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RESEARCH ARTICLE

Safe application of prosulfocarb in faba-bean fields in China

Yuan Gao[1], Zhaoliang Gao[2], Guifang Duan[3], Teng Sun[3], Guohui Shen[1*], and Zhihui Tian[1*]

[1]Eco-Environmental Protection Research Institute, Shanghai Academy of Agricultural Sciences, Shanghai 201403, China.
[2]Forest & Fruit Tree Research Institute, Shanghai Academy of Agricultural Sciences, Shanghai 201403, China.
[3]GreenTec Laboratory, Shanghai Xiaoming Testing Technology Service Co. Ltd., Shanghai 201612, China.
*Corresponding author (zb5@saas.sh.cn; tianzhihui@saas.sh.cn).
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ABSTRACT

Although faba-bean (*Vicia faba* L.) is not a major crop in China, many farmers like to cultivate faba-bean in autumn as a fresh food or animal feed for next spring. However, no herbicides are available to control weeds in the faba-bean fields. Although prosulfocarb is a candidate herbicide, some farmers have reported that prosulfocarb can often cause harm to faba-bean. Our laboratory experiment results revealed that Tongcanxian No. 6, a faba-bean cultivar with large seeds, showed the lowest level of growth inhibition and Qidou No. 2, a faba-bean cultivar with small seeds, showed the highest level of growth inhibition when treated with 3960 and 7920 g ai ha^{-1} prosulfocarb. The growth inhibition rate of faba-bean was 0% when 3960 and 7920 g ai ha^{-1} prosulfocarb was sprayed within 6 d after sowing, indicating that prosulfocarb treatment should be applied as soon as possible after sowing. When the temperature exceeded 20 °C and soil moisture content exceeded 20%, it might have aggravated the phytotoxicity of 'Tongcanxian No. 6' and 'Qidou No. 2' caused by prosulfocarb. Two round of field experiments showed visually that the control effect of 3960 g ai ha^{-1} prosulfocarb on the total weeds exceeded 90% and safe to faba-bean at the same time. This study indicated that prosulfocarb can be safely used in faba-bean fields by improving agronomic methods, which can also provide a reference for weed management in faba-bean fields worldwide.

Key words: Application time, control effect, faba-bean, prosulfocarb, safety, *Vicia faba*.

INTRODUCTION

Faba-bean (*Vicia faba* L.) is a legume crop originated in the Middle East in prehistoric times, and is an important agricultural crop worldwide (Singh et al., 2013; Heuzé et al., 2021; Maalouf et al., 2021). Faba-bean is usually used as a food crop and fodder crop globally, such as the Middle East, Central and East Asia, and North Africa, and is also recently receiving renewed interest in European countries, North America, and Australia (Muehlbauer and Tullu, 1997; Satovic et al., 2013). The planting area and production of faba-bean ranks third among edible bean worldwide (White et al., 2015). There are two subspecies of faba-bean, *Vicia faba* L. unranked *major* (Harz) Beck (broad bean, Chinese bean) which produces large seeds (650-850 g per 1000 seeds) and *V. faba* unranked *minor* (Harz) Beck (horse bean, field bean) which produces small seeds (250-350 g per 1000 seeds) (Muehlbauer and Tullu, 1997), both of which are grown in China.

Chinese farmers also prefer to plant faba-bean in the fall, which make the bean popular fresh foods in the following year. Faba-bean is also used in large quantities as feed and green manure. China has the highest production of faba bean, accounting for more than 30% of world production (http://faostat.fao.org). Many farmers plant faba-bean in the autumn as overwintering crops, mainly in the Yangtze River Basin of China. Weeds found in faba-bean fields are more serious and similar to those in winter wheat fields, including weeds in the Poaceae family and various broad-leaved weeds (Yang et al., 2020). The herbicides currently used in faba-bean fields are relatively rare. Faba-bean is an important crop, and exploring a safe and low-cost herbicide, with simple application methods is urgently needed to control weeds in faba-bean fields. However, most herbicides in winter crop fields are safe for gramineous

crops, with acetyl-CoA carboxylase and acetolactate synthase inhibitors causing severe resistance to many weeds (Deng et al., 2015; Liu et al., 2015; Xia et al., 2015; Gao et al., 2017; Zhang et al., 2017). Therefore, prosulfocarb is a good candidate herbicide.

Prosulfocarb (*S*-benzyl *N*,*N*-dipropylcarbamothioate) is a compound of thiocarbamates, classified as Group N, synthesised by Syngenta (Basel, Switzerland). It is a broad-spectrum systemic and soil-acting herbicide mainly used for weed control in wheat fields (Busi and Powles, 2013). Although the specific target of prosulfocarb is not clear, it is considered to be a "multi-site" herbicide to a certain extent and is believed to inhibit lipid synthesis (Andreasen et al., 2020; http://www.weedscience.com/Pages/Herbicide.aspx). Unlike other single-site herbicides, reports on prosulfocarb resistance in weeds are rare.

Compared with hand-pulling weeding, chemical weeding has the advantages of a higher yield and lower labour costs (Adigun et al., 2017; Kolo et al., 2020). However, improper herbicide application can result in crop poisoning and yield losses (Pereira et al., 2015; Langaro et al., 2017). Herbicides should be applied judiciously, because the smaller weeds are easily destroyed by herbicides; however, crops may fail to avoid the damage induced by herbicides when they are very young. For example, crops are usually able to endure benzoic acid herbicides such as 2,4-D and MCPA after the late tillering stage (Way et al., 1984; Idziak et al., 2012). Therefore, the damage induced by herbicides to crops is not absolute, because the ability of crops to cope with damage induced by herbicides can be improved by changing the application dosage of herbicides or agronomic methods (Davies et al., 2017; Gao et al., 2024).

In this study, the results of pre-experiment showed that faba-bean was sensitive to prosulfocarb, especially when prosulfocarb was first applied and then faba-bean was sown. To reduce the sensitivity, herbicide application or proper agronomic methods should be attempted for the faba-bean to successfully survive. Thus, we conducted laboratory experiments to demonstrate the effect of faba-bean cultivars, prosulfocarb treatment time, temperature, and soil moisture content on safety and then verified the conclusions by conducting two round of field experiments to determine the effective methods for prosulfocarb safely used in faba-bean fields.

MATERIALS AND METHODS

Chemicals and faba-bean cultivars

Prosulfocarb EC (80%) (*S*-benzyl *N*,*N*-dipropylcarbamothioate) was provided by CAC Nantong Chemical Co., Ltd., Nantong city, Jiangsu Province, China. Six faba-bean (*Vicia faba* L.) cultivars often sown in China were selected for this study, including three large seed cultivars, Tongcanxian No. 6 (marked as TC-6), Tongcanxian No. 8 (marked as TC-8), and Qidou No. 9 (marked as QD-9), and three types of small seed cultivars, Hunan regular high-quality faba-bean (marked as HNRH), Qidou No. 2 (marked as QD-2), and Xiaoqing Hudou (marked as XQH).

Laboratory experiments to screen prosulfocarb-resistant faba-bean cultivars

The seeds of all cultivars of faba-bean were planted in plastic pots containing a 5:1 (w/w) mixture of loam and seedling substrate (Huai'an Fenglin Agricultural Technology Co., Ltd., Huai'an, Jiangsu, China). The seeds were sown at 8-10 cm below the soil surface. After sowing, prosulfocarb at 3960 and 7920 g ai ha^{-1} was applied to all cultivars of faba-bean, respectively, using a 3WP-2000 walking spraying system (Nanjing, China). The spraying system was equipped with a 390 mL min^{-1} flow nozzle with a pressure of 3.0 kg cm^{-2} at the time of spraying. When spraying, pots were placed in the spraying system, and then 30 mL diluted prosulfocarb solution was sprayed onto the soil at a forward speed 291 mm s^{-1} through the nozzle. All pots were arranged in randomized block design and placed in a growth chamber at 20/15 °C with 12 h light (20000 lx) and 12 h darkness each day after spraying, and watered every day. After 3 wk, the visual inhibition rate of each treatment was determined. There were 3-4 biological replicates for each treatment, and the experiment was repeated twice.

Laboratory experiments to determine safe time of prosulfocarb application

Two faba-bean cultivars, the most and least sensitive to prosulfocarb, obtained in "Laboratory experiments to screen prosulfocarb-resistant faba-bean cultivars" section, were selected for this study. The spraying method, dosage of prosulfocarb, and experimental design were the same as described in "Laboratory experiments to screen prosulfocarb-resistant faba-bean cultivars" section, except that the spraying time was set at 0, 3, 6, 9, 11 (pre-emergence), and 13 (post-emergence) d after sowing. They were placed in a plastic greenhouse for cultivation after spraying. The watering time was changed to once every 5 d. On the 21th day after sowing, the visual inhibition rate of each treatment was investigated. Each treatment contained at least three biological replicates, and the experiment was repeated twice.

Laboratory experiments to determine safe temperature of prosulfocarb application

Two faba-bean cultivars, the most and least sensitive to prosulfocarb obtained in "Laboratory experiments to screen prosulfocarb-resistant faba-bean cultivars" section, were selected for this study. To determine the effect of temperature on the safety of prosulfocarb on faba-bean, three groups of culture temperature, 5/10 °C, 15/20 °C, and 25/30 °C after prosulfocarb treatment were set for testing. In each group, the low temperature was in the dark period, and the high temperature was during the light period. The dosages of prosulfocarb were 0 and 7920 g ai ha^{-1}, and the application method and experimental design was the same as that described in "Laboratory experiments to screen prosulfocarb-resistant faba-bean cultivars" section. After prosulfocarb treatment, plants were placed in light incubators, set to 12 h light and 12 h darkness. On the 21th day after sowing, the visible inhibition rate of each treatment was investigated. Each treatment contained at least three biological replicates, and the entire experiment was repeated twice.

Laboratory experiments to determine safe soil moisture content of prosulfocarb application

Two faba-bean cultivars, the most and least sensitive to prosulfocarb obtained in "Laboratory experiments to screen prosulfocarb-resistant faba-bean cultivars" section, were selected for this study. To determine the effect of soil moisture content on the safety of prosulfocarb on faba-bean, three groups of soil moisture content, 20%, 40%, and 60% after prosulfocarb treatment were set for testing. The dosages of prosulfocarb were 0 and 7920 g ai ha^{-1}, and the application method and experimental design was the same as that described in "Laboratory experiments to screen prosulfocarb-resistant faba-bean cultivars" section. After spraying, they were placed in a plastic greenhouse for cultivation. During the cultivation process, a small amount of water (< 1 cm water layer) was added to the pot tray every 5 d from the fifth day after prosulfocarb application. On the 21th day after sowing, the visible inhibition rate of each treatment was investigated. Each treatment contained at least three biological replicates, and the entire experiment was repeated twice.

Field experiments verification

Two round of field experiments were carried out to verify whether prosulfocarb can be safely used in faba-bean fields, and the least sensitive faba-bean cultivar to prosulfocarb obtained in "Laboratory experiments to screen prosulfocarb-resistant faba-bean cultivars" section. The application method involved spraying prosulfocarb onto the soil after hole sowing, that is, pre-emergence treatment. The dosage of prosulfocarb was set to four dosages of 2040, 3000, 3960, and 6000 g ai ha^{-1}. For each treatment, four test plots (20 m^2 per plot, 5 m × 4m) were set as biological replicates, and the test was conducted twice in two years. All plots were completely randomized design. The results were investigated three times for each experimental trial. The faba-bean plant height was measured in the field, and a total of 20 plants were selected (five plants were randomly selected in each replicate). When the faba-bean matured, the middle 20 holes of faba-bean from each plot were harvested to determine the fresh weight, which was then converted into the yield of faba-bean per hectare. At the same time, the control effect of 3960 g ai ha^{-1} prosulfocarb on weeds was visually investigated. Other specific information is presented in Table 1.

Statistical analysis

All data were subjected to significance analysis using SPSS version 20 software (IBM, Armonk, New York, USA) by Duncan's multiple range test ($p < 0.05$).

Table 1. Information on two field experiments of prosulfocarb. DAT: Days after treatment.

Location	Starting time	Spraying time	Number of sowing faba-bean holes per plot	Pretreatment of test plot before sowing	Time and content of the first investigation	Time and content of the second investigation	Time and content of the third investigation
Nantong City, Jiangsu Province, China	1st November 2019	1st November 2019	70	Ploughing and ridging (about 10 cm)	34 DAT, measuring plant height and evaluating the control effect on weeds	124 DAT, evaluating the control effect on weeds	199 DAT, measuring the yield of faba bean
	29 October 2020	29 October 2020	88	Ploughing and ridging (about 15 cm)	35 DAT, measuring plant height and evaluating the control effect on weeds	125 DAT, evaluating the control effect on weeds	197 DAT, measuring the yield of faba bean

RESULTS

Faba-bean cultivars with large seeds are less sensitive to prosulfocarb

The laboratory experiment results (Figure 1) showed that faba-bean cultivars with large seeds, TC-6, TC-8, and QD-9 exhibited growth inhibition rates of 16%, 18%, and 18%, respectively, whereas the growth inhibition rates of faba-bean cultivars with small seeds, HNRH, QD-2, and XQH were 20%, 42%, and 38%, respectively, after 21 d of treatment with 3960 g ai ha^{-1} prosulfocarb. The growth inhibition rates of faba-bean cultivars with large seeds, TC-6, TC-8, and QD-9 were 29%, 32%, and 42%, respectively, while the growth inhibition rates of faba-bean cultivars with small seeds HNRH, QD-2, and XQH were 50%, 66%, and 56%, respectively, after 21 d of treatment with 7920 g ai ha^{-1} prosulfocarb. The TC-6 was the most resistant to prosulfocarb, and QD-2 was the most sensitive to prosulfocarb.

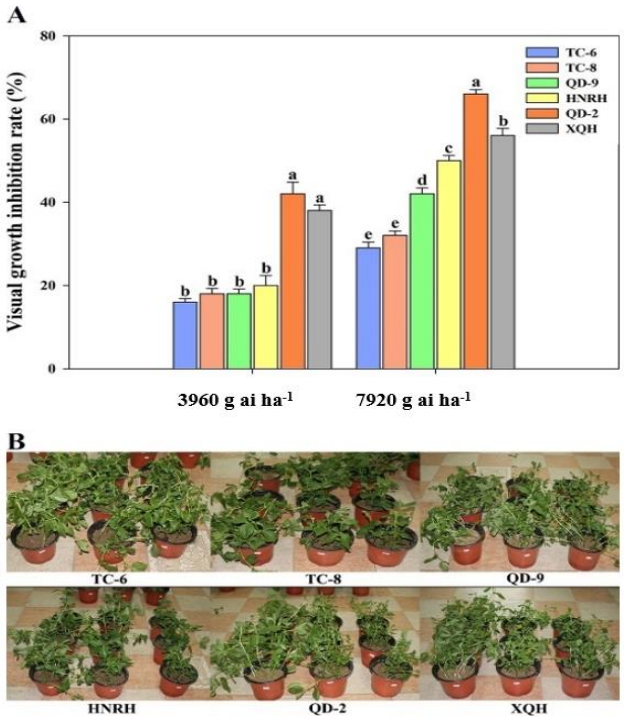

Figure 1. Sensitivity (A) and morphology (B) of different faba-bean cultivars to two doses of prosulfocarb according to growth inhibition rate. Different letters mean significant difference ($p < 0.05$).

Prosulfocarb applied immediately after faba-bean sowing is safer

The TC-6 (the most resistant to prosulfocarb) and QD-2 (the most sensitive to prosulfocarb) were used as laboratory experimental materials to determine the effect of the interval between faba-bean seeding and prosulfocarb application on the safety of faba-bean. The results (Figure 2) showed that when the interval between faba-bean sowing and prosulfocarb application was 0, 3, 6, 9, and 11 (pre-emergence) and 13 (post-emergence) d, the growth inhibition rates for 3960 g ai ha^{-1} prosulfocarb on TC-6 were 0%, 0%, 0%, 4.5%, 9.3%, and 17.9%, respectively; the growth inhibition rates on QD-2 were 0%, 0%, 0%, 4.9%, 18.6%, and 41.8%, respectively; the growth inhibition rates for 7920 g ai ha^{-1} prosulfocarb on TC-6 were 0%, 0%, 0%, 6.1%, 18.3%, and 26.6%; the growth inhibition rates on QD-2 were 0%, 0%, 0%, 0%, 19.0%, 27.0%, and 62.1%. The earlier the application time of prosulfocarb, the lower the growth inhibition rate of faba-bean was observed.

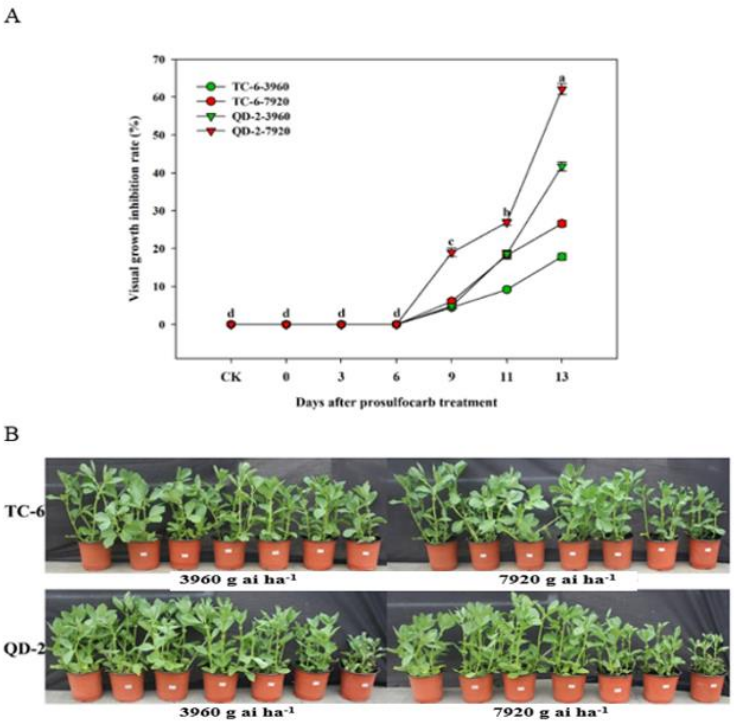

Figure 2. Effect in growth inhibition (A) and faba-bean morphology (B) of prosulfocarb application at different times. Shown from left to right is control, prosulfocarb was applied on the 0, 3, 6, 9, 11 (emergence), and 13 (post-emergence) d after sowing of faba-bean, in each picture. Different letters mean significant difference of the same cultivar treated with the same dosage of prosulfocarb at different time ($p < 0.05$).

Lower temperatures are more beneficial

Laboratory experiments were conducted to determine the effect of temperature on the safety of prosulfocarb on faba-bean. The results (Figure 3A) showed that when the culture temperature was set to 5/10 °C, 15/20 °C, and 25/30 °C after prosulfocarb application, the growth inhibition rate for 7920 g ai ha^{-1} prosulfocarb on TC-6 was 20.5%, 22.8%, and 30.2%, respectively, the growth inhibition rate of QD-2 was 19.3%, 30.0%, and 55.0%, respectively (Figure 3A). Lower temperature caused a lesser effect of prosulfocarb in inhibiting the growth of faba-bean.

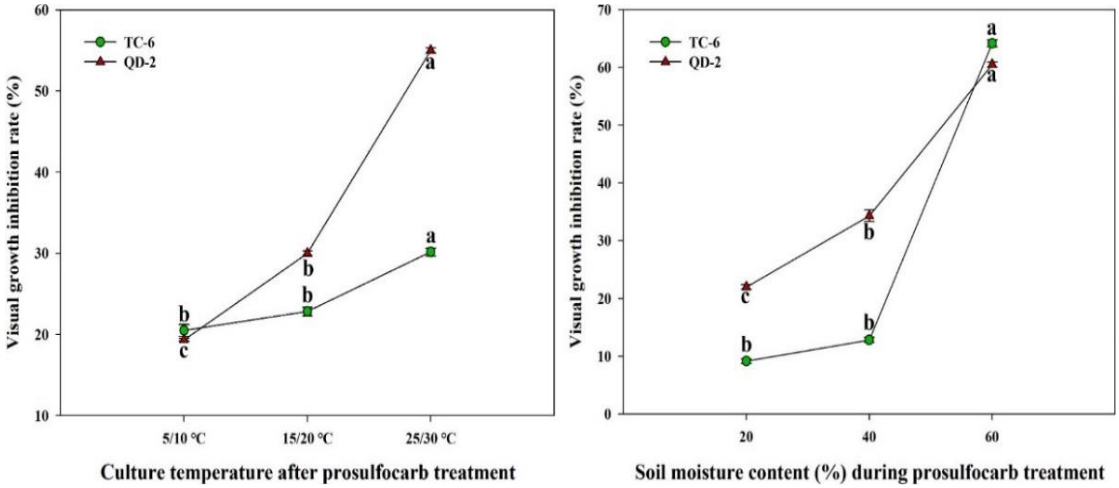

Figure 3. Influence of temperature (A) and soil moisture content (B) on the safety of prosulfocarb application on faba-beans. Different letters mean significant difference of the same cultivar treated with the same dosage of prosulfocarb at different soil moisture content ($p < 0.05$).

Lower soil moisture content is more beneficial

Laboratory experiments were conducted to determine the effect of soil moisture content on the safety of prosulfocarb on faba-bean. The results (Figure 3B) showed that when the soil moisture content was set to 20%, 40%, and 60% after prosulfocarb application, the growth inhibition rate for 7920 g ai ha^{-1} prosulfocarb on TC-6 was 9.2%, 12.8%, and 64.2%, respectively, and the growth inhibition rate of QD-2 was 22.0%, 34.3%, and 60.5%, respectively (Figure 3B). Less soil moisture content caused a lesser effect of prosulfocarb in inhibiting the growth of faba-bean.

Prosulfocarb is safe to faba-bean in field experiments

In this study, two field experiments were conducted to verify the results of the laboratory experiments and to determine whether prosulfocarb is safe on faba-bean in the field (Figures 4 and 5). The results of the experiments carried out in 2019 and 2020 showed that although the height of faba-bean was reduced by 9.5% and 9.0% about 1 mo after 6000 g ai ha^{-1} prosulfocarb treatment, there was nonsignificant difference in plant height between the prosulfocarb-treated groups and the untreated control group ($p < 0.05$). By the time of harvest, the yields of the prosulfocarb-treated groups were significantly higher than the untreated control group and the hand-pulling weeding treatment group ($p < 0.05$). The yield was increased by approximately 72.6% and 32.9% in 6000 g ai ha^{-1} prosulfocarb-treated group and by approximately 52.2% and 27.5% in 3960 g ai ha^{-1} prosulfocarb-treated group in 2019 and 2020 experiments, respectively. The control effect of weeds in 3960 g ai ha^{-1} prosulfocarb-treated group of the two experiments was visually estimated, and showed that on the 34th and 124th days after prosulfocarb treatment in the experiment in 2019, the control effects on *Galium aparine*, *Veronica didyma*, *Alopecurus aequalis*, *Phleum paniculatum* and total grasses were all greater than 90%, except *Beckmannia syzigachne* which was greater than 80%. On the 35th and 125th days after prosulfocarb treatment in 2020 experiment, the control effects on *G. aparine*, *Gnaphalium affine*, *Cerastium arvense*, *A. aequalis*, *B. syzigachne* and total grasses were all greater than 90%.

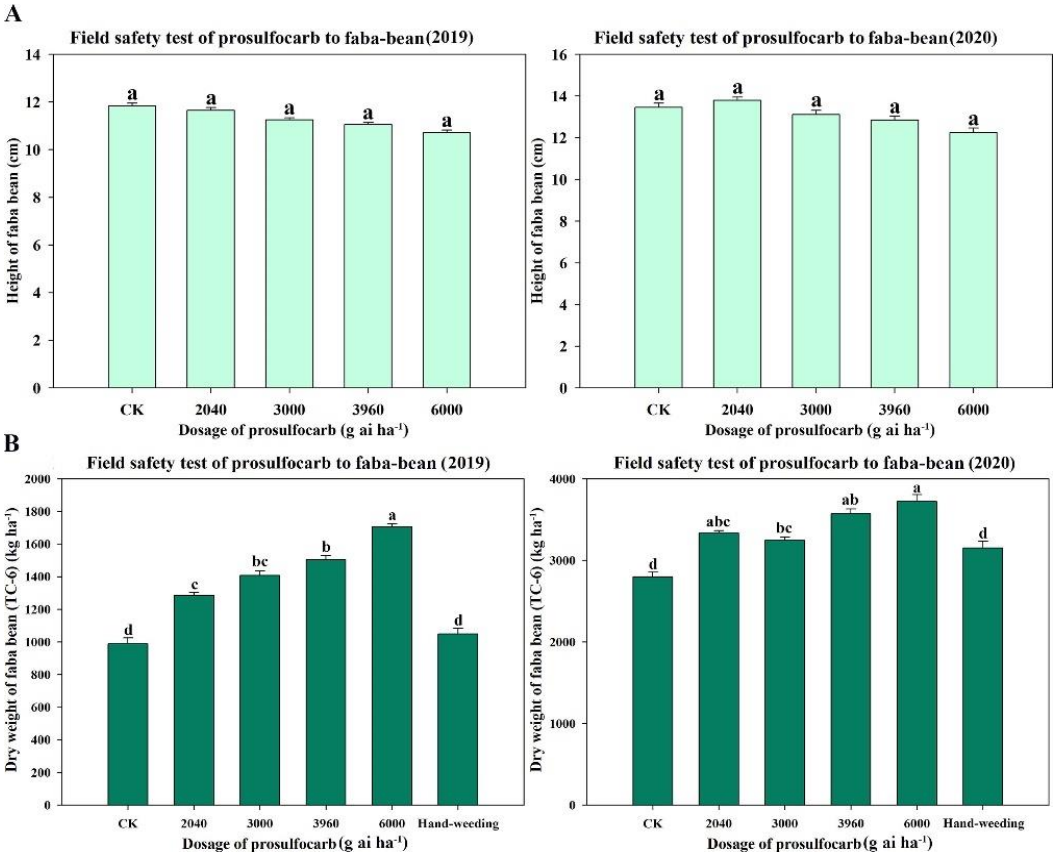

Figure 4. Effects on height (A) and yield (B) of faba-bean of different dosage of prosulfocarb treatment in the field. Different letters mean significant difference of different treatments ($p < 0.05$).

Figure 5. Morphology of faba-bean under different dosage (g ai ha^{-1}) of prosulfocarb treatment in the field experiments. A. Experiment in 2019. B. Experiment in 2020.

DISCUSSION

The safety of herbicides to crops is the key to their application, which is why herbicides have to be evaluated for safety (Schneider, 2008; Zhang et al., 2019; Qu et al., 2021). Herbicide product labels usually contain strict application dosages, clear crop specifications, and detailed application methods. The function of herbicides is to eliminate weeds from the target plants, which makes the selectivity of herbicides a science (Da Costa et al., 2017; Qu et al., 2021). Crops can successfully cope with the stress caused by herbicides by changing the application methods and other factors (Way et al., 1984; Idziak et al., 2012). We speculate that prosulfocarb can also be safely used in faba-bean fields. Therefore, some factors that may affect the safety of prosulfocarb in faba-bean were studied and analysed.

The size of faba-bean is likely to affect the tolerance to herbicides. There is no evidence to suggest a direct relationship between seed size and stress resistance. However, according to our observation, faba-beans with small seeds generally germinate faster than those with large seeds, indicating that the young shoots of faba-beans with small seeds may have come into contact with prosulfocarb earlier. Moreover, the earlier the exposure to herbicides, the higher the concentration. This study further validated this view, because faba-bean cultivars with large seeds were significantly more tolerant to prosulfocarb than faba-bean cultivars with small seeds (Figure 1). Therefore, we can reveal that faba-bean cultivars with large seeds should be chosen for sowing, if prosulfocarb is used for weed control.

The time of application is a key factor in determining whether herbicide can be safely used in crop fields (Way et al., 1984; Idziak et al., 2012). Herbicides can be divided into pre-emergence and post-emergence herbicides according to the time of application. As a pre-emergence herbicide, prosulfocarb mainly acts on the synthesis of lipids, which in turn affects the normal growth of plants (Andreasen et al., 2020). However, it takes about 10 d from the time of sowing to emergence for faba-bean, therefore, it is worth exploring when prosulfocarb application is the safest. This study revealed that prosulfocarb is safer if it is applied at an early stage during the sowing of faba-bean (Figure 2). In addition, the application of prosulfocarb to faba-bean before planting them in the field caused serious damage to the bean (data not shown). This indicated that faba-bean that had not sprouted out of the soil or were in the embryonic stage must not be directly exposed to prosulfocarb. Although in this study, no phytotoxicity on faba-bean was found in the application of prosulfocarb within 6 d after sowing (Figure 2), we recommend that prosulfocarb be applied immediately after the faba-bean.

Waterlogged and high temperatures aggravate the phytotoxicity of herbicides on crops (Baxter et al., 2016; Mueller et al., 2021; Luo et al., 2023). Faba-bean is more sensitive to drought and heat stress than other grain legumes (Amede and Schubert, 2003). The faba-bean sown in autumn has to go through the winter and it is a relatively low-temperature tolerant plant. High soil moisture content can cause root rot in faba-bean (Li and Nan, 2000). If rainfall causes excessive water accumulation in poorly drained fields, faba-bean would be severely damaged (Figure 6A). Although temperature and soil water content are difficult to control, some measures can be taken to avoid phytotoxicity in faba-bean. For example, according to the results of this study, faba-bean can be sown until the temperature drops in autumn and ridges can be used to facilitate drainage before planting faba-bean in a large area. Ridging is essential for improving the prosulfocarb tolerance of faba-bean (Figure 6). In our study, the yield of faba-bean experimental field in 2020 was generally higher than that in the experimental field in 2019 (Figure 4), which was speculated to be caused by higher ridges and better drainage effects in the experimental field of 2020 (Figure 6).

Crop growth in a field environment is different from laboratory growth, and field-grown crop is usually more resistant to stress (Takayanagi et al., 2010; Gao et al., 2021). The field experiments in this study were designed based on the results of laboratory experiments. The cultivated land was ploughed and ridged before the field experiments (Table 1). Two round of field tests (in 2019 and 2020) were carried out to meet the temperature requirements, and prosulfocarb was sprayed immediately after sowing (Table 1). The two round of experiments all achieved the expected positive safety effects (Figures 4 and 5). It has been reported that prosulfocarb is mainly used to control winter weeds in wheat fields (Busi and Powles, 2013; Boutsalis et al., 2015). In this study, the control effect of prosulfocarb was visually estimated, which indicated that it could effectively control *Galium aparine*, *Veronica didyma*, *Gnaphalium affine*, *Cerastium arvense*, *Alopecurus aequalis*, *Beckmannia syzigachne*, *Phleum paniculatum* and the validity period could be as long as 4 mo (Table 2). Prosulfocarb can be safely used in faba-bean fields and faba-bean can grow normally without interference from weeds.

Figure 6. Comparison of morphology of faba-bean treated with prosulfocarb under different ridging conditions. The treatment dosage of prosulfocarb was 7920 g ai ha^{-1}. A. No ridging (pre-experiment). B. Low ridging (experiment in 2019). C. High ridging (experiment in 2020).

Table 2. Visual control effect (%) of prosulfocarb at high-dosage (3960 g ai ha^{-1}) on weeds. - No such weed in this test location.

Investigation and weeds	*Galium aparine*	*Veronica didyma*	*Gnaphalium affine*	*Cerastium arvense*	*Alopecurus aequalis*	*Beckmannia syzigachne*	*Phleum paniculatum*	Total grasses
Experiment in 2019, first investigation	95.00	96.25	-	-	91.25	81.25	90.00	92.50
Experiment in 2019, second investigation	93.75	91.25	-	-	88.75	82.50	91.25	91.25
Experiment in 2020, first investigation	98.75	-	97.50	100.00	92.50	91.25	-	95.00
Experiment in 2020, second investigation	98.75	-	98.75	97.50	91.25	90.00	-	93.75

CONCLUSIONS

This study showed that it was possible to safely applicate prosulfocarb in faba-bean fields by ridging for easy drainage, planting large-seed cultivars after cooling, and applying prosulfocarb immediately after sowing, and achieve good control effects on weeds concurrently. However, prosulfocarb is used in a relatively large amount, which does not meet the requirements for pesticide reduction advocated in recent years. Future research is expected to focus on reducing the use of prosulfocarb. The methods of biological and physical control are the future development direction and are also worth exploring.

Author contribution

Conceptualization: Y.G., Z-L.G. Methodology: Y.G., G-F.D. Software: T.S., Z-L.G. Validation: Y.G., G-F.D. Formal analysis: Y.G., G-F.D., T.S. Investigation: Y.G., G-F.D., T.S., Z-H.T. Resources: Y.G., G-F.D. Data curation: Z-H.T. Writing-original draft: Y.G. Writing-review & editing: Z-H.T., Z-L.G., G-H.S. Visualization: Y.G. Supervision: Z-H.T., G-H.S. Project administration, Y.G., Z-H.T., G-H.S. Funding acquisition: Y.G., Z-H.T., G-H.S. All co-authors reviewed the final version and approved the manuscript before submission.

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