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The role of cytochrome P450 monooxygenase in the different responses to fenoxaprop-P-ethyl in annual bluegrass (*Poa annua* L.) and short awned foxtail (*Alopecurus aequalis* Sobol.)

Hongchun Wang [a,b,c], Jun Li [a,b], Bo Lv [d], Yuanlai Lou [c], Liyao Dong [a,b,*]

[a] College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China
[b] Key Laboratory of Integrated Management of Crop Diseases and Pests (Nanjing Agricultural University), Ministry of Education, Nanjing 210095, China
[c] Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China
[d] College of Science, Nanjing Agricultural University, Jiangsu Key Laboratory of Pesticide Science, Nanjing 210095, China

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ABSTRACT

Herbicide resistance or tolerance in weeds mediated by cytochrome P450 monooxygenase is a considerable problem. However, cytochrome P450 mediated resistance or tolerance in weeds was less studied. Thus, in this work, the role of the cytochrome P450 monooxygenase in the different responses of *Poa annua* and *Alopecurus aequalis* to fenoxaprop-P-ethyl was studied. We found that the effect of fenoxaprop-P-ethyl could be synergized by piperonyl butoxide (PBO) in *P. annua*, but not by malathion. After being treated with fenoxaprop-P-ethyl (containing mefenpyr-diethyl), the contents of cytochrome P450 and cytochrome b_5 in *P. annua* increased significantly compared to plants treated with mefenpyr-diethyl only or untreated plants. However, the increase was less in *A. aequalis*, which was susceptible to fenoxaprop-P-ethyl. The activities of ρ-nitroanisole O-demethylase (PNOD), ethoxyresorufin O-deethylase (EROD), ethoxycoumarin oxidase (ECOD) and NADPH-dependent cytochrome P450 reductase mediated by cytochrome P450 monooxygenase increased in *P. annua* after treatment with fenoxaprop-P-ethyl, especially the activities of ECOD and cytochrome P450 reductase. Besides this, cytochrome P450 monooxygenase activity toward fenoxaprop-P-ethyl in *P. annua* increased significantly compared to untreated or treated with mefenpyr-diethyl plants and treated or untreated *A. aequalis*. Cytochrome P450 monooxygenase may play an important role in the different responses to fenoxaprop-P-ethyl in *P. annua* and *A. aequalis*.

1. Introduction

The relative rate of herbicide degradation in crops and competing weeds is a major determinant of herbicide selectivity [1]. In crops, several herbicides are rapidly detoxified by glutathione S-transferase (GST), cytochrome P450 monooxygenase and other enzymes. But the frequent application of herbicides has resulted in the evolution of resistant weed biotypes and the exacerbation of tolerant weeds [2–7]. In weeds, the insensitive target enzyme based resistance is now well understood [6–10]. In contrast, the role of detoxifying enzyme, especially the cytochrome P450 monooxygenase, in endowing herbicide resistance or tolerance in weeds is poorly understood. Herbicide-resistant *Alopecurus myosuroides* and *Lolium rigidum* biotypes that displayed non-target herbicide resistance across several herbicide modes of action were first reported in 1985 and 1986, respectively [11,12]. Subsequently, *in vivo* studies on herbicide metabolism and cytochrome P450 inhibitors in resistant biotypes found that cytochrome P450 monooxygenase enhanced rates of metabolism of several herbicides [7,13]. The evolution of non-target herbicide resistance due to enhanced rates of herbicide metabolism catalyzed by cytochrome P450 monooxygase has been demonstrated in resistant biotypes of several weeds, such as *L. rigidum*, *A. myosuroides*, *Amaranthus hybridus*, *Bromus tectorum*, *Avena sterilis*, *Phalaris minor*, *Echinochloa phyllopogon*, *Stellaria media*, *Digitaria sanguinalis* and *Sinapis arvensis* [13–21]. However, biochemical studies to characterize herbicide resistance or tolerance mediated by cytochrome P450 monooxygenase in weed species have been less successful. To date, cytochrome P450 monooxygenases that degrade herbicides have not yet been successfully isolated from herbicide-resistant *L. rigidum* or *A. myosuroides* [7], but they have been isolated from *E. phyllopogon* [21], *Oryza sativa* and *Cyperus serotinus* [22]. Studies have shown that induction of cytochrome P450 monooxygenase was involved in multiple herbicide resistance in *E. phyllopogon* [21]. The increase in herbicide resistance, mediated by cytochrome

* Corresponding author at: College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China.
E-mail address: dly@njau.edu.cn (D. Liyao).

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P450 monooxygenase, will have important negative consequences in crop production [7].

Fenoxaprop-P-ethyl is an aryloxyphenoxypropinate (AOPP) herbicide that is used at the postemergence stage to control grass weeds in *Triticum aestivum* (wheat), *Arachis hypogaea* (peanut), *Gossypium hirsutum* (cotton), and *Brassica campestris* (oilseed rape) fields [23]. *Poa annua*, a grass that is found worldwide, which used to be a sporadic species, has recently become a problematic weed [24]. Studies have shown that *P. annua* was tolerant to aryloxyphenoxypropionate (AOPP) and cyclohexanedione (CHD) herbicides [8,25]. The insensitive ACCase and the Leu-1781 codon play a considerable role in resistance to some of ACCase-inhibiting herbicides in *P. annua* [8,26,27]. However, the role of cytochrome P450 monooxygenase in tolerance to fenoxaprop-P-ethyl in *P. annua* is not fully understood.

In this study, microsomal fractions isolated from fenoxaprop-P-ethyl tolerant *P. annua* and fenoxaprop-P-ethyl susceptible weed were used to determine cytochrome P450 content, cytochrome b_5 content and cytochrome P450 monooxygenase activity. The effects of cytochrome P450 monooxygenase inhibitors on the dose response to fenoxaprop-P-ethyl in *P. annua* and *Alopecurus aequalis* were also investigated.

2. Materials and methods

2.1. Chemicals

Fenoxaprop-P-ethyl (69 g L^{-1} emulsion in water (EW, containing 19 g L^{-1} mefenpyr-diethyl used as a safener)) and mefenpyr-diethyl (96%) were provided by Bayer Crop Science. Resorufin, ρ-nitroanisole, ρ-Nitrophenol, 7-hydrocycoumarin, 7-ethoxyresorufin and 7-ethyoxycoumarin were purchased from Sigma (St Louis, MO) and an inhibitor of cytochrome P450, piperonyl butoxide (PBO) (97%), was obtained from Aladdin (Shanghai, China). Malathion (95%) was obtained from Jiangsu Fengshan Group Co. Ltd. (Jiangsu, China).

2.2. Plant materials

P. annua seeds were collected from different oilseed rape and wheat fields in Shanghai, Jiangsu, Henan and Anhui provinces where fenoxaprop-P-ethyl had been applied for various lengths of time. Some weed seeds were also collected from fallow fields or mountain areas where fenoxaprop-P-ethyl had never been applied. The *P. annua* seed collection sites and their herbicide application histories are shown in Table 1.

A. aequalis, *Alopecurus japonicus*, *Beckmannia syzigachne*, and *Selerochloa kengiana* seeds were collected from Zijin Mountain, Nanjing where fenoxaprop-P-ethyl has never been applied.

2.3. Whole plant pot experiments

Seeds were placed on two filter papers (Whatman #1) with 5 mL of deionized water in plastic Petri dishes (9 cm diameter) and put in an incubator at 4 °C in the dark for 24 h. Then the seeds were sown in 11 cm diameter plastic pots filled with a 2:1 (wt/wt) mixture of sand and soil. Soil pH was 5.6 and the organic matter content was 1.4%. The pots had holes in the bottom to allow them to absorb water from dishes placed under each pot. All the pots were placed in a growth chamber at 20/15 °C, with a 12 h light/12 h dark cycle, a photon flux density of 151 μMol m^{-2} s^{-1} and at 85% relative humidity. After emergence, the seedlings were thinned to 25 plants per pot. Three weeks after planting, the plants were sprayed with different doses of fenoxaprop-P-ethyl (containing mefenpyr-diethyl) or mefenpyr-diethyl, at the three to four-leaf stage, using a laboratory sprayer equipped with a flat-fan nozzle calibrated to deliver 480 L ha^{-1} at 230 kPa. They were then returned to the controlled environment. Fenoxaprop-P-ethyl was applied at doses of 0, 1125, 2250, 4500, 9000 and 18,000 g ai ha^{-1} for *P. annua* and 0, 3.75, 7.50, 15.0, 30.0 and 60.0 g ai ha^{-1} for *A. aequalis*, *A. japonicus*, *B. syzigachne* and *S. kengiana*. Mefenpyr-diethyl was applied at doses of 287.5, 574.5, 1149, 2298 and 4596 g ai ha^{-1} for *P. annua* and 1.04, 2.08, 4.16, 8.32 and 16.64 g ai ha^{-1} for *A. aequalis*, *A. japonicus*, *B. syzigachne*, and *S. kengiana*, which were matched with the rates of it in the doses of fenoxaprop-P-ethyl. Mefenpyr-diethyl (96%) was formulated in a mixture of acetone, emulsifier (special ingredient for 69 g L^{-1} fenoxaprop-P-ethyl EW) and water. Reference plants were treated with a mixture of acetone, emulsifier (special ingredient for 69 g L^{-1} fenoxaprop-P-ethyl EW) and water. Two weeks after treatment, the above ground parts of the plants were harvested and the fresh weight was determined. The experiments were conducted four times using a completely randomized design with four replications. The effective dose of fenoxaprop-P-ethyl causing 50% inhibition in fresh weight (ED_{50}) was calculated using non-linear regression analysis with OriginPro® software (version 8.5, Origin Lab Corporation, MA, USA) [28]. Data sets from repeated experiments were pooled and analyzed using ANOVA. When the variance between repeated experiments was not significant, the pooled data were used for subsequent analyses. The data were fitted to the log–logistic model:

$$y = c + [(d - c)/[1 + (x/ED_{50})^{b}]$$

Where c = the lower limit, d = the upper limit, b = the slope, ED_{50} = the dose giving 50% response and x is the herbicide dose. Significant differences among the treatment means were identified using Tukey's test at a 0.05 level of significance.

2.4. Effect of fenoxaprop-P-ethyl and cytochrome P450 inhibitors on weed growth

P. annua (collected from Zijin Mountain) and *A. aequalis* seeds were pre-germinated and cultured as described above. Three weeks after planting, the plants were sprayed with PBO, malathion, fenoxaprop-P-ethyl, mefenpyr-diethyl, PBO plus fenoxaprop-P-ethyl, PBO plus mefenpyr-diethyl, malathion plus fenxoxaprop-P-ethyl or malathion plus mefenpyr-diethyl at the three to four-leaf stage using a laboratory sprayer as described above. Herbicide rates were chosen in order to obtain the ED_{50} value for each treatment. Variable doses of mefenpyr-diethyl were applied so that they matched the amount of it in the variable fenoxaprop-P-ethyl rates applied. PBO was applied in two applications of 2100 g ai ha^{-1} in 97 L ha^{-1} water to give a total application of 4200 g ai ha^{-1} in a total volume of 194 L ha^{-1} water. Malathion was applied at 0.63, 250 or 1000 g ai ha^{-1}. Malathion and PBO were formulated in a mixture of emulsifier (Tween 80, 1 mL L^{-1} in water) and acetone and applied 1 h prior to herbicide application [29]. Reference plants were treated with a mixture of emulsifier and hydrocarbon solvent. Two weeks after treatment, the above ground parts of the plants were harvested and fresh weights were determined. The experiments were conducted four times using a completely randomized design with four replications.

2.5. Herbicide treatment and microsome isolation

P. annua (collected from Zijin Mountain) and *A. aequalis* seeds were placed on two filter papers (Whatman #1) with 5 mL of deionized water in plastic Petri dishes (9 cm diameter) and put in an incubator at 4 °C in the dark for 7 days. Germinated seedlings were transferred to plastic pots and grown in 1/2 strength