The Susceptibility of Pea (*Pisum sativum* L.) to Simulated Mesotrione Residues as Affected by Soil pH Manipulation

Ana Pintar 1,*, Zlatko Svečnjak 2, Josip Lakić 1, Ivan Magdić 3, Dragojka Brzoja 1 and Klara Barić 1

1 Department of Weed Science, Faculty of Agriculture, University of Zagreb, Svetošimunska Cesta 25, 10000 Zagreb, Croatia; jlakic@agr.hr (J.L.); dbrzoja@agr.hr (D.B.); kbaric@agr.hr (K.B.)
2 Department of Field Crops, Forages and Grasslands, Faculty of Agriculture, University of Zagreb, Svetošimunska Cesta 25, 10000 Zagreb, Croatia; svecnjak@agr.hr
3 Department of Soil Science, Faculty of Agriculture, University of Zagreb, Svetošimunska Cesta 25, 10000 Zagreb, Croatia; imagdic@agr.hr
4 Correspondence: apintar@agr.hr; Tel.: +385-1-2393-606

Abstract: Variations in soil pH have been shown to affect mesotrione adsorption, which in turn, may have an impact on crop susceptibility. Therefore, a greenhouse experiment was conducted to evaluate the effect of simulated mesotrione residues on pea crop grown in the typical agricultural soil (gleysol) of north-western Croatia. The soil pH was manipulated to obtain neutral (pH 7.0) and acidic (pH 5.0) values. Simulated mesotrione residues were 1.1, 2.3, 4.5, 9.0, 18, 36 and 72 g a.i. ha\(^{-1}\). Crop visual injuries as well as reductions in chlorophyll fluorescence and aboveground dry biomass were higher at pH 7.0 than at pH 5.0. With increasing mesotrione residues, the reductions in chlorophyll fluorescence ranged from 38.8% to 89.7% at pH 5.0 and from 63.7% to 99.3% at pH 7.0. Compared to chlorophyll fluorescence, the reductions in dry biomass were smaller and ranged from 49.2% to 96.8% at pH 7.0 and from 32.0% to 82.6% at pH 5.0 for the mesotrione residues from 1.1 to 72 g a.i. ha\(^{-1}\). These results indicate that soil pH is an important factor determining the susceptibility of pea crop to simulated mesotrione residues.

Keywords: mesotrione; pea; soil pH; simulated residue carryover; phytotoxicity

1. Introduction

Mesotrione is a p-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting triketone herbicide commonly used for the control of broadleaf and grass weeds in corn pre- and post-emergence [1]. Mesotrione is characterized by favorable physicochemical properties and relatively short degradation time (DT\(_{50}\) = 4–44.3 days under standard laboratory conditions) [2]. It is the third most commonly used herbicide in corn in Croatia [3]. However, extensive use of mesotrione has resulted in its residues remaining in the soil for a year after application, causing significant yield losses in susceptible crops grown in rotation [4,5].

Commercial fields previously treated with mesotrione may occasionally show symptoms of carryover injury. This effect is most commonly observed in overlap areas and is characterized by bleaching of the plants followed by necrosis of the meristematic tissue [6,7]. Twelve months after mesotrione application in a corn, the yield of broccoli, carrot, cucumber and onion was significantly reduced [4]. Similarly, 12 months after mesotrione application in corn a phytotoxic effect was observed on sugar beet, cucumber, bean, pea and soybean [5]. The susceptibility of bean to mesotrione applied 12 months earlier at an application rate of 100 g active ingredient (a.i.) ha\(^{-1}\) differed among cultivars [8]. Higher phytotoxic injury was observed in cranberry (40%) and kidney bean (27%) compared to white and black bean, where injury was less than 6%. Therefore, the authors recommend a replanting interval of 12 months for white and black beans and 24 months for cranberry and kidney bean. In a similar field experiment, no phytotoxic effects were observed in barley, lettuce, pea, sugar beet, sunflower and wheat at mesotrione rates of 168 and 336 g a.i. ha\(^{-1}\) applied 12 months earlier [9].
Several studies have shown that adsorption and degradation of mesotrione are mainly related to soil pH [10,11]. Mesotrione is a weak acid with a dissociation constant (pKa) of 3.12 [2]. Therefore, it is present in acidic soils in neutral form with pronounced affinity for adsorption to soil organic colloids [10,12]. The adsorption coefficient (Kd) of mesotrione was 0.13 L kg\(^{-1}\) in a soil with pH 7.1 and 5.0 L kg\(^{-1}\) in a soil with pH 5.0 [11]. The DT\(_{50}\) of mesotrione varied from 5 days in alkaline (pH 8.1) soil to 34 days in acidic (pH 6.6) soil [11]. The results of laboratory studies on mesotrione degradation showed that the DT\(_{50}\) of mesotrione increased from 13.6 days to 34.7 days when the soil pH decreased from 8.0 to 6.0 [13]. However, the effect of soil pH on the availability of herbicide residues to plants and thus on their susceptibility was not investigated.

In our previous simulated carryover study [14], we found that soil type has a large effect on mesotrione adsorption. Since the studied soils differed in texture and humus content, we assumed that the higher humus content (4.2%) and silty clay loamy texture of hypogley mineral soil compared to humofluvisol (humus 2.7%; silty loamy texture) was the reason for the higher adsorption of mesotrione and thus the lower susceptibility of sugar beet. Therefore, the objective of this study was to determine whether differences in soil pH of gleysol affected the adsorption capacity and thus the susceptibility of pea to simulated mesotrione residues.

2. Materials and Methods

2.1. Sampling and Preparation of Soil Samples

Soil samples were collected in March 2020 and 2021 from a gleysol [15] located in Šašinovecki Lug (north-western Croatia). Samples were collected from the surface layer (0–20 cm) of the untreated parts of the field using a probe (Split Tube Sampler, Ø 53 mm, Eijkelkamp, Giesbeek, The Netherlands). After sampling, the soil was air-dried for 72 h and passed through a 2-mm sieve.

Organic matter (OM) content was measured by the Walkley–Black method, pH was measured in soil suspensions with deionized water at a ratio of 1:2.5 (w/v) and with 1 M KCl according to HRN ISO 10,390:2005, and cation exchange capacity (CEC) was measured according to HRN ISO 11,260:2004. Table 1 shows the main properties of the gleysol.

| Soil     | pH (H\(_2\)O) | pH (KCl) | Humus (%) | CEC (cmol kg\(^{-1}\)) | Soil | pH (H\(_2\)O) (%) | pH (KCl) |
|----------|---------------|----------|-----------|-------------------------|------|------------------|----------|
| Gleysol  | 7.7           | 7.02     | 4.2       | 33.8                    | 1.1  | 59.6             | 39.3     |

2.2. Soil pH Manipulation

In this study a typical cropland soil (gleysol) was collected in north-western Croatia. This soil was slightly alkaline (pH\(_{H2O}\) 7.7) and was adjusted to obtain neutral and acidic pH values by a manipulation procedures similar to [16]. To achieve neutral pH value, a natural air-dried soil was treated with distilled water only. For the purpose of obtaining acidic pH value, the acidified solution (0.1 M HCl) was added to the surface of air-dried soil to ensure uniform distribution. Both (neutral and acidic) soil samples were adjusted to a moisture content of 50% water holding capacity with distilled water, sealed and incubated at room temperature for three weeks to allow the pH to reach equilibrium. The pH was measured in soil suspension with deionized water at a ratio of 1:2.5 (w/v) and the final pH was 7.03 and 5.07 for the neutral and acidic soils, respectively. These pH values are referred to as pH 7.0 (neutral soil) and pH 5.0 (acid soil).
2.3. Greenhouse Bioassay

The greenhouse bioassay was conducted at the University of Zagreb Faculty of Agriculture and repeated over time (2020 and 2021). Each soil sample consisted of 200 g of air-dried soil evenly distributed in 10 cm diameter plastic pots and five pea seeds ('Kelvedon') were sown at a depth of 4 cm. Mesotrione (Callisto® 480 SC) was applied with the CAMAG® TLC sprayer at seven different rates: 1.1, 2.3, 4.5, 9.0, 18, 36 and 72 g a.i. ha\(^{-1}\), or 1/128 R to 1/2 R, where R is the recommended application rate (144 g a.i. ha\(^{-1}\)). These rates simulated the expected mesotrione residues in the soil over time, considering the half-life under field conditions. After application, pots containing pea seeds were exposed to natural conditions of temperature and photoperiod, with a minimum temperature of 10 \(^\circ\)C during the experiment. Each mesotrione rate, including the control (0 g a.i. ha\(^{-1}\)), was set in four replicates, and the experimental design was a randomized complete block design. The pots with both (neutral and acidic) soil samples were watered with 60 mL of distilled water every other day. The volume of distilled water and irrigation dynamics were determined in the preliminary experiment.

2.4. Evaluation of Pea Susceptibility

Pea plants were visually assessed for percentage of injury at 14, 28 and 35 days after treatment (DAT) using a scale from 0 (no phytotoxic effect) to 100% (plant death) [17]. Chlorophyll fluorescence intensity was measured 35 DAT using a Plant Stress Kit (ADC BioScientific Ltd., Hoddesdon, UK) and five measurements were taken for each plant. The aboveground biomass of pea was harvested from each pot 35 DAT and then dried in an oven (UF 260, Memmert GmbH + Co. KG, Schwabach, Germany) at 70 \(^\circ\)C for 72 h. At the time of harvest, the untreated pea plants were at the stage of six extended internodes (BBCH 36).

2.5. Statistical Analysis

Statistical analysis of results was performed by analysis of variance in SAS 8.0 using Mixed Procedure Model (SAS Institute, Cary, NC, USA). Block and its interaction with the year, soil pH and mesotrione rate were random effects. Fixed effects were year, soil pH and mesotrione rate. Year effect was nonsignificant so that data were pooled across years. After a significant F-test, the Least Significant Difference (LSD) test was used to compare the means for \(p \leq 0.05\).

3. Results

The susceptibility of pea at both soil pH values to simulated mesotrione residues did not differ significantly between years, so data were pooled across years.

3.1. Visual Injuries of Pea Plants at 14, 28 and 35 Days after Treatment

The susceptibility of pea to mesotrione differed depending on the level of simulated mesotrione residues and soil pH. The progression of phytotoxic symptoms was observed with increasing DAT at both pH values. At 14 DAT, phytotoxic symptoms on pea were observed at the lowest level (1.1 g a.i. ha\(^{-1}\)) of simulated mesotrione residues and it was 16.0% at pH 5.0 and 31.3% at pH 7.0 (Figure 1a). The highest injuries of 55.0% at pH 5.0 and 67.5% at pH 7.0 were observed at a simulated mesotrione residues of 72 g a.i. ha\(^{-1}\).

Similarly, at 21 DAT, phytotoxic injuries increased with an increasing level of simulated mesotrione residues and were significantly lower in pea plants at pH 5.0 compared to plants at pH 7.0 (Figure 1b). At pH 7.0, pea injury exceeded more than 50% at 4.5 g a.i. ha\(^{-1}\), while at pH 5.0, similar injuries (51.0%) were observed at 18 g a.i. ha\(^{-1}\). At both pH, the injuries manifested as bleaching followed by necrosis of aboveground biomass of pea.
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developed on 60.5% of pea plants at an simulated mesotrione residues of 2.3 g a.i. ha\(^{-1}\). When mesotrione was applied to pea plants at pH 5.0, similar injury symptoms (61.8%) were observed after an application of 18 g a.i. ha\(^{-1}\). Almost completely injured (>80%) pea plants were observed at 36 g a.i. ha\(^{-1}\) at pH 7.0 and at 72 g a.i. ha\(^{-1}\) at pH 5.0.

Figure 1. Visual injury (% of control) evaluation of pea at 14 (a), 21 (b) and 35 (c) days after treatment with simulated mesotrione residues applied to soil at pH 7.0 and pH 5.0. Vertical lines indicate a statistical difference according to Fisher’s LSD test at \(p \leq 0.05\).
The highest injuries at both pHs were observed at 35 DAT (Figure 1c). However, pea susceptibility was again most pronounced at pH 7.0. At this pH, injury symptoms developed on 60.5% of pea plants at an simulated mesotrione residues of 2.3 g a.i. ha\(^{-1}\). When mesotrione was applied to pea plants at pH 5.0, similar injury symptoms (61.8%) were observed after an application of 18 g a.i. ha\(^{-1}\). Almost completely injured (>80%) pea plants were observed at 36 g a.i. ha\(^{-1}\) at pH 7.0 and at 72 g a.i. ha\(^{-1}\) at pH 5.0.

3.2. Chlorophyll Fluorescence Reduction at 35 Days after Treatment

The reduction in chlorophyll fluorescence in pea varied greatly depending on the soil pH and level of simulated mesotrione residues (Table S1). Chlorophyll fluorescence was exponentially reduced with increasing levels of simulated mesotrione residues at both pH values (Figure 2). Moreover, the percentage of reduction in chlorophyll fluorescence increased with increasing soil pH. A significantly higher reduction in chlorophyll fluorescence, especially at lower levels of simulated mesotrione residues, was observed at pH 7.0. Compared to untreated soil, there was a reduction in chlorophyll fluorescence from 63.7% to 99.3% for simulated mesotrione residues from 1.1 to 72 g a.i. ha\(^{-1}\), with greater than 90% reduction observed at 36 g a.i. ha\(^{-1}\) at pH 5.0. At pH 5.0, the reduction in chlorophyll fluorescence ranged from 38.8% to 89.7% for the same levels of simulated residues.

![Figure 2. Chlorophyll fluorescence reduction (% of control) in pea plants 35 days after treatment with simulated mesotrione residues applied to soil at pH 7.0 and pH 5.0. Vertical lines indicate a statistical difference according to Fisher’s LSD test at \(p \leq 0.05\).](image)

3.3. Aboveground Dry Biomass Reduction

A significant interaction between simulated mesotrione residues and soil pH (Table S2) indicates that the reduction in pea aboveground dry biomass by the simulated mesotrione residues was influenced by soil pH. Pea dry biomass was greatly reduced following the application of increased levels of simulated mesotrione residues at both pHs. Similar to the reduction in chlorophyll fluorescence, the reduction in pea dry biomass was less at pH 5.0 (Figure 3). At pH 5.0, a 50% reduction in dry biomass was observed at 9 g a.i. ha\(^{-1}\), while at pH 7.0, a similar percentage of reduction (49.2%) was observed at the lowest (1.1 g a.i. ha\(^{-1}\)) level of simulated residues. Dry biomass of pea was almost completely (>80%) reduced at 36 g a.i. ha\(^{-1}\) at pH 7.0 and at 72 g a.i. ha\(^{-1}\) at pH 5.0.
3.2. Chlorophyll Fluorescence Reduction at 35 Days after Treatment

Figure 3. Reduction in aboveground dry biomass (% of control) of pea plants treated with simulated mesotrione residues applied to soil at pH 7.0 and pH 5.0. Vertical lines indicate a statistical difference according to Fisher’s LSD test at \( p \leq 0.05 \).

4. Discussion

It is well known that adsorption of herbicides to soil particles is the most important process affecting the behavior of organic herbicides after their application. The intensity of adsorption depends on a variety of factors, and it is usually difficult to estimate which factor is crucial. Some authors emphasize the importance of soil pH on the adsorption of mesotrione [10,11]. Mesotrione is a weak acid with a pKa value of 3.12 [2]. Therefore, regardless of soil composition and texture, the adsorption of mesotrione is more pronounced under acidic soils. Under acidic soils, mesotrione is present as a neutral molecule with a pronounced affinity for adsorption, making it less susceptible to degradation than when it is present as an anion in alkaline soils [10]. The molecular form of weak acids is less soluble in water due to greater adsorption, while the anionic form is more soluble [18]. The adsorption coefficient (Kd) of mesotrione in neutral (pH 7.1) soil was 0.13 L kg\(^{-1}\), while in acidic (pH 5.0) soil it was 5.0 L kg\(^{-1}\) [10]. This is also evident from the results of this study, where significantly higher phytotoxic injury to pea was found at soil pH 7.0 compared to soil pH 5.0. Therefore, mesotrione in anionic form is predominantly in the liquid phase of the soil from where it can be taken up by the plant [19], which is the reason for the higher phytotoxicity of pea at pH 7.0. In contrast to our results, less phytotoxic injury was observed in oat after application of simazine and atrazine on soil with pH 5.8 compared to soil with pH 6.7 [20]. Atrazine and simazine are weak bases, so they protonate and form cations in acidic soil. As such, they are adsorbed on negatively charged soil colloids, leading to a decrease in their availability in the liquid phase of the soil [21].

In connection with the above, the degradation of mesotrione is more pronounced under neutral and alkaline soils. This is confirmed by the results of a laboratory study [13], which investigated the time for 50% degradation of the applied mesotrione rate under constant conditions of temperature (25 °C) and soil moisture (60%), but different pH (6, 7 and 8). The results of their study showed that acidic soil (pH 6.0) favored lower degradation rate (DT\(_{50}\) = 34.7 days), neutral soil (pH 7.0) favored faster degradation rate (DT\(_{50}\) = 20.4 days) and alkaline soil (pH 8.0) favored the fastest degradation rate (DT\(_{50}\) = 13.6 days).

The results of the determination of chlorophyll fluorescence are in agreement with the results of another study [22], in which it was found that the application of mesotrione leads to a reduction in photosynthetic capacity in two species of algae, Microcystis sp. and Scenedesmus quadricauda. Moreover, a significantly higher reduction in chlorophyll fluorescence intensity was observed at a soil pH of 7.0 compared to a soil pH of 5.0. In our previous study [14], we investigated the effect of simulated mesotrione residues applied to hypogoley mineral soil and humofluvisol on total carotenoid content in sugar beet. The soils
did not differ in pH (both soils were alkaline (pH 7.7–8.2)) but they differed in adsorption intensity ($K_f$). A higher adsorption intensity ($K_f = 1.396$) was found in the hypogley mineral soil than in humofluvisol ($K_f = 0.544$). Therefore, the lower adsorption capacity of humofluvisol indicates a higher possibility of the uptake of non-adsorbed mesotrione by sugar beet, resulting in a higher reduction of total carotenoid content for the same amount of mesotrione residue in the soil.

A significant reduction in pea dry biomass was observed at both soil pH values, with a higher reduction at soil pH 7.0. Our results are in agreement with a previous study that reported a 16 to 51% reduction in pea dry biomass after the application of simulated mesotrione residues at a rate of 28 to 51 g a.i. ha$^{-1}$ at soil pH 6.7 [5]. Moreover, the injury resulted in a significant reduction in pea yield of 39 and 47% at 42 and 56 g a.i. ha$^{-1}$ mesotrione, respectively.

From the results of the study, it is evident that the same amount of simulated mesotrione residues applied at the time of sowing caused higher reduction in chlorophyll fluorescence than reduction in aboveground dry biomass of pea. Herbicide residues from the imidazolinone group (acetolactate synthase herbicides) caused a reduction in photosynthetic intensity even before visible phytotoxic injury to rice plants [23]. Previous studies reported significant reduction of photosynthetic intensity in various crops by bensulfuron-methyl residues at concentrations of 50 µg kg$^{-1}$ [24]. Although the inhibition of photosynthesis is not the primary mechanism of action of bensulfuron-methyl, the inhibition of other photosynthesis-related metabolic processes in the plant results in disruption of plant activity [25,26].

The overall results of this study show that pH plays an important role in the susceptibility of pea to simulated mesotrione residues. In general, mesotrione is more adsorbed at lower pH values, resulting in less phytotoxic injury to pea plants 35 DAT. However, due to higher adsorption, the persistence of mesotrione could be more pronounced in acidic soils, which could limit the sowing of susceptible crops in the rotation.

5. Conclusions

This study showed that pea was susceptible to simulated mesotrione residues even at the lowest value (1.1 g a.i. ha$^{-1}$ applied at the time of sowing). The extent of injury was also significantly affected by soil pH manipulation. The higher plant visual injury and reductions in chlorophyll fluorescence and aboveground dry biomass at 35 DAT were found in neutral (pH 7.0) compared to acidic (pH 5.0) soil reaction. These results indicate that soil pH is an important factor determining the susceptibility of pea crop to simulated mesotrione residues, and should be considered in crop rotation practices.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture11080688/s1, Table S1: Two-way analysis of variance for the reduction in chlorophyll fluorescence (% of control) of pea plants treated with simulated mesotrione residues applied to soil at pH 7.0 and pH 5.0, Table S2: Two-way analysis of variance for the reduction in aboveground dry biomass (% of control) of pea plants treated with simulated mesotrione residues applied to soil at pH 7.0 and pH 5.0.

Author Contributions: Conceptualization, A.P.; methodology, A.P., J.L. and I.M.; software, A.P. and Z.S.; formal analysis, A.P.; investigation, A.P., J.L. and D.B.; resources, K.B.; data curation, A.P.; writing—original draft preparation, A.P.; writing—review and editing, A.P., Z.S., I.M. and K.B.; visualization, A.P.; supervision, A.P.; funding acquisition, K.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.
Conflicts of Interest: The authors declare no conflict of interest.

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