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Crop Protection

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Differential sensitivity of field muskmelon (*Cucumis melo* L. var. *agrestis* Naud.) populations to nicosulfuron, imazapic, fomesafen and bentazon



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ARTICLE INFO

Keywords: Cucumis melo Resistance Nicosulfuron Imazapic Fomesafen Bentazon

ABSTRACT

Cucumis melo L. var. agrestis Naud. (field muskmelon), a troublesome weed affecting corn, soybean and peanut fields, is difficult to control and drastically reduces crop yields. Farmers have reported that nicosulfuron, imazapic, fomesafen, and bentazon, which are common herbicides used in corn, soybean and peanut fields, do not control field muskmelon effectively. This lack of control might be due to the evolution of resistance to these herbicides in field muskmelon. Thus, this study was designed to determine the sensitivity of field muskmelon to nicosulfuron, imazapic, fomesafen and bentazon. Eleven putative resistant populations and 1 sensitive population were used to evaluate the sensitivity of field muskmelon to these herbicides. Further, we analyzed the cross resistance and multiple resistance in field muskmelon plants. Among the 11 putative resistant populations, 10 populations evolved resistance to nicosulfuron, 6 populations were resistant to imazapic, and 4 populations evolved resistance to fomesafen, whereas none of the populations were resistant to bentazon. Moreover, field muskmelon has evolved resistance to the ALS-inhibiting herbicides nicosulfuron and imazapic, and to the PPO-inhibiting herbicide fomesafen. Fortunately, most of the resistance levels were low. The findings indicate that effective measures are needed to control resistant field muskmelon and avoid the further development of resistance in this plant.

1. Introduction

In recent years, overdependence on chemical herbicides has led to the development and progression of herbicide-resistant weeds. There are currently 480 unique cases of herbicide-resistant weeds globally, which involve 252 species (147 dicots and 105 monocots). Weeds have evolved resistance to 23 of the 26 known herbicide sites of action and to 161 different herbicides (Heap, 2016). Resistance has become an important problem for the control of field weeds (Gherekhloo et al., 2016). Since the first report in 1990, the cases of herbicide-resistance weed species has rapidly increased (\geq 42) in China (Heap, 2016), which increasing the difficulty of weed control and seriously affecting crop yields.

Cucumis melo L. var. agrestis Naud. (field muskmelon) is an annual, monoecious, trailing-vine weed that belongs to the Cucurbitaceae family. It is an invasive species in Asia and has been found in Shandong, Jiangsu, Anhui, Henan, and Shanghai Provinces in China. The flowering time is May to July, and its fruits mature from July to September. Fruits

are elliptical and have a length of 3–3.5 cm and a width of 2–3 cm. They have numerous seeds that are oviform, yellowish white, 4-5 mm long, 2 mm wide and 1 mm thick (Zhang et al., 2016).

Field muskmelon was planted as a crop during the 1960s and 1970s in China to press oil from its seeds and make wine from its flesh, among other uses. However, it currently invades economic crop fields, such as corn, soybean, and peanut fields (Xu et al., 2017). It has a tendril that can be used to entwine itself around other plants or leaves, which reduces crop photosynthesis and can decrease grain yield. The individual biomass of field muskmelon is extremely high. At a density of 10–15 plants/m², field muskmelon can mulch an entire corn row (Zhang et al., 2016). Seed numbers per individual are also high, typically greater than 1200 seeds (Zhang et al., 2016).

Field muskmelon mainly occurs in corn, soybean, and peanut fields. Nicosulfuron is a common herbicide used in corn fields to control broadleaf weeds (Shigeo et al., 2000). Imazapic, fomesafen and bentazon have been widely used in peanut and soybean fields (Zhang et al., 1997; Peachey et al., 2012; Han and Wang, 2002). Farmers have

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Table 1
Sampling locations of field muskmelon populations.

Population	Location			Crop field
	Province	City	County	
1	Henan	Nanyang	Zhenping	Roadside
2	Henan	Xinxiang	Huojia	Corn
3	Henan	Xinxiang	Yuanyang	Corn
4	Henan	Puyang	Puyang	Corn
5	Henan	Luohe	Yancheng	Corn
6	Henan	Zhoukou	Taikang	Peanut
7	Hebei	Baoding	Tangxian	Soybean
8	Hebei	Shijiazhuang	Lingshou	Soybean
9	Hebei	Cangzhou	Cangxian	Soybean
10	Hebei	Xingtai	Liuying	Peanut
11	Hebei	Shijiazhuang	Lingshou	Peanut
12	Shandong	Heze	Yancheng	Corn

reported that these herbicides do not effectively control field muskmelon and that field muskmelon might have evolved resistance to these herbicides. Thus, the objective of this study was to determine whether field muskmelon has evolved resistance to nicosulfuron, fomesafen, imazapic, and bentazon, and to provide theoretical support for the control of field muskmelon in fields.

2. Materials and methods

2.1. Plant materials

In 2015, seeds of field muskmelon were collected from different provinces in China (Table 1). Population 1 was collected from a roadside that had never been treated with herbicide and served as a reference sensitive population. Others were collected from individual fields in different crop lands. The characteristics of the studied populations are summarized in Table 1. Mature fruits of field muskmelon were collected randomly from several plants. Seeds were removed from the fruits by cutting them into two parts. The seeds were cleaned and dried for 7 day at room temperature (25 °C) and stored in paper bags until use (Tanveer et al., 2012).

2.2. Herbicides

The herbicides used for the sensitivity evaluation tests were nicosulfuron (40 g/L OD, Huifeng Agrochemical, Jiangsu, China), imazapic (240 g/L AS, Basf, Jiangsu, China), fomesafen (250 g/L AS, Syngenta, Shanghai, China), and bentazon (480 g/L AS, Basf, Jiangsu, China).

2.3. Whole-plant pot experiments for sensitivity evaluation

A dose-response assay was conducted to evaluate the sensitivity of 12 field muskmelon populations (Pan et al., 2015; Xu et al., 2013). Experiments were conducted at Henan Research and Development Base for Modern Agriculture, Henan Academy of Agricultural Sciences (Xinxiang City, Henan Province, 35°0′16.35" N, 113°42′6.08"E), under greenhouse conditions from May to October in 2016. During this period, the temperature fluctuated between 35 \pm 5 °C in the day and 25 \pm 5 °C at night. Fifteen seeds were sown in 12 \times 11 cm (diameter×height) plastic pots filled with a 2:1 (wt/wt) mixture of sand and soil. Pots were placed in the greenhouse and watered as necessary. After emergence, the seedlings were thinned to 8 plants per pot. Approximately 20 days after planting, plants at the 2- to 4-leaf stage were sprayed with herbicide by using a sprayer equipped with a flat-fan nozzle to deliver 370 L/ha at 0.8 MPa (3WP-2000, Nanjing Research Institution for Agricultural Mechanization at Ministry of Agriculture). For each population, 8 herbicide doses (Table 2) and four replicate trays per dose were sprayed, and the experiment was conducted twice. After spraying, the plants were returned to the greenhouse. Both replicate

Table 2
Herbicide rates used in the Whole-plant pot dose–response experiments.

Herbicide	Doses (g a.i./ha)
Nicosulfuron	0, 5.25, 10.5, 21, 42, 84, 168, 336
Imazapic	0, 90, 180, 360, 720, 1440, 2880, 5760
Fomesafen	0, 46.875, 93.75, 187.5, 375, 750, 1500, 3000
Bentazon	0, 9, 18, 36, 72, 144, 288, 676

experiments were arranged as a randomized complete block. Fourteen days after treatment, the plants were cut at the level of the soil surface, and the fresh weights were determined. The effective dose of herbicide that caused 50% growth reduction (GR_{50}) relative to the fresh weight of untreated check plants was calculated.

2.4. Data analysis

Combined data over the two experimental runs were subjected to an analysis of variance (ANOVA).

Analysis of the dose-response data was performed using a three-parameter log-logistic model in R (R Studio) (Knezevic et al., 2007). GR₅₀ values were computed with the following equation:

$$Y = d/1 + \exp(b(\log x - \log e))$$

where e is the GR_{50} value, d is the upper limit, and the parameter b denotes the relative slope around e.

The resistance index (RI) was calculated as the GR_{50} of the suspected resistant population divided by the GR_{50} of the sensitive population to indicate the level of resistance for the suspected resistant population. Resistance levels were classified by the RIs, and sensitivity was categorized as follows: S = not resistant (< 2), L = low resistance (2-5), M = moderate resistance (6-10), H = high resistance (10-100), VH = very high resistance (> 100) (Beckie and Tardif, 2012).

3. Results

In the whole-plant pot experiments, 11 putative resistant populations and 1 sensitive population were used to evaluate sensitivity to nicosulfuron, imazapic, fomesafen, and bentazon. The responses of field muskmelon to the four herbicides varied.

3.1. Sensitivity evaluation for nicosulfuron

The responses of the 12 populations to nicosulfuron differed (Table 3 and Fig. 1). Population 1 was sensitive to nicosulfuron, in this

Table 3 GR_{50} values of different field muskmelon populations to nicosulfuron, and resistance levels were indicated by the resistance index (RI).

Population	GR ₅₀ (g a.i./ha) (SE)	RI ^a
1	1.48 (0.50)	1.00 S
2	6.19 (1.97)	4.18 L
3	4.75 (0.93)	3.21 L
4	18.53 (5.64)	12.52 H
5	6.03 (1.17)	4.07 L
6	4.05 (1.44)	2.74 L
7	6.98 (2.51)	4.72 L
8	4.41 (0.81)	2.98 L
9	3.93 (1.66)	2.66 L
10	5.08 (0.99)	3.43 L
11	5.86 (1.24)	3.96 L
12	1.90 (0.62)	1.28 S

SE = Standard error

 $[^]a$ RIs were calculated as the ratio of the GR_{50} of the resistant population to the GR_{50} of the sensitive population: S= not resistant (< 2), L= low resistance (2-5), M= moderate resistance (6-10), H= high resistance (10-100), VH= very high resistance (> 100).

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