dose–response assay

Whole-plant dose-response experiments were conducted in a greenhouse at Zhejiang Chemical Industry Research Institute (30°15′34″N, 120°03′53″E), Hangzhou, Zhejiang province, China to confirm and characterize clodinafop, fenoxaprop, quizalofop, fluazifop, haloxyfop, pinoxaden, clethodim and sethoxydim resistance in *P. fugax* populations. Seeds were sown in separate 7.5-cm diameter plastic pots containing a sterile potting medium (mixed vegetable garden soil/coversoil, 2:1, v/v). Plants were maintained in the greenhouse with temperature at approximately 20 °C, 75% humidity and natural sunlight and thinned to 10 evenly sized plants per pot before treatment. The soil was maintained wet throughout the experiment by adding water to saucer under the pots.

Herbicide treatments were applied at the three-leaf stage *P. fugax* plants using a compressed air laboratory spray tower equipped with a Teejet 9503EVS flat fan nozzle to deliver 450 L ha^{-1} at 0.28 MPa. Plants were treated with the eight ACCase-inhibiting herbicides mentioned above, 10% clodinafop, 10% fenoxaprop, 10% quizalofop, 10% fluazifop, 10% haloxyfop, 0.1% pinoxaden, 1% clethodim and 1% sethoxydim. The recommended dose and treated dose of each herbicide are shown in Table 1. All herbicides were provided by Zhejiang Chemical Industry Research Institute. Plants were returned to the greenhouse after herbicide application.

The experiment was in a completely randomized design with three replications and was repeated. Twenty days after treatment, plants were cut at the soil surface and oven-dried for 48 h at 60 °C to determine above-ground biomass, and dry weight was expressed as a percentage of the untreated control to standardize comparisons between populations. The least significance (LSD) between the two experiments was calculated by using one-way analysis of variance for the values. The ANOVA showed no significant difference between the two experiments, means of treatments were averaged over the two experiments. A non-linear regression using the following four-parameter log-logistic equation was used to fit data [22]:

$$Y = C + (D - C)/[1 + (x/GR_{50})^b]$$

where C = the lower limit, D = the upper limit, b = the slope at the GR_{50}, and GR_{50} = the herbicide dose required for 50% growth reduction. The level of resistance for the *P. fugax* populations was determined by the resistance ratio (R/S), which was calculated as the GR_{50} of the R population divided by the GR_{50} of the susceptible control (S) population.

2.3. Molecular basis of resistance

P. fugax leaves of individual resistant and susceptible plants were harvested at 25 DAT and their DNA was extracted with a plant DNA kit (Generay Biotech Co., Ltd., Shanghai, China).

Two forward and reverse overlapping primers (Sangon Biotech Co., Ltd., Shanghai, China) were designed to amplify the CT domain of ACCase gene of *P. fugax* in two segments according to the sequence of Japanese Foxtail (*Alopecurus japonicus*) (GenBank accession JX502888) (Table 2). Primers F719 and R2070 were used to amplify a 1262bp fragment (GenBank accession numbers