Studies on the tolerance to new herbicide pyribambenz-propyl in oilseed rape

JIN Zonglai[1], MA Jiayu[1], ZHANG Fan[1], ZHANG Wenfang[1], TANG Qinghong[2], LU Long[2], YE Qingfu[1], ZHOU Weijun[1]

[1] *College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310029, China*
[2] *Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China*
Email: wjzhou@zju.edu.cn

Abstract

Experiments were conducted to investigate the tolerance, its mechanism and the best concentration of PP (Pyribambenz-propyl) and Bispyribac-sodium (BS, Nominee) in *Brassica rapa* (including 4 varieties), *B. juncea* and *B. napus* determined as the activity of acetolactate synthase (ALS). The results indicated that the ALS activities of PP treatments under higher concentration (500 and 1000 mg/l) were significantly lower than that of the control, while the treatment of PP at low concentration (100 mg/l) showed the higher ALS activity. *B. rapa* was more sensitive to PP than other two *Brassica* species (which was 7.7% lower than that of *B. juncea*), and *B. napus* showed the medium tolerance. Within 4 cultivars of *B. rapa*, Changshaguihuazi and Xiaoyoucai were more sensitive than the other two cultivars. Cultivar Jianghuang showed the highest resistance, with the 6.5% higher of ALS activity compared to Changshaguihuazi, the most susceptible variety. Further study indicated that the ALS activity increased at 14 days, then began to fall at 28 days (except for 1000 mg/l). Among the all treatments, the 100 mg/l PP played an important role in the promotion of the enzyme effect and improved the growth of plants.

Key words: New herbicide Pyribambenz-propyl, oilseed rape, Brassica species, ALS activity, tolerance

Introduction

The Pyribambenz-propyl {Propyl 4-[2-(4,6-dimethoxy-2-pyrimidinyloxy) benzyl amino] benzoate, PP, formerly ZJ273} is a novel oilseed rape field herbicide with the advantages of low dosage, low mammalian toxicity, broad weeding spectrum and compatible to environment. This novel herbicide derived from a precursor compound (as a derivative of 2-pyrimidinyloxy-N-aryl-benzoate) possessing new structure and efficient biological activity (Wu et al., 2003). This new herbicide was supposed to be one kind of herbicides with the inhibition in biosynthesis (Cobb et al., 2000; Heim et al., 1993; Scarponi et al., 1997; Erksson et al., 1996). Previous results of the biological activity and harmful symptom of the treated plant showed the similar effects as treated by the ALS (acetolactate synthase) inhibiting herbicides (Tang et al., 2005). Nevertheless, there is some difference between them, maybe the PP is a precursor herbicide and its inhibitory effect was functioned through the metabolization within the plant (Chen et al., 2005).

ALS (syn. acetohydroxyacid synthase, AHAS) catalyses the first step of parallel reactions in the biosynthesis of the branched-chain amino acids valine, leucine and isoleucine in plants, bacteria and yeast, which reaction is responsible for the formation of 2-acetolactate and CO_2 by condensation of two molecules of pyruvate or pyruvate and 2-ketobutyrate (Leyval et al., 2003). This anabolic enzyme has already been purified and investigated in several fields of research, like recombinant bacterial strains mentioned by Xing & Whitman (1994). This enzyme seemed to be a key enzyme in the pathway of valine biosynthesis because of the feed-back inhibition by branched-chain amino acids (Eggeling et al., 1987; Durner et al., 1988; Bryan et al., 1991).

Until now, there is no report about this new herbicide regarding its mechanism in the plant. Understanding its mode of action is vital for the successful application in the field. This paper presents the tolerance of new herbicide PP in oilseed rape through its influence on the anabolic enzyme ALS, compared to the herbicide Bispyribac-sodium (BS), a known ALS inhibitor (Pu et al., 2003).

Materials and Methods

Plant material: Six cultivars of oilseed rape from *B. rapa* (syn. *B. campestris*, including 4 varieties), *B. juncea* and *B. napus* were used in the experiments. Field trials were conducted at the Zhejiang University farm, Hangzhou (30^0 10′N, 120^0 12′E). In a rotational cropping system after harvest of rice (*Oryza sativa* L.), rapeseeds were sown in early October of 2004 and 2005 in the field of a silt-loam soil with initial 0.18% total nitrogen, 1.75% organic matter and 63 mg/kg soil available phosphorus. Each plot was 1.0 m long and 0.8 m wide, and the experiment was laid out in a randomized complete block design with 3 replications in each case. The herbicide PP was supplied by Dr. Lu (Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, China), and the control herbicide BS was purchased from the local market. Various concentrations of PP (0, 100, 500 and 1000 mg/l) and BS (0, 100 and 500 mg/l) were foliar applied at 5 leaf stage at the quantity of 750 kg/ha. Conventional crop management was used during the growing period (Zhou, 2001; Zhang et al., 2006).

ALS/AHAS assays: Physiological measurements were sampled from the plants in the central rows of each plot at various growth intervals (7, 14 and 28 days after the treatment). The fresh leaf samples were stored at -80 °C until use. The ALS/AHAS assay was based on the method of Yang et al. (2000) and Leyval et al. (2003) with modifications. Enzyme ALS/AHAS specific activity was determined by the absorbance of the reddish reaction mixture that measured at 530 nm, and was expressed in absorbance of 2-acetolactate formed per mg protein per hour.

Statistical analysis: In all experiments, data were analyzed using the statistical programme SAS and the analysis of variance (ANOVA) was followed by Fisher's protected LSD test to identify homogenous groups within the means. Significant differences among treatments were considered at the $P < 0.05$ level.

Results

The activity of ALS from leaf extracts of *B. juncea*, *B. napus* and *B. rapa* was compared in the presence of different herbicides and tolerance values were determined. The results indicated that the ALS activities of PP treatments under higher concentration (500 and 1000 mg/l) were significantly lower than that of the control, while the treatment of PP at low concentration (100 mg/l) showed the higher ALS activity (Fig. 1). In general within the *Brassica*, *B. rapa* was more sensitive than other two species (which was 7.7% lower than that of *B. juncea* on the average of *B. rapa* 4 cultivars), and *B. napus* showed the medium tolerance. Compared in 4 cultivars of *B. rapa,* cultivars Changshaguihuazi and Xiaoyoucai were more sensitive than the other two varieties Jianghuang and Youbai (Table 1). Jianghuang showed the highest resistance, with the 6.5% higher of ALS sensitivity compared to that of Xiaoyoucai, the most susceptible variety. There was no significant difference between cultivars Changshaguihuazi and Xiaoyoucai in the sensitivity of ALS.

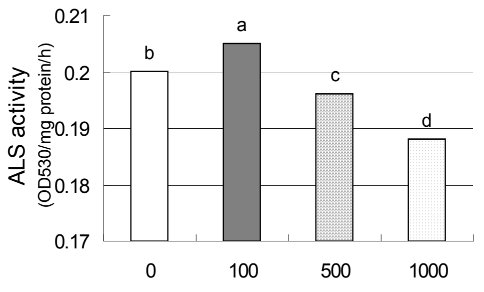

Fig. 1. Leaf ALS activity (OD_{530}/mg protein/h) of ZJ273 treatments under different concentrations (0, 100, 500 and 1000 mg/l) for 6 varieties from 3 species of oilseed rape pooled from the measurements of 3 sampling intervals.

Table 1. The sensitivity for PP herbicide using the activity of ALS isolated from leaves of 6 oilseed rape varieties. The tolerance value was expressed as the average of activity in the all treatments. Data are means (SE) within a column followed by the same letter do not differ significantly according to Fisher's protected LSD test (P < 0.05).

Species	Cultivar	Tolerance value
B. juncea	Hongyejie	0.209 *(0.025)* a
B. napus	ZS 758	0.197 *(0.013)* b
B. rapa	Jianghuang	0.201 *(0.046)* a
	Youbai	0.194 *(0.016)* b
	Changshaguihuazi	0.189 *(0.014)* c
	Xiaoyoucai	0.188 *(0.026)* c

Further study indicated that in general the ALS activity of PP treatment increased at 14 days and then began to fall to 28 days, while the ALS activity of BS treatment was not detectable due to the deterioration of the plant (Fig. 2). At the first 7 days, high activity of ALS was observed at the 100 mg/L PP, except for cv. Hongyejie (Fig. 2A), because of its resistance to the herbicide. The subsequent 7 days until 14 days, nearly all the ALS activity of plants that were applied by herbicide PP was recovered, even better than that of the control except for *B. juncea* (Fig. 2A). Based on the data from *B. juncea* it was found that the activity of enzyme was declining with the increasing of PP concentrations, while they were still relatively higher than the others in the rest of cultivars. The data obtained from the 28 days after PP treatment were lower than that observed from 14 days, respectively.

There was no obvious change in activity of ALS (around 0.220 OD_{530}/mg protein/h) of *B. juncea* between 7 and 14 days, except for the treatment of PP at high concentration (1000 mg/L) which also showed a decrease along with the prolongation of growth intervals. At the 28 days, ALS activity of 100 mg/l PP treatment did not decrease significantly as compared to the other concentrations (Fig. 2A).

Like as *B. juncea,* the same trend was found in *B. napus* that there was no obvious difference in ALS activity of PP treatments at the first 14 days, and then fell to 0.168 OD_{530}/mg protein/h in the control (the lowest at 28 days) (Fig. 2B). Surprisingly, treatment of 100 mg/l PP still maintained higher ALS activity at 28 days which was significantly higher than that at 7 and 14 days as well as higher than that of other treatments.

The data from the experiments in 4 cultivars of *B. rapa* denoted that it was similar with other two species *B. juncea* and *B.*

napus, though *B. rapa* was relatively more sensitive species in *Brassica*. However, variation existed among various PP treatments and cultivars of *B. rapa* (Fig. 2C-F). Compared with the ALS activity at 14 days, lower enzyme activities at 7 and 28 days were observed from most of the PP treatments. But the ALS activities of PP treatments at 100 and 500 mg/l were gradually decreased with the prolongation of growth intervals in cultivar Jianghuang (Fig. 2C). In cultivar Changshaguihuazi, low concentration (100 mg/l) of PP had similar ALS activity with the control at 7 and 28 days, and all PP treatments had higher ALS activities than that of the control at14 days (Fig. 2D). Higher ALS activities were also observed from the 100 and 500 mg/l PP treatments at 14 days and no obvious difference between PP treatments and the control at 28 days in variety Xiaoyoucai (Fig. 2E). In cultivar Youbai, 100 and 500 mg/l PP treatments showed higher ALS activities at 28 Youbai, 100 and 500 mg/l PP treatments showed higher ALS activities at 28 days (Fig. 2F).

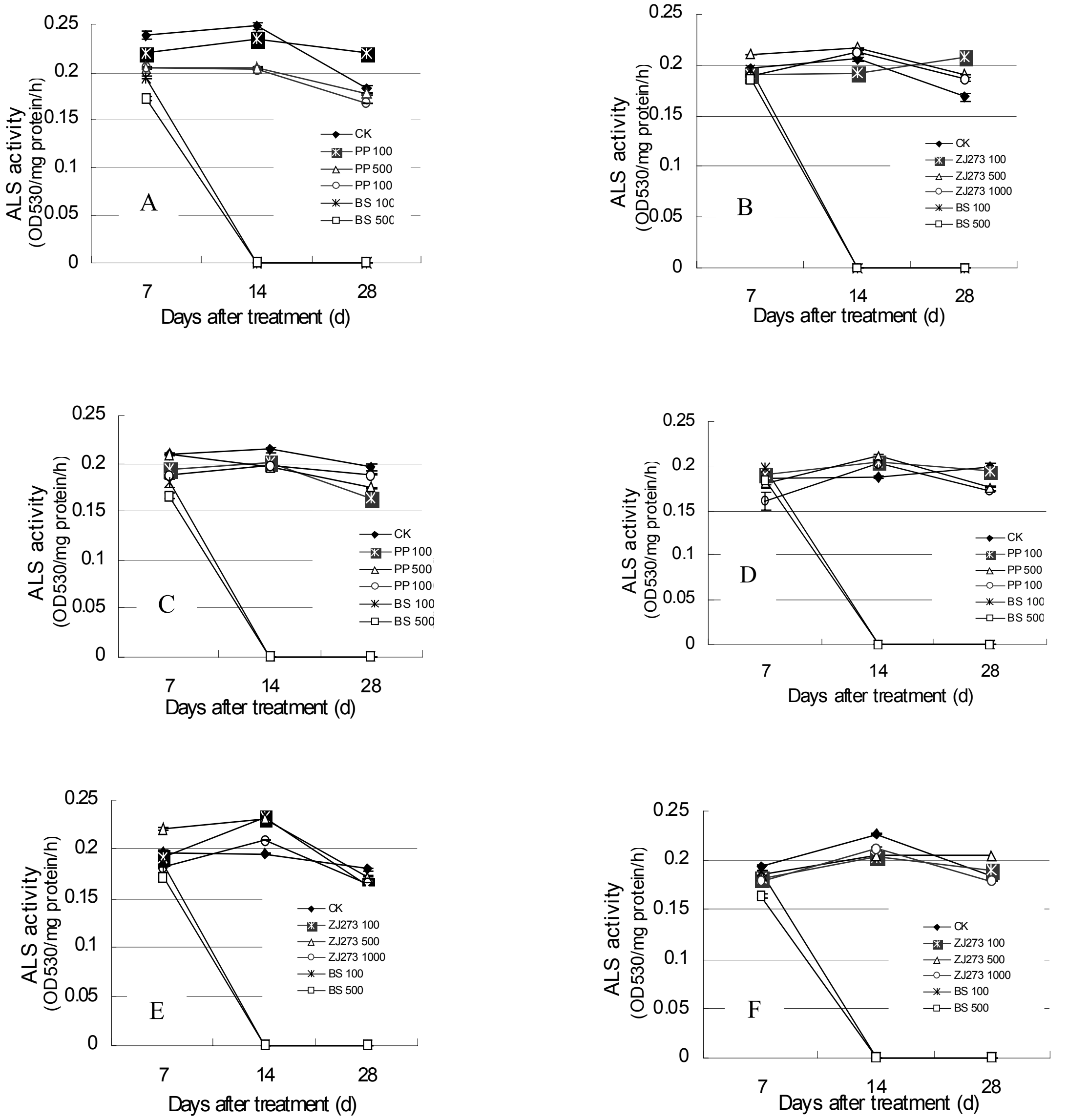

Fig. 2. Leaf ALS activity (OD_{530}/mg protein/h) of PP treatments under different concentrations (0, 100, 500 and 1000 mg/l) for 6 varieties from 3 species of oilseed rape measured at 3 sampling intervals (7, 14 and 28 days after the treatment). (A) Hongyejie (*B. juncea*), (B) ZS 758 (*B. napus*), (C) Jianghuang (*B. rapa*), (D) Changshaguihuazi (*B. rapa*), (E) Xiaoyoucai (*B. rapa*), and (F) Youbai (*B. rapa*). Bars indicate SE.

Discussion

The valine biosynthetic pathway is strongly regulated, essentially at the ALS/AHAS level, by its ultimate products, the branched-chain amino acids, valine and leucine. The ALS/AHAS activities of the pathway are inhibited by its end products, the branched-chain amino acids (Beyer et al., 1998; Wright et al., 1998; Florence & Bastide, 1997). In this study, the activity of ALS from the treatment of 100 mg/l PP seemed to be the optimal concentration, which enhanced the enzyme activity and promoted the growth of plants, while other two higher concentrations of PP had the inhibiting effect. Compared to the BS, an ALS-inhibitor herbicide, much less damage of PP to oilseed rape was observed. Plants treated by high concentration of BS (500 mg/l) displayed serious injury after 3 days, and died at 14 days at both concentrations (100 and 500 mg/l).

As a new herbicide of rape field, the safety of rape to PP seemed to be more important than any other aspects. The experiments indicated that *B. rapa* was more sensitive to PP than other two *Brassica* species, and *B. napus* showed the medium tolerance. Within 4 cultivars of *B. rapa*, Changshaguihuazi and Xiaoyoucai were more sensitive than the other two cultivars. Cultivar Jianghuang showed the highest resistance, with the 6.5% higher of ALS activity compared to Changshaguihuazi, the most susceptible variety. Further research is under the way to clarify the mechanism of this new herbicide through physiological, biochemical and molecular approaches.

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