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## Soil solution pH can affect the response of the common bean (*Phaseolus vulgaris* L.) to mesotrione residues

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**Abstract:** Soil pH can affect the adsorption of mesotrione and exacerbate crop injury under non-acidic conditions. Soil samples collected from the same location were irrigated with water solutions of pH 7.5, 6.5, 5.5, and 4.5 and treated with 72, 36, 24, 18, 9, 4.5, 2.3, and 1.1 g a.i. (active ingredient) of mesotrione/ha. Bean growth was monitored over 28 days. Soil pH solution did not influence the effect of mesotrione on plant fresh weight, while herbicide-induced visual injury and reduction in carotenoid content were significantly mitigated under acidic conditions. The lowest rate (1.1 g a.i./ha) applied in slightly acidic soil (pH 6.5) caused visual injury of 45% 28 days after treatment, while visual injuries on plants grown in soils with pH 4.5 were only 20%. Further, bean plants grown at pH 4.5 showed only 3.3% lower carotenoid content compared to control plants since for those grown in a slightly alkaline environment (pH 7.5) reduction of this pigment was 35.5%. The mean effective dose ( $ED_{50} \pm$  standard error) of mesotrione for inhibition of carotenoids were  $5.25 \pm 0.61$  g a.i./ha at pH 7.5,  $9.57 \pm 0.74$  g a.i./ha at pH 6.5,  $13.07 \pm 0.91$  g a.i./ha at pH 5.5, and  $14.98 \pm 0.94$  g a.i./ha at pH 4.5. Results indicate that the common bean is highly susceptible to the presence of mesotrione residue and that this sensitivity strongly depends on soil pH solution.

**Keywords:** phytotoxicity; crop rotation; dose-response; triketones; spectrophotometry; carotenoid inhibition

Herbicides should effectively inhibit the growth of weeds without affecting the target crops or the crops used in rotation. Triketones are widely used as pre and postemergence herbicides to control a wide range of weeds in maize fields (Goršić et al. 2008, Romdhane et al. 2019). Among the triketones, mesotrione is soil and foliar herbicide used to control annual broadleaf weeds and certain annual grasses in maize fields (Young et al. 1999). It has been used in more than 50 countries across the world (Carles et al. 2017) and is typically administered on its own or mixed with other herbicides. Mesotrione inhibits the activity of 4-hydroxyphenylpyruvate dioxygenase, an essential enzyme in the carotenoid biosynthesis pathway (Mitchell et al. 2001), thus preventing

normal plant development. Although mesotrione is rapidly metabolised in maize (Wichert et al. 1999), it can have adverse effects on rotation crops, such as bleaching symptoms followed by necrosis of the leaf tissue (Felix et al. 2007, Soltani et al. 2007, Robinson 2008, Riddle et al. 2013, Pintar et al. 2020b). Given that some weed species are resistant to frequently used acetolactate synthase herbicides (Heap 2022), optimising the use of triketones such as mesotrione for weed control must be explored further.

Herbicide residues in the soil can be determined using high-resolution gas or liquid chromatography (Chen et al. 2012, Barchanska et al. 2015, Pang et al. 2016), although the preparation of soil samples for such instrumental analyses can be expensive and

time-consuming. However, liquid chromatography may fail to detect phytotoxic levels of mesotrione in the soil (Pintar et al. 2020c). Thus, suitably sensitive field tests are needed to detect changes in herbicide dosage (Santelmann 1977). In addition, the half-life of mesotrione in soil strongly depends on pedoclimatic conditions (Dyson et al. 2002, Felix et al. 2007, Chaabane et al. 2008, Su et al. 2017), especially soil organic carbon content and pH (Shaner et al. 2012). Thus, crop rotation decisions should take into account soil properties. For example, the adsorption of mesotrione onto soil colloids is more pronounced in acidic soils, where the herbicide degrades more slowly (Dyson et al. 2002). The estimated half-life of mesotrione is 32 days in soils at a pH of 5.0, but only 4.5 days in soils at a pH of 7.1 (Chaabane et al. 2008).

In field experiments, it is difficult to assess the influence of specific pedoclimatic factors on mesotrione adsorption, as well as their subsequent phytotoxic effects on crops in rotation. A recent study proposed that the potential phytotoxic effects of mesotrione on plants can be determined by irrigating the soil with water solutions of different pH (Pintar et al. 2021). Based on this approach, the present study hypothesised that the common bean can be used as a suitable test crop to evaluate the herbicidal effects of different mesotrione treatments. After irrigating the soil using water solutions with a range of pH values (4.5, 5.0, 6.5, and 7.5), visual injury, fresh weight of aboveground biomass, and content of chlorophyll and carotenoids were assessed. Previous studies have shown that the common bean is sensitive to mesotrione residue (Riddle et al. 2013). Therefore, the results of the present study can contribute to our understanding of the effect of soil pH on mesotrione absorption. As far as we know, this is the first study aiming to identify the best predictor of mesotrione phytotoxicity in the common bean under different soil pH conditions.

## MATERIAL AND METHODS

**Soil samples and bioassay.** In September 2020, soil samples were collected from the surface layer (0–20 cm) in untreated fields at the Šašinovec Experimental Station (45.51005.200; 16.10034.100) at the University of Zagreb Faculty of Agriculture. All samples were collected using a probe (Split Tube Sampler, Ø 53 mm, Eijkelkamp, Giesbeek, The Netherlands). The soil was identified (Pintar 2020a) as silty clay loam and consisted of sand (1.1%), silt (59.6%), clay (39.3%),

soil organic carbon (2.5%), and calcium carbonate (2.5%) at a pH of 7.5 (water; H<sub>2</sub>O) and 7.04 (potassium chloride; KCl). The mechanical soil properties, soil organic carbon, and soil pH were determined according to the ISO 11277 (2004), ISO 14235 (2004), and 10390 (2005), respectively.

After being dried at room temperature for 72 h, soil (200 g per container) was filled into a total of 144 plastic containers (volume, 0.4 L; diameter, 10 cm; depth, 4–5 cm). Reduced rates of mesotrione (Callisto 480 SC<sup>®</sup>, Syngenta Crop Protection AG, Basel, Switzerland), referred to as simulated mesotrione rates (SMRs) (Pintar et al. 2021), were applied using a TLC sprayer (CAMAG<sup>®</sup>, Muttens, Switzerland). After dilution in distilled water, mesotrione was applied at the following rates (in g a.i. (active ingredient)/ha): 1.1, corresponding to 1/128 the recommended rate (R) of mesotrione of 144 g a.i./ha; 2.3 (1/64 R); 4.5 (1/32 R); 9.0 (1/16 R); 18 (1/8 R); 24 (1/6 R); 36 (1/4 R); and 72 (1/2 R). Soils irrigated with water were used as a control. All treatments (including the control) were performed in four replicates based on the randomised complete block design.

After the mesotrione treatment, six beans (Trešnjevac, cv. Ferguson) seeds were planted in each container, which was then placed in a growth chamber. Plant growth was monitored for 28 days under controlled conditions of temperature (25 °C during the day, 15 °C at night), photoperiod (12 h day/12 h night), relative humidity (70%), and photosynthetically active radiation (250 μmol/m<sup>2</sup>/s).

The natural slightly alkaline soil pH was adjusted by an acidification procedure to obtain three different pH values of the soil solution: 6.5, 5.5, and 4.5. To obtain these pH values, 0.1 mol/L HCl was added to distilled water, and the pH was continuously monitored using a pH meter with a combined FE20/EL20 electrode (Mettler Toledo, Greifensee, Switzerland). To obtain the pH values of the acidic soil solution, the soil samples were irrigated every two days with 60 mL of the acidified solution (6.5, 5.5, 4.5). A natural, slightly alkaline soil was treated with distilled water. The final pH of the soil solution for the acidic soils was 6.46, 5.48, and 4.45, further referred to as soil pH 6.5, 5.5, and 4.5, respectively.

**Evaluation of sensitivity to SMR.** The sensitivity of the bean plants to the SMRs was evaluated using a visual injury assessment of aboveground bean biomass at 7, 14, 21, and 28 days after treatment (DAT). The fresh weight of aboveground biomass and carotenoid content was measured at 28 DAT.

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Each bean plant was removed at the soil level using scissors, and the weight of all plants per replicate was calculated. Plants corresponding to each treatment were then packed separately in plastic bags and stored at  $-80\text{ }^{\circ}\text{C}$  for total carotenoid content analysis.

The visual injury was assessed using a percentage scale on which 0% indicated no visible injury and 100% indicated complete necrosis and plant death (EPPO 2014). Spectrophotometric analysis was performed to measure the carotenoid content in bean plants treated with simulated mesotrione rates (SMRs) between 1.1 and 24 g a.i./ha. Plants treated with SMRs of 36 or 72 g a.i./ha showed complete necrosis and were excluded from the analysis. Plant leaves were thawed, cut into small pieces with scissors, and homogenised in a blender. Plant tissue (0.1 g) was mixed with 2 mL of acetone (99%) in a test tube; this step was performed in triplicate. The tissue was then further homogenised (Ultra Turrax T-10, IKA, Staufen, Germany) and centrifuged at 4 000 rpm for 5 min, after which the supernatant was transferred into a 25 mL flask using a plastic Pasteur pipette. The green-coloured solid residue was repeatedly extracted with acetone until a colourless extract was obtained. The extracts were pooled and analysed using a Helios Gamma UV/VIS spectrophotometer (Thermo Electron Corporation, Rugby, UK). The absorbance of the samples was measured at wavelengths of 662, 644, and 440 nm, and acetone was used as a blank. The carotenoid content (mg/g) in each sample was calculated from the chlorophyll content based on Holm (1954) and von Wettstein (1957).

**Statistical analysis.** The values obtained for the fresh weight of aboveground biomass and carotenoid content in the bean plants were expressed using the Abbot reduction coefficient (Püntener 1981) and analysed using R (version 4.0.0, Vienna, Austria). Data were checked for normality, and homogeneity of variance was assessed using Levene's test. Two-

way analysis of variance (ANOVA) was used to assess differences in growth parameters, after which means were compared using Tukey's *HSD* (honestly significant difference) test at  $P \leq 0.05$ . The block and its interaction with solutions of different pH and SMRs were considered as random effects, while solutions of different pH and SMRs were considered as fixed effects. Dose-response curves were developed and effective dose (ED) values were calculated using the *drc* package (Ritz et al. 2015). ED values were calculated using best-fit models. A three-parameter log-logistic model for reduction of fresh weight of aboveground biomass and a four-parameter Weibull model for the reduction in carotenoid content were selected by comparing different models using the Akaike information criterion.

The correlation between the spectrophotometrically determined decrease in chlorophyll and carotenoid content (28 DAT) was explored using the CORR procedure in SAS 9.4 (SAS Institute, Cary, USA).

## RESULTS AND DISCUSSION

The effect of mesotrione on bean plants differed significantly depending on the soil pH solution and the SMR treatment applied (Table 1;  $P < 0.001$ ). A significant interaction between SMR and soil pH solution was observed for all measured parameters except reduction in fresh weight of aboveground biomass (Table 1;  $P < 0.001$ ). These results indicate that different rates of mesotrione have significantly different effects on bean plants irrigated with different pH solutions.

**Visual injury.** Soil pH (4.5–7.5) and SMR treatments (1.1–72 g a.i./ha) caused significant visual injury to the bean plants. Visual injury symptoms were typical of herbicides that inhibit carotenoid biosynthesis and included bleaching followed by necrosis of leaf tissue. The extent of injury increased

Table 1. Two-way ANOVA to examine the effects of simulated mesotrione rate (SMR) and soil pH on bean plants in terms of visual injury, fresh weight of aboveground biomass, and content of carotenoids

Parameter	$n - 1$	Visual injury				Fresh weight	Carotenoid content
		7 DAT	14 DAT	21 DAT	28 DAT		
SMR	8	11 662.375***	1 563.762***	1 690.703***	1 912.752***	84.596***	471.362***
pH	3	1 300.949***	105.348***	78.546***	141.266***	9.523***	4.419**
Replicates	3	3.201*	0.779 <sup>ns</sup>	0.434 <sup>ns</sup>	1.413 <sup>ns</sup>	0.193 <sup>ns</sup>	0.132 <sup>ns</sup>
SMR $\times$ pH	24	167.454***	8.253***	13.452***	19.485***	0.908 <sup>ns</sup>	9.883***

\* $P = 0.05$ ; \*\* $P = 0.01$ ; \*\*\* $P < 0.001$ ; ns – not significant; ANOVA – analysis of variance; DAT – days after treatment

Table 2. Effect of soil pH on bean plants treated with different simulated mesotrione rates (SMRs), based on visual injury and reduction in carotenoid content

Soil pH	SMR (g a.i./ha)	Visual injury				Carotenoid content (%)
		7 DAT	14 DAT	21 DAT	28 DAT	
7.5	72	61.25 <sup>a</sup>	70.00 <sup>a</sup>	92.50 <sup>ab</sup>	98.75 <sup>ab</sup>	100 <sup>a</sup>
	36	51.25 <sup>c</sup>	63.75 <sup>ab</sup>	86.25 <sup>bcd</sup>	96.50 <sup>abcd</sup>	100 <sup>a</sup>
	24	40.00 <sup>e</sup>	56.25 <sup>cd</sup>	78.75 <sup>defg</sup>	88.75 <sup>def</sup>	69.19 <sup>bc</sup>
	18	35.00 <sup>f</sup>	48.75 <sup>efg</sup>	76.25 <sup>fg</sup>	87.50 <sup>ef</sup>	68.10 <sup>bc</sup>
	9	25.00 <sup>h</sup>	35.00 <sup>j</sup>	66.25 <sup>h</sup>	76.25 <sup>gh</sup>	55.20 <sup>cde</sup>
	4.5	15.00 <sup>j</sup>	21.25 <sup>kl</sup>	53.75 <sup>i</sup>	63.75 <sup>ij</sup>	50.85 <sup>def</sup>
	2.3	10.00 <sup>k</sup>	15.50 <sup>lmn</sup>	47.50 <sup>ijkl</sup>	56.25 <sup>jk</sup>	31.01 <sup>hijk</sup>
	1.1	5.00 <sup>l</sup>	11.25 <sup>nop</sup>	33.75 <sup>m</sup>	47.50 <sup>lm</sup>	35.52 <sup>ghij</sup>
6.5	0	0.00 <sup>m</sup>	0.00 <sup>q</sup>	0.00 <sup>p</sup>	0.00 <sup>o</sup>	0.00 <sup>p</sup>
	72	60.00 <sup>a</sup>	65.00 <sup>ab</sup>	95.00 <sup>a</sup>	100.00 <sup>a</sup>	100 <sup>a</sup>
	36	51.25 <sup>c</sup>	55.00 <sup>cde</sup>	92.75 <sup>ab</sup>	96.75 <sup>abcd</sup>	100 <sup>a</sup>
	24	45.00 <sup>d</sup>	48.75 <sup>efg</sup>	83.75 <sup>cdef</sup>	90.00 <sup>cdef</sup>	74.74 <sup>b</sup>
	18	35.00 <sup>f</sup>	42.50 <sup>gh</sup>	77.50 <sup>efg</sup>	82.50 <sup>fg</sup>	68.93 <sup>bc</sup>
	9	30.00 <sup>g</sup>	34.25 <sup>j</sup>	63.75 <sup>h</sup>	71.25 <sup>hi</sup>	41.53 <sup>efgh</sup>
	4.5	15.00 <sup>j</sup>	18.50 <sup>klm</sup>	51.25 <sup>ij</sup>	56.25 <sup>jk</sup>	27.36 <sup>ijkl</sup>
	2.3	10.00 <sup>k</sup>	12.75 <sup>mno</sup>	45.00 <sup>kl</sup>	50.00 <sup>klm</sup>	22.42 <sup>klm</sup>
5.5	1.1	5.00 <sup>l</sup>	8.50 <sup>op</sup>	40.00 <sup>lm</sup>	45.00 <sup>m</sup>	20.71 <sup>klmn</sup>
	0	0.00 <sup>m</sup>	0.00 <sup>q</sup>	0.00 <sup>p</sup>	0.00 <sup>o</sup>	0.00 <sup>p</sup>
	72	55.00 <sup>b</sup>	59.25 <sup>bc</sup>	95.00 <sup>a</sup>	98.50 <sup>ab</sup>	100 <sup>a</sup>
	36	45.00 <sup>d</sup>	56.25 <sup>cd</sup>	88.75 <sup>abc</sup>	96.50 <sup>abcd</sup>	100 <sup>a</sup>
	24	35.00 <sup>f</sup>	45.00 <sup>efgh</sup>	85.00 <sup>bcde</sup>	92.50 <sup>abcde</sup>	68.54 <sup>bc</sup>
	18	30.00 <sup>g</sup>	35.50 <sup>ij</sup>	65.00 <sup>h</sup>	70.00 <sup>hi</sup>	48.14 <sup>defg</sup>
	9	20.00 <sup>i</sup>	24.00 <sup>k</sup>	48.75 <sup>ijk</sup>	51.25 <sup>klm</sup>	35.02 <sup>ghij</sup>
	4.5	15.00 <sup>j</sup>	17.75 <sup>klm</sup>	42.50 <sup>kl</sup>	45.00 <sup>m</sup>	31.32 <sup>hijk</sup>
4.5	2.3	10.00 <sup>k</sup>	13.50 <sup>mno</sup>	25.00 <sup>n</sup>	27.50 <sup>n</sup>	15.23 <sup>lmno</sup>
	1.1	5.00 <sup>l</sup>	7.75 <sup>op</sup>	20.00 <sup>no</sup>	25.00 <sup>n</sup>	7.40 <sup>nop</sup>
	0	0.00 <sup>m</sup>	0.00 <sup>q</sup>	0.00 <sup>p</sup>	0.00 <sup>o</sup>	0.00 <sup>p</sup>
	72	45.00 <sup>d</sup>	56.00 <sup>cd</sup>	88.75 <sup>abc</sup>	97.50 <sup>abc</sup>	100 <sup>a</sup>
	36	35.00 <sup>f</sup>	51.00 <sup>def</sup>	81.25 <sup>cdef</sup>	91.25 <sup>bcde</sup>	100 <sup>a</sup>
	24	25.00 <sup>h</sup>	46.00 <sup>efgh</sup>	77.50 <sup>efg</sup>	82.50 <sup>fg</sup>	57.99 <sup>cd</sup>
	18	20.00 <sup>i</sup>	41.75 <sup>hi</sup>	71.25 <sup>gh</sup>	76.25 <sup>gh</sup>	49.99 <sup>def</sup>
	9	15.00 <sup>j</sup>	16.00 <sup>lmn</sup>	52.50 <sup>ij</sup>	53.75 <sup>kl</sup>	38.13 <sup>fghi</sup>
4.5	4.5	12.00 <sup>k</sup>	13.50 <sup>mno</sup>	50.00 <sup>ijk</sup>	52.50 <sup>klm</sup>	18.67 <sup>klmn</sup>
	2.3	10.00 <sup>k</sup>	10.50 <sup>nop</sup>	42.50 <sup>kl</sup>	48.75 <sup>klm</sup>	11.73 <sup>mno</sup>
	1.1	5.00 <sup>l</sup>	6.00 <sup>pq</sup>	15.00 <sup>o</sup>	20.00 <sup>n</sup>	3.31 <sup>op</sup>
	0	0.00 <sup>m</sup>	0.00 <sup>q</sup>	0.00 <sup>p</sup>	0.00 <sup>o</sup>	0.00 <sup>p</sup>

Different letters in each column for each separate soil pH indicate significant differences between group means of measured parameters ( $P = 0.05$ ; Tukey's *HSD* test). Spectrophotometry of carotenoid content was performed in triplicate. Visual injury on bean plants was assessed on a scale from 0 (no visible injury) to 100% (complete necrosis and plant death). DAT – days after treatment; SMR – simulated mesotrione rate

from 7 DAT to 28 DAT for all SMR treatments at all pH values (Table 2).

At 7 DAT, minimal visual injury (5%) was observed for SMR of 1.1 g a.i./ha at all soil pH, while the highest

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SMR (72 g a.i./ha) caused 61.25% injury in soil pH of 7.5 (Table 2). The higher visual injury was seen at SMR  $\geq$  9 g a.i./ha in slightly alkaline and slightly acidic soils than on plants grown in soil irrigated with water solutions of pH 5.5 or 4.5. No such differences were observed between plants grown in soil at different pH values under lower SMR treatments.

The visual injury was much more pronounced at 14 DAT, where the significant injury was observed at an SMR of 72 g a.i./ha at pH 7.5 (70%) and 6.5 (65%). Bean injury continued to increase exponentially with mesotrione treatments at 21 DAT and 28 DAT. Due to a strong herbicidal effect (81.2–100%), there was no significant difference in visual injury at 28 DAT across soil pH groups when SMRs were higher (72 or 36 g a.i./ha). However, this was not the case when the plants were treated with lower SMRs. Under soil conditions close to neutral, visual injury at an SMR of 1.1 g a.i./ha was 47.5% (pH 7.5) and 45% (pH 6.5), while under acidic conditions, injury was 25% (pH 5.5) and 20% (pH 4.5; Table 2). Taken together, our results indicate that bean plants are more vulnerable to injury in slightly alkaline soils (pH 7.5) than in acidic soils (pH 4.5).

**Fresh weight and content of carotenoids.** At 28 DAT, the fresh weight of bean plants was substantially determined by the amount of mesotrione applied: the fresh weight of beans decreased as the amount of mesotrione increased. The values reported for the fresh weight of aboveground biomass are averages of all calculated values for mesotrione treatments

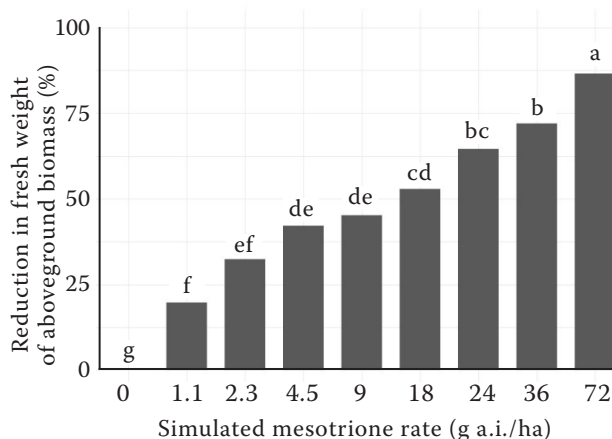


Figure 1. Effect of simulated mesotrione rates on average fresh weight of common bean aboveground biomass. Means followed by the same letter are not significantly different ( $P > 0.05$ ) according to the Tukey *HSD* (honestly significant difference) test. a.i. – active ingredient

and at all soil pH values tested, as no SMR  $\times$  pH interaction was detected for this parameter (Table 1; Figure 1). There was a 19.8% decrease in fresh weight at the lowest SMR (1.1 g a.i./ha) and an 86.4% decrease at the highest SMR (72 g a.i./ha; Figure 1). In contrast, the post hoc test reported no significant differences in the reduction of fresh weight of bean plants as a function of soil pH. However, similar to the trends observed for visual injury, mesotrione reduced fresh weight more under slightly alkaline conditions (56.3% at pH 7.5) than under acidic conditions (42.5% at pH 4.5).

The Pearson correlation coefficient ( $r = 0.963$ ,  $P < 0.0001$ ) showed a strong positive correlation between chlorophyll (data not shown) and carotenoid content determined spectrophotometrically. The spectrophotometric quantification of carotenoid content at 28 DAT was strongly affected by SMR treatment but also soil solution pH. Across all pH treatments, the greatest carotenoid reduction was observed at SMR  $>$  9 g a.i./ha, and the smallest reduction was almost always observed in the most acidic environments. For example, 58% carotenoid reduction was observed in beans grown in soil at pH 4.5, compared to 68.5% reduction at pH 5.5. At the lowest SMR (1.1 g a.i./ha), beans grown in an acidic environment (pH 4.5) showed a 3.3% reduction in carotenoid content, while those grown in a slightly alkaline environment (pH 7.5) showed a 35.5% reduction.

The findings of the present study confirm that the common bean is highly susceptible to the presence of mesotrione residues in the soil. Across all soil pH, even the lowest SMR treatment (1.1 g a.i./ha) triggered significant phytotoxic effects compared to untreated plants. These results are consistent with previous work (Riddle et al. 2013) and imply that the common bean may be a suitable test crop to assess levels of mesotrione residues in soils at different pH values.

The best predictor of yield loss in soybean plants treated with mesotrione is a visual assessment at 28 DAT (Young et al. 2003). Furthermore, Felix et al. (2007) showed that 43% damage of bean plants at 28 DAT with mesotrione was associated with a yield loss of 81%. The present study also showed that a rate of mesotrione as low as 1/128 of the recommended application rate can significantly reduce bean yield in terms of the fresh weight of aboveground biomass. Consequently, bean plants grown near maize crops can experience mesotrione drift (Young et al. 2003) resulting in damaged bean

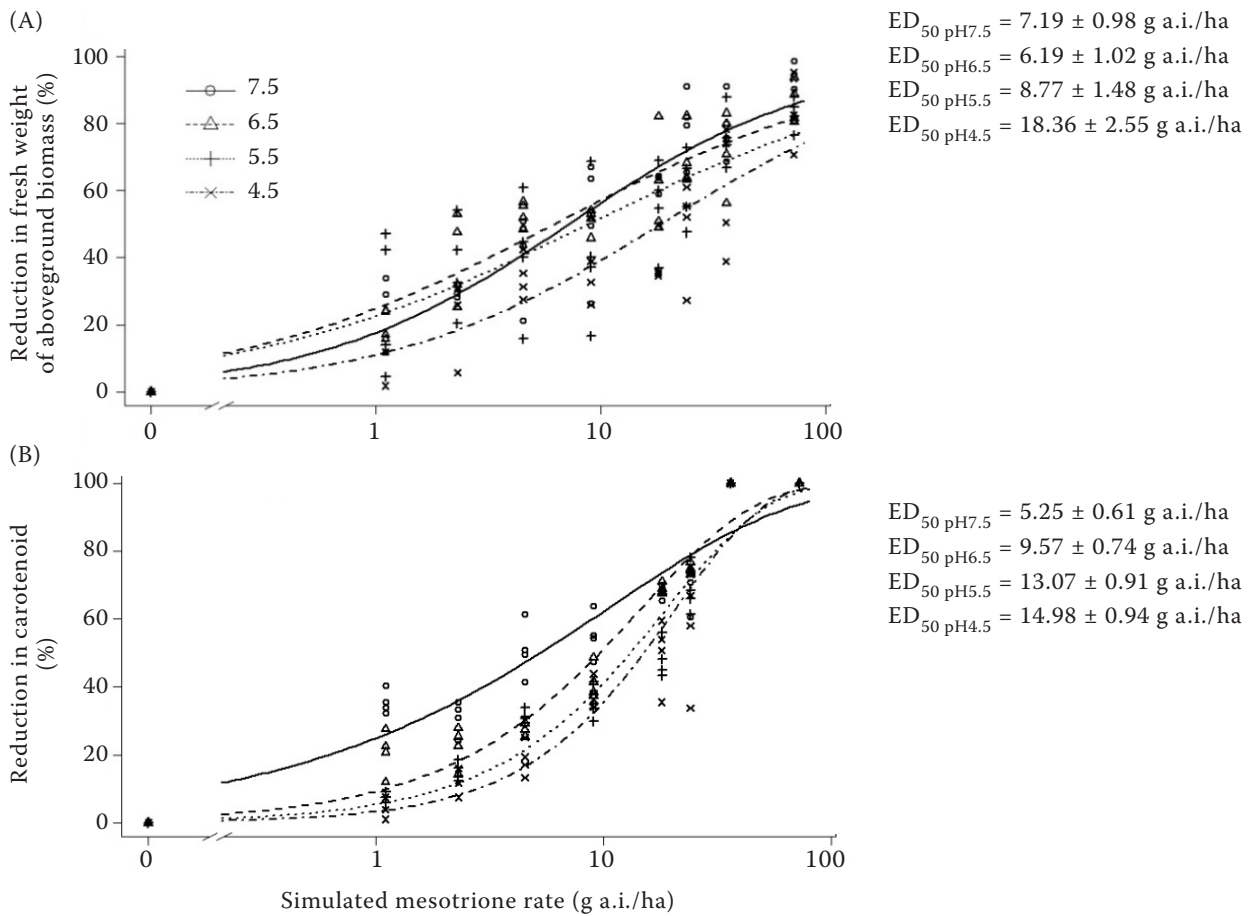
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Figure 2. Dose-response curves for (A) fresh weight of aboveground biomass and (B) carotenoid content of bean plants grown in soil at different pH values. The estimated mean effective dose ( $ED_{50}$ ) of mesotrione for each soil pH is shown below the graphs as estimated dose  $\pm$  standard error; a.i. – active ingredient

plants and consequently lower pigment content in their leaves. Considering that the half-life of mesotrione increases in acidic soils due to better adsorption (Su et al. 2017), the herbicide may also harm crops in rotation. In a field experiment, Felix et al. (2007) reported that the greatest damage to beans grown in acidic soils (pH 5.5) was observed one year after mesotrione application. Therefore, further field trials should be conducted to determine the exact timing of common bean seeding after mesotrione application at different soil pH levels.

The results of the present study show that the pH of soil solution strongly influences the susceptibility of bean plants to mesotrione: acidic pH appears to protect the plants from the herbicide's phytotoxic effects (Figure 2). In a highly acidic environment (pH 4.5), the following rates of mesotrione were required to cause a 50% reduction ( $ED_{50}$ ) in measured parameters:  $18.4 \pm 2.6 \text{ g a.i./ha}$  for fresh weight, and  $15.0 \pm 0.9 \text{ g a.i./ha}$  for carotenoid content. In the case

of bean plants grown in soils at pH 7.5, 6.5 or 5.5,  $ED_{50}$  was similar for the reduction in fresh weight ( $6.2 \pm 1.0$ – $8.8 \pm 1.5 \text{ g a.i./ha}$ ). In contrast,  $ED_{50}$  for reduction in carotenoid content varied substantially with pH:  $5.3 \pm 0.6 \text{ g a.i./ha}$  for pH 7.5,  $9.6 \pm 0.7 \text{ g a.i./ha}$  for pH 6.5,  $13.1 \pm 0.9 \text{ g a.i./ha}$  for pH 5.5, and  $15.0 \pm 0.9 \text{ g a.i./ha}$  for pH 4.5. These findings imply that the effect of soil pH on bean growth may be better predicted by carotenoid analyses than by fresh weight. Since the application of mesotrione inhibits 4-hydroxyphenylpyruvate dioxygenase in the carotenoid biosynthesis pathway, bioassays of this enzyme may be more accurate if they take into account its mechanism of action as suggested earlier by Jovanović (2011).

These findings emphasise the importance of considering soil pH when using test plants to detect herbicide residues in soil. Soil organic carbon is believed to be the main component of soil that retains herbicide molecules, which it does through

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hydrophobic interactions and hydrogen bonding. Adsorption of compounds that act as weak acids, such as mesotrione, is greater under acidic conditions when the compounds are neutral (Đurović 2011). There should be abundant interactions to adsorb such compounds as long as the organic carbon content in the soil is higher than 1% (Lehman et al. 1992). This may explain the association between acidic pH of soil solution and less severe bean plant injury in the present study, which involved silty clay loam soil with an organic carbon content of 2.5%. In contrast, 47.5% visual injury and 35.5% reduction in carotenoid content were observed at 28 DAT with the lowest rate of mesotrione (1.1 g a.i./ha) at soil pH 7.5 (Table 2). This may reflect the higher bioavailability of the anionic form of mesotrione, which predominates in neutral-alkaline soil (Boesten 1993).

In conclusion, our results suggest that the common bean is highly susceptible to the presence of mesotrione residues in the soil, especially in neutral-alkaline environments. Therefore, it can be used as a suitable model for optimising crop rotation after mesotrione application.

Furthermore, our results support spectrophotometry as a reliable method to detect the effects of mesotrione on plant growth. Although the spectrophotometrically determined chlorophyll and carotenoid content are strongly positively correlated, follow-up work with liquid chromatography is needed to accurately quantify the amount of both pigments after applying simulated mesotrione rates.

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