



Article

Herbicidal Activity of Cinmethylin against Grass Weeds and Its Safety for Use with Different Wheat Varieties

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Abstract: Cinmethylin is a potential pre-emergence herbicide that could be used to control grass weeds in winter cereals. To determine the herbicidal activity of cinmethylin against common graminaceous weeds in wheat fields in China and its level of safety on wheat, we conducted the following experiments: (i) assessing the efficacy of cinmethylin against 11 grass weeds and (ii) determining its safety against 19 wheat varieties. The results showed that cinmethylin had good herbicidal efficacy against annual bluegrass (*Poa annua* L.), shortawn foxtail (*Alopecurus aequalis* Sobol.), slender meadow foxtail (*Alopecurus myosuroides* Huds.), Japanese foxtail (*Alopecurus japonicus* Steud.), Italian ryegrass (*Lolium multiflorum* Lam.), British timothy (*Phleum paniculatum* Huds.), Asia Minor bluegrass (*Polygogon fugax* Nees ex Steud.), *Helictotrichon tibeticum* (Roshev.) Holub., and wild oat (*Avena fatua* L.), with a GR₅₀ (the herbicide dose resulting in 50% growth inhibition) value of 4.50–99.21 g a.i. ha^{−1} in plant height and 1.43–70.34 g a.i. ha^{−1} in fresh weight. However, cinmethylin cannot control Japanese brome (*Bromus japonicus* L.) or Tausch's goatgrass (*Aegilops tauschii* Coss.) at a dose of 200 g a.i. ha^{−1}. Different wheat varieties varied in their phytotoxicity to cinmethylin. Overall, there is a phytotoxicity risk when using cinmethylin on wheat, mainly to wheat roots, with a reduction in root length of 40.81–64.09% at a dose of 400 g a.i. ha^{−1}. These findings indicate that the pre-emergence herbicide cinmethylin provides good efficacy against most grass weeds and may possess potential for weed management in wheat fields. However, attention should be given to the application dosage and the sensitivity of wheat varieties when using cinmethylin in wheat fields.

Keywords: pre-emergence herbicide; grass weeds; root; control efficacy; phytotoxicity



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1. Introduction

Wheat (*Triticum aestivum* L.), one of the three major staple grains, plays a pivotal role in China's food structure. However, weeds, throughout the entire wheat growth period, can significantly reduce wheat yield [1,2]. Weeds in farmland will not only compete with crops for water, growth space, sunlight, fertilizer, and other necessary growth conditions, but also act as a disease and pest vector, directly or indirectly exacerbating their occurrence [3]. The loss of wheat grain yield is approximately 4 billion kg every year due to weed interference in China [4]. Therefore, weed management in wheat fields is necessary to ensure wheat yield and quality. At present, chemical herbicides are widely used on agronomic crops as the predominant and most successful method of controlling weeds because of their economic and effective properties [5,6]. Herbicides can be divided into pre-emergence herbicides and post-emergence herbicides. Post-emergence herbicides for wheat fields that have been historically used for a long time in China mainly include acetyl-CoA carboxylase inhibitor herbicides and acetolactate synthetase inhibitor herbicides [7]. The long-term use of these herbicides has resulted in serious problems, such as increased herbicide resistance,

the succession of weed communities, and phytotoxicity to wheat [8,9]. With the extensive and repeated use of herbicides, herbicide-resistant weeds quickly followed [10,11]. There are currently 530 herbicide-resistant reported weed biotypes globally, with 272 species (155 dicots and 117 monocots) as of March 2024 [12]. For example, Zhang et al. reported that the presence of multiple resistance mechanisms within the Italian ryegrass population has resulted in the development of resistance to various herbicides, including high resistance to quizalofop-p-ethyl and haloxyfop-r-methyl, moderate resistance to clodinafop-propargyl and sethoxydim, and low resistance to pinoxaden and clethodim [13]. Bi et al. showed that AH-15 (*A. japonicus* population) has developed resistance to the ACCase herbicides sethoxydim, clodinafop-propargyl, fenoxaprop-p-ethyl, clethodim, and pinoxaden, as well as the ALS herbicides mesosulfuron-methyl, pyroxsulam, flucarbazone-Na, and imazethapyr [14]. In contrast, there are lower levels of resistance risks to pre-emergence herbicides due to the smaller number of weed cohorts affected by many pre-emergence herbicides [15]. Therefore, pre-emergence herbicides are playing an increasingly important role in controlling weeds in wheat fields. However, traditional pre-emergence herbicides such as pretilachlor, diflufenican, and isoproturon have a narrow spectrum and strong volatility and are prone to phytotoxicity [16–19]. Addressing the significant problems caused by the resistance of post-emergence herbicides and the drawbacks of traditional pre-emergence herbicides, it is imperative to explore novel pre-emergence herbicides for weed control in wheat.

Cinmethylin, which is a benzyl ether derivative of the natural terpene 1,4-cineole, is a new pre-emergence herbicide for controlling grass weeds in wheat fields [20,21]. Originally, it was first described in 1981 by Shell Chemical Company and introduced to the market in 1989 by American Cyanamide Company as a herbicide for the control of grass weeds in rice [20]. Cinmethylin binds to acyl-ACP thioesterase and inhibits the fatty acid thioesterase (FAT) in plastids, which prevents the release of both unsaturated and saturated fatty acids from the plastid to the endoplasmic reticulum. Thus, very-long-chain fatty acid biosynthesis is blocked [20]. Similar to other pre-emergence herbicides, this compound is primarily absorbed by plant roots [22]. Cinmethylin, which obtained the first classification of action mechanism given by the Herbicide Resistance Action Committee (HRAC) in 1985, is presently classified as Group 30 of the HRAC [20]. This means that cinmethylin has FAT inhibition, which can destroy the germination and establishment of grass weeds. To date, cinmethylin is the first and only registered pre-emergence herbicide for wheat for controlling Wimmera ryegrass (*Lolium rigidum* Gaudin) [23]. As cinmethylin has only recently been introduced and has limited use, there have been no confirmed cases of resistance observed so far, and its herbicidal spectrum is still unclear [24]. In seedling-thrown transplanted rice fields, cinmethylin, compounded with bensulfuron-methyl, exhibits effective control against barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], heartshape false pickerelweed [*Monochoria vaginalis* (Burm. f.) C. Presl ex Kunth], pygmy arrowhead (*Sagittaria pygmaea* Miq), and Indian toothcup [*Rotala indica* (Willd.) Koehne] [25]. In wheat fields, it effectively controls multiple-herbicide-resistant Wimmera ryegrass and appears to be phytotoxic to wheat when the wheat seed is placed on the soil surface [26]. It has also been reported that cinmethylin can effectively control slender meadow foxtail in winter cereals [27]. The efficacy and safety of cinmethylin against major grass weeds in China are unknown. Thus, the aims of this study were to clarify the herbicidal efficacy of cinmethylin against 11 common grass weeds in wheat fields in China and its safety against 19 wheat varieties. This study will provide new insights for weed management in wheat and theoretical guidance for the better utilization of cinmethylin in wheat fields for weed control.

2. Materials and Methods

2.1. Plant Material

Eleven common grass weeds in wheat fields and 19 wheat varieties were used in this study. Seed samples of these weeds were randomly collected from wheat fields in May 2020, and they were transferred to the laboratory, air-dried, and stored in paper bags at

room temperature until use. The seed sample details of the weeds are given in Table 1. The wheat varieties are Zhengmai 101, Zhengmai 113, Zhengmai 119, Zhengmai 618, Zhengmai 925, Zhengmai 0926, Zhengmai 0943, Zhoumai 27, Zhoumai 36, Zhongmai 578, Xinmai 45, Chunmai 21, Jimai 22, Jimai 224, Heima 2, Zhongzhi 13, Taishan 27, Wanfeng 269, and Xinong 9718. All wheat seeds were supplied by the Wheat Research Institute of Henan Academy of Agricultural Sciences.

Table 1. Seed sample details of grass weeds.

Weeds	Location			Longitude and Latitude
	Province	City	County/District	
<i>Helictotrichon tibeticum</i>	Henan	Jiaozuo	Qinyang	112°50' E, 35°1' N
<i>Avena fatua</i>	Henan	Jiaozuo	Qinyang	112°51' E, 35°4' N
<i>Poa annua</i>	Henan	Kaifeng	Xiangfu	114°31' E, 34°48' N
<i>Alopecurus myosuroides</i>	Henan	Pingdingshan	Wugang	113°36' E, 33°26' N
<i>Phleum paniculatum</i>	Henan	Shangqiu	Sui	115°8' E, 34°16' N
<i>Aegilops tauschii</i>	Henan	Shangqiu	Yucheng	115°50' E, 34°18' N
<i>Alopecurus japonicus</i>	Henan	Xinyang	Luoshan	114°30' E, 32°12' N
<i>Alopecurus aequalis</i>	Henan	Xinyang	Pingqiao	114°18' E, 32°21' N
<i>Polypogon fugax</i>	Henan	Xinxiang	Yuanyang	113°41' E, 35°00' N
<i>Lolium multiflorum</i>	Henan	Zhumadian	Yicheng	113°58' E, 32°51' N
<i>Bromus japonicus</i>	Henan	Zhumadian	Zhengyang	114°17' E, 32°37' N

2.2. Herbicides

An amount of 750 g L⁻¹ cinmethylin EC (emulsifiable concentrate) (Luximax, BASF, Melbourne, Australia) was used to evaluate the control efficacy on 11 grass weeds and the safety for use on 19 wheat varieties. The herbicide dosage chosen for the dose–response experiments was based on preliminary experiments and the literature [26]. Cinmethylin was applied at 0, 6.25, 12.5, 25, 50, 100, and 200 g a.i. ha⁻¹ in the dose–response experiment on the efficacy against weeds. It was applied at 0, 400, and 1600 g a.i. ha⁻¹ in the wheat-tolerance experiment (recommended label rate in Australia is 375 g a.i. ha⁻¹ of cinmethylin [26]).

2.3. Efficacy of Cinmethylin for Controlling Grass Weeds

In this study, pot-based dose–response experiments were implemented in a greenhouse to evaluate the sensitivity of 11 grass weeds. Preliminary testing indicated that the germination rates of the weeds were all greater than 90%. Fifteen full and uniform seeds of each weed species were sown in 6.8 cm × 6.8 cm × 7.0 cm pots filled with soil consisting of a mixture of farmland soil (0.54% organic matter, pH 8.38) and cultivation substrate mixed in a mass ratio of 3 to 2 and then covered with an additional 3 cm of soil. Pots were then placed in a plastic tray filled with water to saturate the soil with water. After the water had seeped to the soil surface, pots were transferred to a greenhouse for cultivation with a 12 h photoperiod at 25 °C and a 12 h dark period at 20 °C, 30,000 lx light intensity, and 65–75% relative humidity. Cinmethylin was applied to the soil surface 1 day after sowing using a spray tower (Nanjing Agricultural Mechanization Research Institute of the Ministry of Agriculture, 3 WP-2000 walking spray tower, TP6501 flat-spray nozzle, liquid volume 450 L ha⁻¹). There were four replicate pots for each dose of cinmethylin. Each pot was an experimental unit. Pots were placed in a greenhouse to continue cultivation after spraying. The experiment was designed in a completely randomized way and was repeated twice. The data were pooled for analysis.

2.4. Selectivity of Cinmethylin to Wheat

The determination of herbicide selectivity was conducted according to the soil spray method [8]. Twelve wheat seeds were buried in pots and covered with 2 cm deep soil before cinmethylin was applied. The application method was the same as in the weed efficacy

experiments described earlier. Pots of seeded wheat were transferred to a greenhouse after application for cultivation with a 12 h photoperiod at 25 °C and a 12 h dark period at 20 °C, 30,000 lx light intensity, and 65–75% relative humidity. Each line treatment combination was replicated four times. Each pot was an experimental unit. The experiment was conducted in a completely randomized way, and it was repeated twice during the trial. The data were pooled for analysis. The wheat seeds in the two runs of experiment were sown on 13 October 2021 and 16 February 2022, respectively. Cinmethylin was sprayed onto the soil surface using a spray tower 1 day after sowing.

2.5. Plant Assessments

Plant height, fresh weight, and survival rate were determined with reference to Xu et al. [8] and Busi et al. [26], with modifications. Twenty-one days after the application of cinmethylin, the number of weed seedlings that emerged from the soil and attained a height greater than 1 cm was counted to determine survival percentage. Additionally, the aboveground parts of the weeds were clipped to measure plant height and fresh weight. Similarly, the aboveground parts of the wheat plants were clipped to measure plant height and fresh weight 21 days after spraying [28]. The root lengths of the wheat plants in each pot were also evaluated. The method for determining wheat root length was based on the approach of Hu et al. [29], with improvements made. After careful removal from each pot, roots were rinsed and cleaned with water. Roots were dried using filter paper. Root length was measured using a ruler, taking into account the longest length of the main root.

2.6. Statistical Analysis

All regression analyses in this study were performed using SigmaPlot software (Version 10.0; Systat Software Inc., Santa Clara, CA, USA). The dose–response curves were obtained by nonlinear regression through a four-parameter logistic response equation [30]. The four-parameter logistic model is described in Equation (1):

$$Y = c + (d - c) / \{1 + \exp [b (\log x - \log GR_{50})]\} \quad (1)$$

where b represents the relative slope of the herbicide dose resulting in 50% growth inhibition (GR_{50}), and c and d represent the lower limit and upper limit, respectively. The herbicide dose is the independent variable (x) and the growth response (percentage of the nontreated control) is the dependent variable (y) in the regression equation. The statistical analysis was conducted with GraphPad Prism (Version 7.0; GraphPad Software, Inc., La Jolla, CA, USA). One-way analysis of variance (ANOVA) and multiple comparisons between plant height, fresh weight, and root length reduction in wheat varieties were conducted by Tukey's test to compare the differences among the different doses of cinmethylin. Significant differences are represented by different lowercase letters at the $p < 0.05$ level.

The standard for determining the safety to wheat in this study is as follows: safe: reduction < 10%; slight phytotoxicity: reduction 10 to 20%; phytotoxic or unsafe: reduction > 20%.

3. Results

3.1. Efficacy of the Herbicide Cinmethylin in the Control of Grass Weeds

With an increasing dose of cinmethylin, the percentages of plant height and fresh weight of the different weeds all decreased (Figure 1). Seven weeds, *P. annua*, *A. aequalis*, *A. myosuroides*, *A. japonicus*, *L. multiflorum*, *P. paniculatum*, and *P. fugax*, were comparably more sensitive to cinmethylin than the other four weeds tested. At a dose of 50 g a.i. ha^{−1}, the plant growth of these seven weeds was inhibited, with percentages of <20% in plant height and <13% in fresh weight. The percentages of plant height and fresh weight all reached zero at the maximum dose of 200 g a.i. ha^{−1}. The GR_{50} values of plant height and fresh weight were 4.50–14.74 g a.i. ha^{−1} and 1.43–11.19 g a.i. ha^{−1}, respectively, for these seven weeds (Table 2). Cinmethylin also influenced the survival of the seven weed species. The percentages of plant survival of these seven weeds all reached almost zero at a dose of

200 g a.i. ha⁻¹ (Figure 1). The GR₅₀ value of plant survival was 5.44–55.62 g a.i. ha⁻¹ (Table 2). *H. tibeticum* and *A. fatua* were less sensitive to cinmethylin. At a dose of 200 g a.i. ha⁻¹, the percentages of plant height of *H. tibeticum* and *A. fatua* were 20.08% and 30.75%, respectively, and the percentages of fresh weight of the two weeds were 18.25% and 24.43%, respectively (Figure 1). The GR₅₀ values of the plant height of *H. tibeticum* and *A. fatua* were 68.15 g a.i. ha⁻¹ and 99.21 g a.i. ha⁻¹, respectively, and the fresh weight GR₅₀ values were 40.59 g a.i. ha⁻¹ and 70.34 g a.i. ha⁻¹, respectively (Table 2). These results suggest that cinmethylin has good herbicidal efficacy against these nine weeds. However, at the maximum dose of 200 g a.i. ha⁻¹, the plant height, fresh weight and plant survival of *B. japonicus* and *A. tauschii* were more than 75%, 42% and 93%, respectively (Figure 1). The GR₅₀ value of *B. japonicus* was 80.44 g a.i. ha⁻¹ for fresh weight and more than 200 g a.i. ha⁻¹ for plant height and plant survival. The GR₅₀ values of plant height, fresh weight, and plant survival of *A. tauschii* were all more than 200 g a.i. ha⁻¹ (Table 2). Thus, the herbicidal efficacy of cinmethylin against *B. japonicus* and *A. tauschii* was poor. The above results suggest that cinmethylin, applied as a pre-emergence herbicide, is effective in controlling most of the grass weeds infesting wheat fields in China, especially small seeds, such as *P. annua*, *A. aequalis*, and *A. myosuroides*, but less effective against *B. japonicus* and *A. tauschii*.

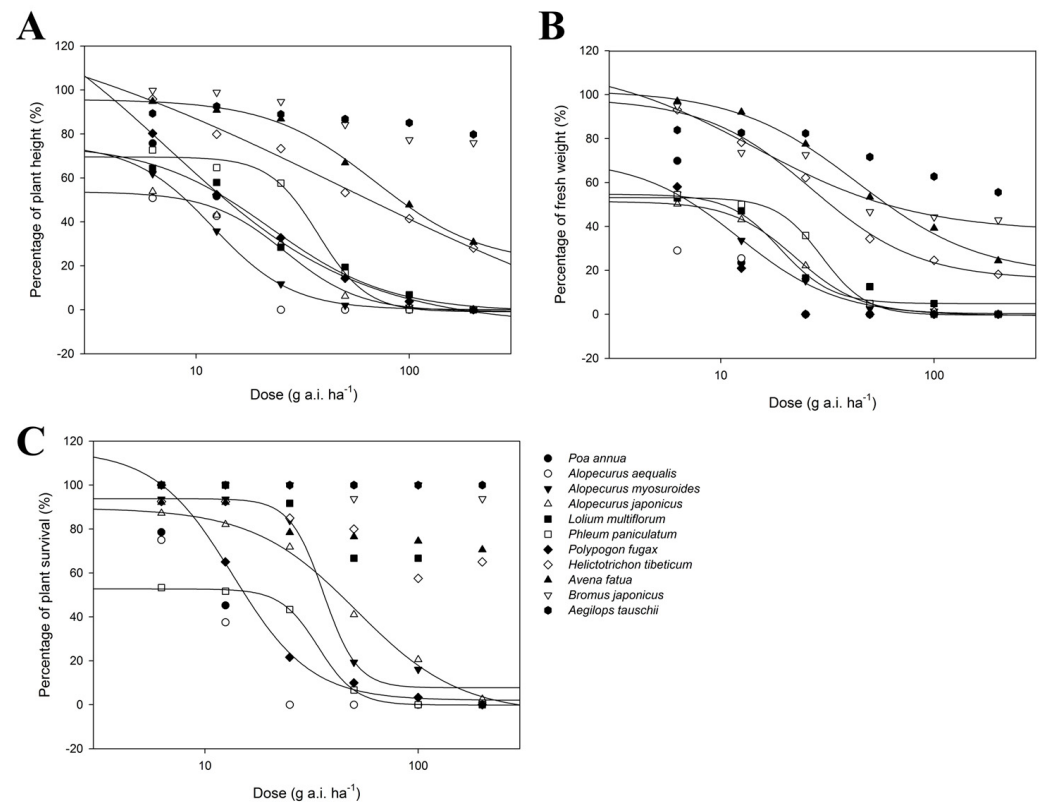


Figure 1. Aboveground plant height (A), fresh weight (B), and plant survival (C) as percentages of plant emergence of 11 grass weeds in a greenhouse at various doses of cinmethylin applied to the soil surface pre-emergence. Values are means. The fit of dose–response data to a four-parameter log-logistic curve model is shown by the lines (Equation (1)). Plant height and fresh weight are expressed as a percentage of the nontreated control.

Table 2. Estimated GR₅₀ values (95% confidence interval, CL) for 11 weeds treated with different doses of cinmethylin. Nonlinear regression analysis was used to fit the herbicide dose to plant height, fresh weight, and plant survival data. GR₅₀ = herbicide dosage causing a 50% reduction in the relative plant height, fresh weight, and plant survival. ^a: 95% confidence interval.

Weeds	GR ₅₀ (g a.i. ha ^{−1}) (95% CL) ^a		
	Plant Height	Fresh Weight	Plant Survival
<i>Poa annua</i>	5.93 (1.51–23.21)	4.69 (1.10–20.13)	5.89 (1.52–22.79)
<i>Alopecurus aequalis</i>	4.50 (−0.02–1.33)	3.10 (0.52–19.85)	5.44 (1.35–21.83)
<i>Alopecurus myosuroides</i>	9.71 (5.50–17.15)	9.34 (4.82–18.11)	26.92 (15.18–47.73)
<i>Alopecurus japonicus</i>	11.13 (5.31–23.35)	1.43 (1.21–1.66)	32.97 (24.77–43.88)
<i>Lolium multiflorum</i>	14.04 (6.17–31.96)	10.96 (4.41–27.26)	55.62 (34.52–89.61)
<i>Phleum paniculatum</i>	14.74 (7.62–28.55)	11.19 (5.75–21.76)	11.60 (5.68–23.68)
<i>Polypogon fugax</i>	15.21 (8.28–27.95)	4.08 (0.88–19.03)	27.57 (17.40–43.70)
<i>Helictotrichon tibeticum</i>	68.15 (52.51–88.44)	40.59 (32.61–50.52)	>200
<i>Avena fatua</i>	99.21 (78.20–125.85)	70.34 (61.30–80.72)	>200
<i>Bromus japonicus</i>	>200	80.44 (43.50–148.75)	>200
<i>Aegilops tauschii</i>	>200	>200	>200

3.2. Selectivity of Cinmethylin to Wheat

The results showed that different wheat varieties had different sensitivities to cinmethylin. At a dose of 400 g a.i. ha^{−1}, the reduction in plant height of Zhoumai 36 was highest (17.76%), significantly higher than that of the other 18 wheat varieties. The reduction in plant height of Heima 2, Zhengmai 101, Zhengmai 0926, Zhengmai 113, Zhongmai 578, Xinong 9718, Zhengmai 925, Zhengmai 119, Zhengmai 0943, Jimai 224, Taishan 27, Zhongzhi 13, Wanfeng 269, and Xinmai 45 was less than 10%, indicating that cinmethylin is safe for these wheat varieties (Figure 2A). The reduction in fresh weight of Jimai 22 was the highest, at 16.36%. Except for Jimai 22, Zhoumai 36, Zhoumai 27, and Chunmai 21, the fresh weight reduction in the other 15 wheat varieties was less than 10% (Figure 2B). Surprisingly, the root length of 19 wheat varieties was significantly inhibited by cinmethylin, with a reduction of 40.81–64.09% (Figure 2C). The results suggest that cinmethylin has little inhibition on the aboveground parts of different wheat varieties and appears to be safe or slightly phytotoxic to wheat. However, notable phytotoxicities were observed on the roots of wheat.

At a dose of 1600 g a.i. ha^{−1}, cinmethylin caused different degrees of phytotoxicity in wheat. The reduction in plant height of Zhengmai 113 was highest, at 23.87%, and the values for the other 18 wheat varieties were less than 20% (Figure 2D). The fresh weight reduction rates of Zhengmai 618, Chunmai 21, Jimai 22, Zhoumai 36, and Wanfeng 269 were all more than 20% (Figure 2E). The reduction in root length of 19 wheat varieties was more than 46% (Figure 2F). In conclusion, the wheat varieties Xinong 9718, Heima 2, Taishan 27, Zhengmai 119, Zhengmai 0926, Zhengmai 113, Wanfeng 269, Zhongzhi 13, Zhengmai 0943, Zhengmai 925, Jimai 224, Zhongmai 578, Zhengmai 101, and Xinmai 45 showed high tolerance to cinmethylin. However, there is a phytotoxicity risk when using cinmethylin on wheat, mainly to wheat roots.

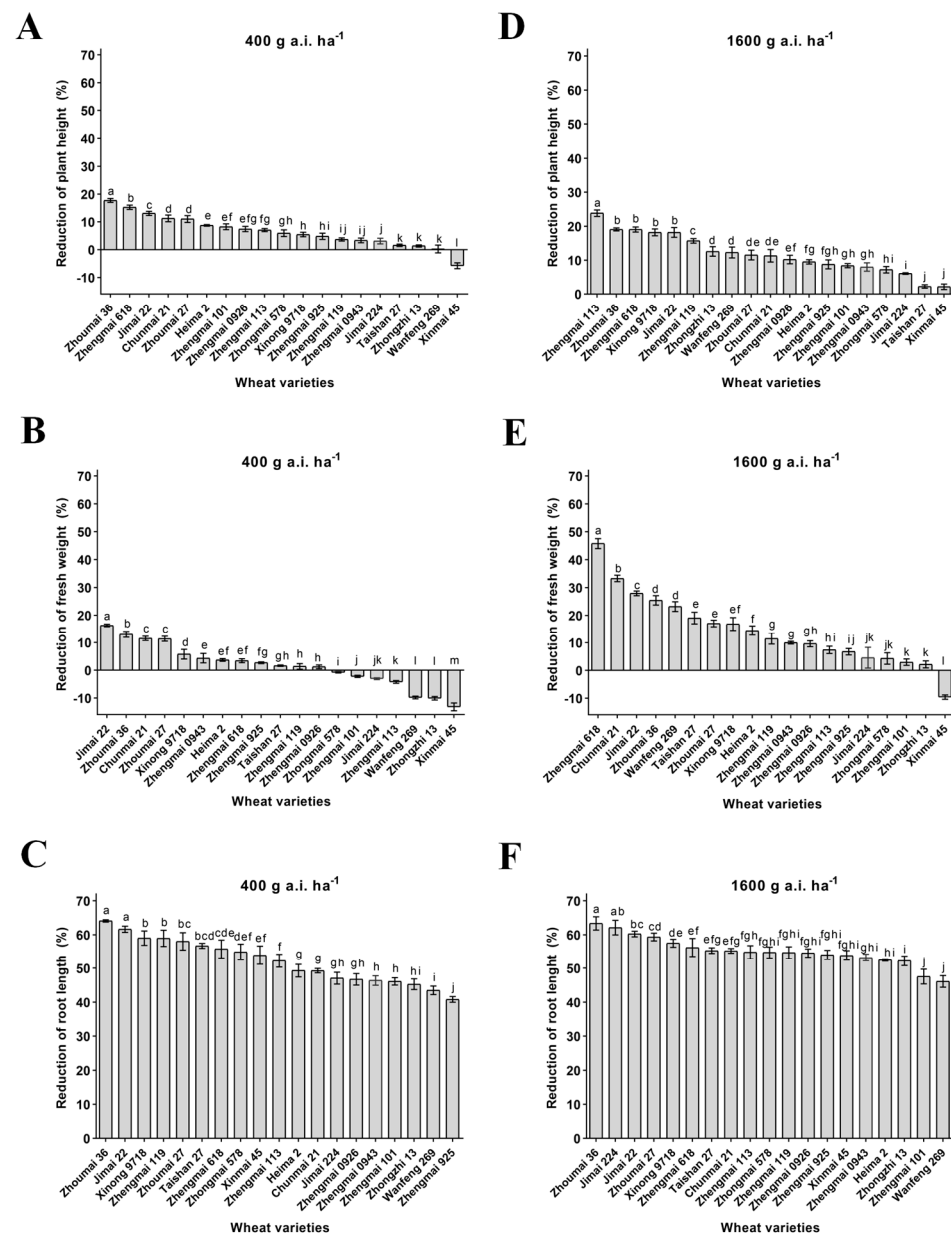


Figure 2. The reduction in wheat varieties under different doses of pre-emergence cinmethylin applied to the soil surface. The reduction in plant height (A), fresh weight (B), and root length (C) of 19 wheat varieties at 400 g a.i. ha⁻¹, respectively. The reduction in plant height (D), fresh weight (E), and root length (F) of 19 wheat varieties at 1600 g a.i. ha⁻¹ dose, respectively. ANOVA and multiple comparisons between plant height, fresh weight, and root length inhibition rates of wheat varieties were conducted by Tukey's test. Values are means $\pm$ SEs (standard errors). Significant differences are indicated by different lowercase letters ($p < 0.05$). Wheat varieties are arranged from large to small according to the reduction.

4. Discussion

Cinmethylin was first used for the control of annual graminaceous grass weeds in rice and has recently been applied in wheat fields to control noxious grass weeds [20,21]. Messelhäuser et al. demonstrated that the highest control efficacy ($>90\%$) against *A. myosuroides* was achieved by cinmethylin at a dose of 495 g a.i. ha⁻¹ in winter cereals [27]. Boutsalis et al. showed that treatment with 200 g a.i. ha⁻¹ cinmethylin had a poor control effect on *L. multiflorum* plants (29% density reduction) [31]. In this study, at a dose of 200 g a.i. ha⁻¹ of 750 g L⁻¹ cinmethylin, the percentages of plant height, fresh weight,

and plant survival of *L. multiflorum* and *A. myosuroides* all reached zero in the greenhouse. These divergences are mostly related to the differences in greenhouse culture conditions, field climate, temperature and humidity, soil, and other conditions [32–34]. Under the greenhouse culture, the reductions in *L. multiflorum* in emergence and aboveground biomass were more than 85% and 90%, respectively, at a dose of 400 g a.i. ha⁻¹ of cinmethylin [26]. In contrast, the results of the present study are consistent with Goggin et al., who showed that ED₅₀ values (7.9–63.1 g ha⁻¹) for all ryegrass populations were far less than the recommended application rate of 375 g ha⁻¹ [35]. A mixture of herbicides can not only broaden the herbicide control spectrum, but also subject weeds to the combined action of two different herbicides used as a mixture to achieve better control effects and potentially slow the evolution of herbicide resistance [26,36]. This study showed that cinmethylin is effective in controlling most of the grass weeds infesting wheat fields in China, except *B. japonicus* and *A. tauschii* (Figure 1, Table 2). Thus, cinmethylin should be used alone or mixed in combination with other herbicides to control different weed communities. For example, in areas with a mixture of broadleaf and grass weeds, a mixture of cinmethylin and a broadleaf control herbicide, such as diflufenican, is used; in areas where grass weeds such as *B. japonicus* and *A. tauschii* are predominant, a mixture of cinmethylin and pyroxasulfone is used. However, the weed control efficacy resulting from combinations of cinmethylin with other herbicides needs further study.

The results of this study can be used to guide weed management in different wheat-growing regions in China, which are divided into 4 main regions and 10 subregions [37]. Due to the differences in crop rotation systems, geographical environments, climate, and other factors in each wheat production area, the distribution of weeds in each wheat production area varies [38]. The results of this study show that cinmethylin could be used to control weeds such as *A. aequalis*, *P. annua*, and *P. fugax*, which mainly infest subregions in the Northern winter (autumn sowing) wheat area, the Huanghuai winter (autumn sowing) wheat area, the Yangtze River winter (autumn sowing) wheat area, the Southwest winter (autumn sowing) wheat area, the South China winter (late-autumn sowing) wheat area, and the Xinjiang winter–spring wheat-planting area. However, the control effect of cinmethylin on the dominant weeds *B. japonicus*, *A. tauschii*, and *A. fatua* of the Huanghuai winter wheat area is poor, and the single use of this herbicide may lead to the expansion of these weeds into malignant weeds. Based on the results of this study, cinmethylin is not recommended for use in wheat fields with *B. japonicus* and *A. tauschii* because the GR₅₀ values of plant height and plant survival were more than 200 g a.i. ha⁻¹ (Table 2). The materials in this study were all collected from Henan Province; thus, these results are directly applicable therein. Henan Province is a major wheat-producing province, with wheat-planting areas and yield ranking first in China [39]. The distribution of weeds in wheat fields varies greatly in different regions of Henan Province due to different climates, planting methods and other factors [38]. According to the results of this experiment, cinmethylin can be effectively used in the control of major weeds such as *A. aequalis*, *A. japonicus*, and *L. multiflorum* in the plain area of Eastern Henan, the hilly area of Western Henan, and the plain area of Southern Henan [38,40]. For the plain areas in the north and middle south of Henan dominated by *A. tauschii*, *A. fatua*, *P. annua*, and *B. japonicus*, and the hilly area in the southwest of Henan dominated by *A. fatua* [38,40], it is recommended that cinmethylin be mixed with other appropriate herbicides before application.

In recent years, *A. aequalis*, *A. myosuroides*, *A. japonicus*, and *L. multiflorum* have become widespread in wheat fields and have developed into malignant and resistant weeds [14,38,41–44]. It has been reported that *A. aequalis* easily results in significant damage to wheat [38,41]. In the Yangtze River winter (autumn sowing) wheat area, the damaged area of *A. aequalis* is approximately 3.33 million ha⁻¹ [38]. *A. japonicus* [14,42] and *A. myosuroides* [43,44] are both invasive grass weeds that severely infest wheat fields. They have evolved resistance to herbicides with different modes of action in recent years. *L. multiflorum*, introduced as a forage grass in China, has spread to wheat fields in recent years, causing serious damage to some wheat fields in Henan, Shanxi, Hubei, Jiangsu, Anhui, and

Shandong [13,36,45]. Therefore, cinmethylin has brought new hope for controlling these malignant and resistant weeds in wheat fields.

The results of this study show that cinmethylin is effective in controlling most of the gramineous weeds infesting wheat fields in China, but cinmethylin poses a phytotoxicity risk to wheat roots. It is reported that the occurrence of wheat phytotoxicity is mainly caused by an inappropriate application amount, period, and method, the influence of surrounding environmental factors, and the different sensitivities of wheat varieties [26,27,31,46–49]. In this study, the application amount and sensitivity of wheat varieties were the main reasons leading to wheat phytotoxicity. Cinmethylin appeared to be safe or slightly phytotoxic to the aboveground parts of 19 wheat varieties at a dose of 400 g a.i. ha⁻¹; the reductions in plant height and fresh weight of 19 wheat varieties were all less than 18%. Increases in dosage caused phytotoxicity to Zhengmai 113, Zhengmai 618, Chunmai 21, Jimai 22, Zhoumai 36, and Wanfeng 269 (Figure 2A,B,D,E). It is reported that cinmethylin appeared to be safe for wheat when seeds were sown at a depth of 1 cm or 2.5 cm at a dose of 188 g a.i. ha⁻¹ or 400 g a.i. ha⁻¹ [26]. Since different wheat varieties were sown at a depth of 2 cm in this study, the phytotoxicity of wheat at the dose of 400 g a.i. ha⁻¹ may be due to different sensitivities of the wheat, and an increase in the dose of cinmethylin would aggravate the phytotoxicity of wheat. In addition, research has shown that cinmethylin caused a significant wheat yield loss for later seeding dates [27]. Therefore, attention should be given to the dosage and sensitivity of wheat varieties when using herbicides in wheat fields. Cinmethylin had a high degree of phytotoxicity to the roots of 19 wheat varieties, with a reduction of 40.81–64.09% at the dose of 400 g a.i. ha⁻¹ (Figure 2C). This high degree of phytotoxicity may be related to the short culture time and the fact that cinmethylin is absorbed by the roots [22], resulting in a higher concentration of cinmethylin in the root, thereby causing a certain degree of herbicide damage to the roots. In addition, phytotoxicity may also be related to the physiological function of wheat roots. In addition to absorbing and transporting water and nutrients for the growth and living needs of the roots and aboveground parts of plants, roots can resist various adverse environments to a certain extent to ensure the normal growth of plants [50]. Since the impact of cinmethylin on wheat roots is greater than that on the aboveground parts, the root length should be taken as an important reference index in the safety test of wheat. It has been reported that once wheat is harmed by herbicides, it will lead to a significant reduction in wheat yield, as well as a serious threat to the quality and safety of wheat [49]. In this study, the pot-based dose–response method, which is widely recognized and the results of which are close to the field application, was used. Therefore, the extent of cinmethylin phytotoxicity to wheat roots needs to be further verified by taking into account the effects of rainfall and soil moisture in the field. Furthermore, the effects of cinmethylin on wheat tillering, yield, and quality, and how to mitigate cinmethylin phytotoxicity need further study. When spraying cinmethylin in the field, the application time and method, dosages, and different sensitivities of wheat varieties should be considered when trying to prevent wheat phytotoxicity [26,27,31,46–49]. At the same time, it is necessary to popularize prevention and remedial measures such as appropriate mixing, scientific herbicide use, and the optimal period of herbicide use to ensure high wheat yield and food security.

5. Conclusions

This study found that cinmethylin, applied as a pre-emergence herbicide, is effective in controlling most of the gramineous weeds infesting wheat fields in China, except for *B. japonicus* and *A. tauschii*. It is especially effective with smaller seeds, such as *P. annua*, *A. aequalis*, and *A. myosuroides*. The wheat varieties Xinong 9718, Heima 2, Taishan 27, Zhengmai 119, Zhengmai 0926, Zhengmai 113, Wanfeng 269, Zhongzhi 13, Zhengmai 0943, Zhengmai 925, Jimai 224, Zhongmai 578, Zhengmai 101, and Xinmai 45 showed high tolerance to cinmethylin. However, there is a phytotoxicity risk when using cinmethylin on wheat, mainly to wheat roots. As this study used the pot dose–response method, the degree of the phytotoxicity of cinmethylin on wheat root and the effects on wheat growth,

yield, and quality need to be verified in the field. Moreover, mitigation methods for wheat phytotoxicity should be further clarified.

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