Effect of soil pH on common bean (Phaseolus vulgaris L.) sensitivity to simulated mesotrione residues

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MASTER'S THESIS

Laura Pismarović

Zagreb, September, 2021.

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University of Zagreb
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Graduate study:

Environment, agriculture and resource management (INTER – EnAgro)

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ON ACADEMIC INTEGRITY

I, **Laura Pismarović**, JMBAG 0119004252, born on 6th of January in Zagreb, declare that I have independently written the thesis under the title of:

Effect of soil pH on common bean (*Phaseolus vulgaris L.*) sensitivity to simulated mesotrione residues

With my signature, I guarantee:

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- that all literature references, both published and unpublished, are adequately cited or paraphrased, and listed at the end of the paper;
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- that electronic version of this thesis is identical to the printed one approved by the mentor;
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Report

ON EVALUATION AND DEFENCE OF MASTER’S THESIS

Master’s thesis written by **Laura Pismarović**, JMBAG 0119004252, under the title of

Effect of soil pH on common bean (*Phaseolus vulgaris L.*) sensitivity to simulated mesotrione residues

is defended and evaluated with the grade ____________________, on

____.

Comittee for thesis evaluation and defense: signatures:

1.	Assoc. Prof. Maja Šćepanović, PhD	mentor	____________________
2.	Assist. Prof. Boris Lazarević, PhD	member	____________________
3.	Assist. Prof. Kristina Kljak, PhD	member	____________________

Acknowledgements

First of all, I would like to thank my mentor, Assoc. Prof. Maja Šćepanović, PhD, for giving me the opportunity to participate in this research. I would also like to thank her for her patience, support, guidance and invested time during my scientific work.

My thanks go to my immediate supervisor, Ana Milanović-Litre, PhD, for all the help in setting up the experiment, making measurements, and guiding me in the analyses and writing of this paper, especially for selflessly helping me with all the hard work during the setup of the experiment.

In addition, I would like to thank Assist.Prof. Boris Lazarević, PhD for providing me with the climate chamber for a month, without which the experiment could not be performed, and for preparing the solutions, which were one of the most important tools for this experiment. I would also like to thank him for allowing me to use the equipment for the chlorophyll and fluorescence measurements.

Thanks to Josip Lakić, mag.ing.agr. for the technical help in setting up the experiment with the applications of mesotrione and for the advice during and after the experiment.

A big thank you to all the staff of the Department of Animal Nutrition, especially toAssist.Prof. Kristina Kljak, PhD, for providing a workspace in their laboratory, for teaching me how to perform the analyses and for their help during my presence.

I would like to thank my better half for all the patience, love and support during my studies and for giving me the opportunity to enrol and complete my studies while sharing life with me.

I would like to thank my brother for always cheering me on to finish my studies, keeping up the enthusiasm for the work and giving me back the idea that nothing is impossible.

My very special thanks go to my mother for her boundless patience and understanding throughout my education and for never losing faith in me while I pursued my interests. I thank her for keeping my dreams alive and for all the love and support she gave me.

Laura Pismarović

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Summary

Of the master’s thesis – student Laura Pismarović, entitled

Effect of soil pH on common bean (*Phaseolus vulgaris L.*) sensitivity to simulated mesotrione residues

Under certain field conditions, mesotrione persists in the soil longer than expected and damages sensitive crops in the rotation. Soil pH has the greatest influence on the persistence of mesotrione in soil. The bioassay was conducted to evaluate the effect of soil pH on the sensitivity of common bean to simulated mesotrione residues (SMR). The natural soil was manipulated to obtain three pH values: 6.5, 5.5 and 4.5 (pH_1-pH_4) and was treated with seven SMR from 1.1 to 72 to g a.i. ha^{-1}. The fresh aboveground bean weight and photosynthetic pigments were reduced, but the extent of the inhibitory effect depended on SMR and soil pH. The highest phytotoxic injury to common bean was recorded in all soils 28 DAT at 72 g a.i. ha^{-1} (96.5 to 100%), with the least damage at pH_4 (20%). Bean fresh weight was reduced most on soil pH_1 where 86.47 % reduction was observed. Carotenoid content was significantly reduced by average of 52.13 %, 45.84 % on soil pH_1 and pH_2, respectively. Common bean was more sensitive to SMR grown on neutral/alkaline soils.

Key words: mesotrione, common bean, soil pH, simulated residue carryover, phytotoxicity

1. Introduction

Herbicides are expected to be effective against weeds, but at the same time, selective for the crops in which they are applied, or for crops in rotation. Triketones are a new generation of herbicides developed from an allelopathic compound first isolated from the bottlebrush plant (*Callistemon citrinus L.*) (Beaudegnies et al., 2009). They are widely used as pre-emergence (pre-em) and post-emergence (post-em) herbicides to control a wide range of weeds in maize (Romdhane et al., 2019). Mesotrione, which belongs to the triketone group, is a selective soil and foliar herbicide used to control annual broadleaf weeds and some annual grasses in maize (Young et al., 1999). Due to its favorable ecological and toxicological properties and high selectivity (Mitchell et al., 2001), mesotrione has been registered in more than 50 countries (Carles et al., 2017).

It is known that a herbicide in crop rotations can have effects on non-target crops. In Croatia, the following crops should not be sown 24 months after application of mesotrione (Callisto 480 SC®): sugar beet (*Beta vulgaris L. var. Saccharifera Alef.*), fodder beet (*Beta vulgaris L. spp. Crassa Alef.*), beetroot (*Beta vulgaris L. subsp. Vulgaris*), lettuce (*Lactuca sativa L.*), spinach (*Spinacia oleracea L.*), peas (*Pisum sativum L.*), beans (*Phaseolus vulgaris L.*) and other species of the genus Phaseolus and Vicia. Many authors reported damage to crops one or two years after applying mesotrione (Riddle et al., 2013; Felix et al., 2007; Soltani et al., 2007; Robinson et al., 2006). However, some of them suggest shorter restriction periods (12 months) for sowing crops (Felix et al., 2007) because the amount of herbicide residues in the soil depends mainly on the adsorption capacity of the herbicide (Dyson et al., 2002) and the rate of degradation (Barchanska et al., 2015). Although mesotrione is defined as a non-persistent herbicide (Lewis et al., 2016), the studies have shown that its half-life (DT_{50}) in soil is highly dependent on pedoclimatic conditions, especially soil pH (Dyson et al., 2002).

It has been found that soil pH had the greatest influence on the DT_{50} of mesotrione because its adsorption is more pronounced in acidic soils (Dyson et al., 2002), with an estimated DT_{50} from 4.5 days (pH 7.1) to 32 days (pH 5.0) (Chaabane et al., 2008).

Based on these facts, this study investigated the effect of simulated mesotrione residues on test plants (common bean) grown in four different soil pH values (4.5, 5.5, 6.5 and 7.5). To exclude other factors (soil texture, soil moisture, microbial activity) that could influence adsorption capacity and thus the susceptibility of bean to simulated mestorione residues, the same soil was used and manipulated to achieve different pH values.

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 the bean to simulated mesotrione residues**.** Simulated mesotrione residues represent the expected mesotrione residues in the soil over a period of time after application. The recommended application rate for mesotrione in Croatia is 144 g a.i. ha^{-1}, so ½ - 1/128 of the recommended rate was used for this study. Common bean (*Phaseolus vulgaris L.*) was selected as the test crop as it is very sensitive to the effects of mesotrione residues (Riddle et al., 2013).

2. Hypotheses and aims of the research

Based on the literature review and the already known findings, hypotheses and research objectives were established.

1. bean sensitivity will vary with the amount of simulated mesotrione residues, with higher sensitivity being at higher simulated mesotrione residues,
2. bean sensitivity to the same amount of simulated mesotrione residues will vary with soil pH, with sensitivity being higher at more alkaline soil pH.

Based on the hypotheses presented, the following research objective was established:

To determine the effect of simulated mesotrione residues on common bean in relation to soil pH based on the visual phytotoxicity assessment and reduction of fresh weight, and chlorophyll, and carotenoid content.

3. Literature review

Mesotrione is a selective soil and foliar herbicide used to control annual broadleaf and some annual grass weeds in maize (Young et al., 1999). It was the second top-selling herbicide in the world in 2010 (Barchanska et al., 2015). It belongs to the new generation of herbicides, triketones, developed by mimicking the structure of leptospermone (2,2,4,4-tetramethyl-6-(3-methyl-1-oxobutyl)-1,3,5-cyclohexanetrione), an allelopathic compound first isolated from the bottlebrush plant (*Callistemon citrinus* L.) (Beaudegnies et al., 2009). Since their introduction to the market in European countries as the replacement for banned triazines, synthetic β-triketones have been widely used as pre-emergence (pre-em) and post-emergence (post-em) herbicides to control a wide range of broadleaf weeds in maize (Romdhane et al., 2019).

Due to its relatively favorable ecological and toxicological properties, mesotrione has been approved in more than 50 countries (Carles et al., 2017). The high **selectivity** of mesotrione in maize was reported by Mitchell et al. (2001). The research showed that the metabolism of mesotrione degradation was slower in *Chenopodium album, Amaranthus retroflexus*, *Ipomoea hederacea* than in maize. Authors explained its selectivity to maize by the lack of translocation of unchanged mesotrione away from the site where the herbicide is initially absorbed after foliar uptake; only 0.0008 µg of mesotrione was detected in maize seven days after application in contrast to 0.002 to 0.3 µg detected in weed species. This suggests that the crop selectivity of mesotrione is due to differential rates of metabolism of mesotrione in maize compared to other plant species. James et al. (2006) conducted six field trials over three growing seasons to evaluate the use of mesotrione for pre- and post-em weed control in maize. As a pre-em treatment, it provided excellent control of broadleaf weeds (>99% reduction in dry matter) but was poor on grass weeds (80% reduction). Post-em applications of mesotrione were very effective on broadleaf weeds (>94%) but less effective on grass weeds (about 85%). Most importantly, mesotrione did not cause apparent injury to maize crops in any trial and grain yields were not significantly different from the standard treatments.

The **herbicidal effect** of mesotrione is due to the inhibition of the enzyme 4-hydroxyphenylpyruvate dioxygenase (4-HPPD) (Lewis et al., 2016), which significantly reduces the level of plastoquinones in plant cells, thereby blocking the process of carotenoid biosynthesis (Beaudegnies et al., 2009). The main role of carotenoids is in the light-harvesting antenna structures of photosynthetic tissue, where they suppress high-energy triplet states of chlorophyll that would otherwise generate singlet oxygen. The depletion of carotenoids caused by herbicides is associated with the light-dependent generation of singlet oxygen, which damages lipids and proteins and causes the breakdown of the photosynthetic complex and the release of free chlorophyll. Free chlorophyll is photodynamically photodestructive and itself further generates singlet oxygen, which eventually leads to the destruction of all leaf pigments (Beaudegnies et al., 2009) and results as bleaching, a typical symptom of mesotrione action (Mitchell et al., 2001).

Mesotrione is classified as a **non-persistent herbicide** with an estimated half-life (DT_{50}) of 4 to 44 days (in the laboratory studies), i.e. 3 to 7 days in the field studies (Lewis et al., 2016). Because soil type and weather conditions strongly influence herbicide persistence, some studies have shown that the half-life of mesotrione can be significantly longer under certain pedoclimatic conditions (Wangcang et al., 2017; Chaabane et al., 2008; Dyson et al., 2002;). Rouchaud et al. (2001) studied the decomposition of mesotrione in soils with approximately the same acidity (pH 6.4 - 6.8) and organic matter content (1.45 - 1.94%), but different **soil texture**. According to the results, 90% of the total applied mesotrione in the surface layer of sandy soils (0-10 cm) was degraded after 3.6 months and in silty loam, loam and clay soils after 4.7 months. In addition, Wangcang et al. (2017) reported the influence of **soil moisture** content on the persistence of mesotrione. For example, 90 days after application, 98% of the total applied mesotrione was degraded at a soil moisture content of 60%, 81% at a soil moisture content of 40%, and only 52% at a soil moisture content of 15%. Soil moisture content also affects the stability of mesotrione. In soils with a temperature of 15 °C, 21 days after application, undegraded amounts of mesotrione were three times higher (81%) than in soils with a temperature of 35 °C, where the proportion of undegraded mesotrione was 25% (Wangcang et al., 2017). However, it has been found that **soil pH** had the greatest influence on the DT_{50} of mesotrione because its **adsorption** is more pronounced in acidic soils (Chaabane et al., 2008; Dyson et al., 2002). Considering the different physicochemical properties of the studied soils, the DT_{50} of mesotrione ranged from 4.5 days (loamy clay, pH 7.1, humus content 3.3%) to 32 days (silty loam, pH 5.0, humus content 2% (Dyson et al., 2002).

Mesotrione is a weak acid (pKa[1] = 3.1), and its adsorption to soil particles is positively correlated with soil organic carbon content: it is more available for transport by leaching and less bioavailable in soil solution for microbiological **degradation** in more acidic soils (Dyson et al., 2002). Barchanska et al. (2015) found no apparent correlation between soil organic carbon content and the stability of mesotrione and its degradation products. However, the half-live of mesotrione was 5–9 days in soils exposed to sunlight and 2–18 days in soils kept in the dark. Soil microflora also affected the degradation of mesotrione by accelerating the process and formation of its degradation product MNBA. Barchanska et al. (2015) studied the degradation and stability of mesotrione and its byproducts in soil. Two mesotrione metabolites, immobile and nonherbicidal, 2-amino-4-methylsulfonylbenzoic acid (AMBA) and 4-methylsulfonyl-2- nitrobenzoic acid (MNBA), were identified in soils (Armel et al., 2005). The mobility of mesotrione and its two metabolites increased with increasing soil pH. It was concluded that the molecular form of mesotrione has the ability to release protons and form negatively charged ions in higher pH environments. At soil pH values between 6.0 and 7.7, the mesotrione molecules were present in a dissociated form and therefore were more available in the soil solution. As a weak acid, mesotrione, as all triketone herbicides, exists in a molecular form at low pH and in an anionic form at neutral or alkaline pH. As pH increases, mesotrione dissociates from the molecular to the anionic form, more resistant to hydrolysis and photolysis (Chaabane et al., 2005; Dyson et al., 2002).

At the outset of the evaluation of the impact of residue on crops in rotation, it was concluded, based on the rapid degradation of mesotrione in the soil, that there was no risk of carryover into crops in rotation even in extremely sensitive crops such as soybean (*Glycine max (L) Merr*), which develop bleaching symptoms when treated with mesotrione at an

[1] The dissociation constant (pKa) represents the pH value of the soil at which half of the compound is in neutral or ionized form (Burnside et al., 1969).

application rate of only 4 g ha^{-1} (Wichert et al., 1999). However, a study by Riddle et al. (2013) suggests that a greater amount of mesotrione remains in the soil one year after application, resulting in greater **phytotoxic injury** to plants. Robinson (2008) studied the effect of mesotrione residues applied pre-em at rates of 140 and 280 g a.i. ha^{-1} on broccoli, carrot, cucumber and onion. Although no rotation restriction data are available for these crops, the above study found that all crops sown on the same field one year after the application of mesotrione showed a significant decrease in yield. Therefore, the author suggests a 24-month period during which these crops should not be sown after the application of mesotrione. A much shorter interval (12 months) for seeding cucumbers is suggested by Felix et al. (2007), who found no phytotoxic injury to cucumber at two (280 g a.i. ha^{-1}) and four (560 g a.i. ha^{-1}) times higher than recommended rate one year after mesotrione application. The soils studied had similar pH values (pH 6.2 and pH 6.7), so differences in organic matter and clay particle content are thought to be the reason for the difference in persistence of mesotrione. The soil in Robinson's (2008) study contained more organic matter (5.2%) and clay particles (21%) compared to the soil (organic matter 3.0%; clay 12%) in the study by Felix et al. (2007). It is therefore hypothesized that due to the higher adsorption potential of the soil in the study by Robinson (2008), the persistence of mesotrione was more pronounced, and consequently, the observed injury was significantly higher.

The official Croatian data on crop rotation restriction after the application of mesotrione states that the following crops should not be sown on the same area 24 months after the application of the mesotrione-based preparation (Callisto 480 SC®): sugar beet (*Beta vulgaris L. var. Saccharifera Alef.*), fodder beet (*Beta vulgaris L. spp. Crassa Alef.*), beetroot (*Beta vulgaris L. subsp. Vulgaris*), lettuce (*Lactuca sativa L.*), spinach (*Spinacia oleracea L.*), peas (*Pisum sativum L.*), beans (*Phaseolus vulgaris L.*) and other species of the genus Phaseolus and Vicia. Studies by various authors suggest that the **degree of susceptibility** of these crops can vary widely under certain conditions (Riddle et al., 2013; Soltani et al., 2007). Soltani et al. (2007) studied the effect of mesotrione applied in the previous year in pre-em (175 - 350 g a.i. ha^{-1}) and post-em (100 - 200 g a.i. ha^{-1}) on cranberry, kidney, black, and white beans. Minimal visual injury (6%) was observed in black and white bean varieties one year after mesotrione application, both pre- and post-em. Visual injury of 23% and 36% was observed on cranberry bean one year after pre-em mesotrione application at rates of 175 and 350 g a.i. ha^{-1}, respectively. The minor visual injury was observed on kidney bean cultivars at both rates during the mentioned period and amounted to 3% (175 g a.i. ha^{-1}) and 15% (350 g a.i. ha^{-1}), respectively. Major phytotoxic injury to cranberry and kidney bean was observed one year after post-em application of mesotrione. At this application, injury to cranberry bean was 40% at a rate of 100 g a.i. ha^{-1} and 42% at a rate of 200 g a.i. ha^{-1}. Injury to kidney bean was less than injury to cranberry bean in the post-em period and was 27% (100 g a.i. ha^{-1}) and 31% (200 g a.i. ha^{-1}), respectively. In view of the observed differences in sensitivity, the authors suggest that crop rotation restrictions should be adapted to the particular cultivar. They, therefore, recommend setting restrictions of 12 months for black and white bean and 24 months for kidney and cranberry bean. Torma et al. (2004) studied the effect of mesotrione at the recommended (168 g a.i. ha^{-1}) and double (336 g a.i. ha^{-1}) rates on wheat, oilseed rape, barley, pea, sugar beet, sunflower and lettuce. According to the research results, none of the applied rates caused phytotoxic damage to the studied crops. In contrast, a study by Riddle et al. (2013) found that mesotrione applied at rates of 140, 280, 420, and 560 g a.i. ha^{-1} in the previous year caused phytotoxic damage to sugar beet, cucumber, bean, pea, and soybean. The phytotoxic injuries varied depending on the crop and application rate. The highest phytotoxic injury was observed on sugar beet and ranged from 8% (140 g a.i. ha^{-1}) to 89% (560 g a.i. ha^{-1}). Injury to cucumber was 10% at a rate of 140 g a.t. ha^{-1} and a maximum of 33% at a rate four times higher (560 g a.i. ha^{-1}). Injury to bean ranged from 11% (140 g a.i. ha^{-1}) to 49% at rates of 280 and 560 g a.i. ha^{-1}. Injury to pea and soybean was 18% and 4%, respectively, one year after application of the recommended mesotrione rate (140 g a.i. ha^{-1}) and reached a maximum of 28% and 26%, respectively, when the highest mesotrione rate (560 g a.i. ha^{-1}) was applied. It is suggested that differences in the chemical properties of the soil used in the study by Riddle et al. (2013) and the soil used in the study by Torma et al. (2004) are the reason for the difference in crop sensitivity. The soil used in the Riddle et al. (2013) study was slightly acidic (pH 6) with an organic matter content of 1.7%, while the soil used in the Torma et al. (2004) study was neutral (pH 7.1) with a higher organic matter content (3.1%).

Riddle et al. (2013) found that sugar beet and green **bean** are very sensitive to mesotrione and that they can be used to evaluate potential residues after mesotrione application in soil. Allemann and Molomo (2016) tested six dry bean cultivars for mesotrione application at eight rates ranging from 0.0 to 51.2 µg kg^{-1}. Authors calculated mesotrione values based on the half-life of mesotrione in the soil and used these values to calculate mesotrione that would be available in the top 15 cm of a sandy loam soil with a bulk density of 1 625 kg m^{-3} every 45 days after an application of 124.8 g a.i ha^{-1} up to 270 days. The results showed highly significant effects on plant height as well as a highly significant interaction effect on dry biomass due to both cultivar and mesotrione rates. The authors concluded that a period of 270 days before sowing dry beans seems to be sufficient to avoid injuries to the tested cultivars on the soil type used, which is different from restrictions prescribed in Croatia.

Pintar et al. (2020) studied the effective mesotrione rate (ED_{50}) for a 50% reduction in fresh weight and carotenoid content of sugar beet on two different soil types. The soils were treated with mesotrione at seven rates that simulated mesotrione concentrations in the soil, considering its half-life in the field. The ED_{50} for 50% reduction in fresh weight was 5.9 and 4.4 g a.i. ha^{-1} while for total carotenoid content ED_{50} was 4.7 and 2.1 g a i ha^{-1} in hipogley and humofluvisol, respectively. The authors concluded that crop rotation must be performed based on soil properties because it affects amount of the bioavailable mesotrione residues in the soil, resulting in different levels of degradation in two soils with the same rate. A more recent study by Pintar et al. (2021) examined how neutral (pH 7) and acidic (pH 5) soils affected pea treated with simulated mesotrione residues at the same rate. Crop visual injuries, 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. The reductions in dry biomass were smaller, ranging from 49.2% to 96.8% at pH 7.0 and from 32.0% to 82.6% at pH 5.0 for mesotrione residues from 1.1 to 72 g a.i. ha^{-1}. Therefore, soil pH had an important effect on pea sensitivity to simulated mesotrione residues.

To summarize literature review, crop susceptibility to mesotrione residues suggests that restrictions on crop rotation following herbicide application should be based on weather conditions, soil type, and applied rates, rather than applying equally to all conditions as stated in herbicide directions for use.

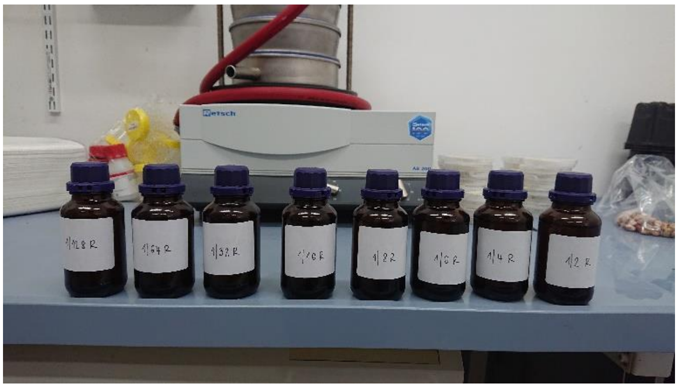
4. Materials and methods

4.1. Sampling and preparation of soil samples

Hipogley soil[2] samples were collected in September 2020 from the surface layer (0 – 20 cm) of the untreated fields located in Šašinovec using a probe (Split Tube Sampler, Ø 53 mm, Eijkelkamp, Giesbeek, The Netherlands). The soil was dried at room temperature for three days and then ground to the appropriate particle size. Plastic containers with a volume of 0.5 L (10 cm in diameter, 4-5 cm deep) were filled with 200 g of the sampled soil.

4.2. Simulated residue carryover study

Reduced application rates of mesotrione, referred as simulated mesotrione rates, were performed using a TLC sprayer (CAMAG®, Switzerland). Mesotrione was diluted in distilled water (Figure 4.1a) and applied at the following rates: 1.1 (1/128 R), 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) g ha^{-1} (Figure 4.1b), where R is the recommended (144 g a.i. ha^{-1}) rate of mesotrione.



b

a

Figure 4.1. Preparation of reduced rates (a) of mesotrione by dilution (b) (Riddle et al., 2013.)

All treatments studied (including control) were laid out in four replicates, and the design of the experiments was a randomized complete block design.

[2] Texture class: silty clay loam, sand=1.1 %, silt=59.6 %, clay=39.3 %, humus=4.2 %, organic matter content=2.5 %, cation exchange capacity= 33.8 cmol kg^{-1}, pH (H_2O) =7.7.

The bean seeds were soaked a day before sowing to promote faster germination once they were in planting containers. The containers, each planted with six bean seeds (Figure 4.2) and treated with mesotrione, were placed in a climatic chamber where the plants developed for four weeks under controlled conditions of temperature (25 °C day/15 °C night), lighting duration (12 h day/12 h night) and relative humidity (70%). During the growth period, the plants were regularly irrigated to maintain moisture at the field water capacity level.

Figure 4.2. Planting of bean seeds

Irrigation was done with water solutions of three different pH values (4.5, 5.5, 6.5) to simulate soil pH for three separate blocks of the experiment. The fourth block was irrigated with distilled water to obtain the original soil pH value (7.5). The planting containers were irrigated with 60 mL of solutions every third day.

4.3. Parameters for determining the sensitivity of beans to simulated mesotrione residues

The sensitivity of the bean to simulated mesotrione residues was determined using the following parameters: visual toxicity assessment of aboveground bean mass, reduction of fresh aboveground weight of the bean, reduction of chlorophyll and carotenoid content.

Visual phytotoxicity assessments of aboveground bean mass were performed 7, 14, 21 and 28 days after treatment (DAT) by using a scale of 0% (no injury) to 100% (plant death) [EPPO standard PP 1/135 (4)]), to detect a progressive or degressive effect trend of mesotrione.

The fresh aboveground weight of the bean was determined at 28 DAT. Each bean plant was removed at soil level with scissors, and then the weight of all plants per container (treatment) was determined using a digital scale. The plants from each treatment were then packed separately in plastic bags and stored at -80 °C until the total carotenoid analysis.

Direct measurement of chlorophyll was performed (Opti - sciences CCM - 200plus) (Figure 4.3) 21 DAT. The measurement provided preliminary data on the chlorophyll content in the plants and consequently information on the variation between treatments and between different soil pH values.

Figure 4.3. Direct measurement of chlorophyll

Determination of the concentration of total chlorophylls and carotenoids was carried 28 DAT at the Department of Animal Nutrition, University of Zagreb, Faculty of Agriculture. The total concentration of photosynthetic pigments was determined for all plants per treatment. Each sample was analysed in triplicate. Plant leaves were cut into small pieces with scissors, homogenised in a blender, and weighed to a total mass of 0.1 g. The plant tissue (0.1 g) was mixed with 2 mL of acetone (99%) in a test tube. The tissue was then further homogenised using a laboratory homogenizer (Ultra Turax T-10, IKA, Germany) and centrifuged at 4000 rpm for 5 minutes (Figure 4.4).

The supernatant was pipetted into a 25-mL flask, and the solid residue of green colour was repeatedly extracted with acetone until the colourless extract (Figure 4.5). The samples were analysed using a UV/VIS spectrophotometer (Helios Gamma, Thermo Electron

Corporation, United Kingdom). The absorbance of the samples was measured at wavelengths of 662, 644 and 440 nm using acetone as a blank probe.

a 

b 

Figure 4.4. Laboratory homogenizer (a) and centrifuge (b).

The formulas of Holm (1954) and Wettstein (1957) were used to calculate the concentration of photosynthetic pigments in mg g^{-1} in each sample:

- chlorophyll a = 9.784 A662 - 0.990 A644
- chlorophyll b = 21.426 A644 - 4.65 A662
- chlorophyll a + b = 5.134 A662 + 20.436 A644
- carotenoids = 4.695 A440 - 0.268 (chlorophyll a + b).

The values obtained with fresh aboveground weight, chlorophyll and carotenoid content measurement to determine the sensitivity of bean to simulated mesotrione residues were expressed by Abbot reduction coefficient (Puntener, 1981):

$$\% \text{ reduction} = 100 - \left(\frac{\text{control plants} - \text{treated plants}}{\text{control plants}}\right) \times 100$$

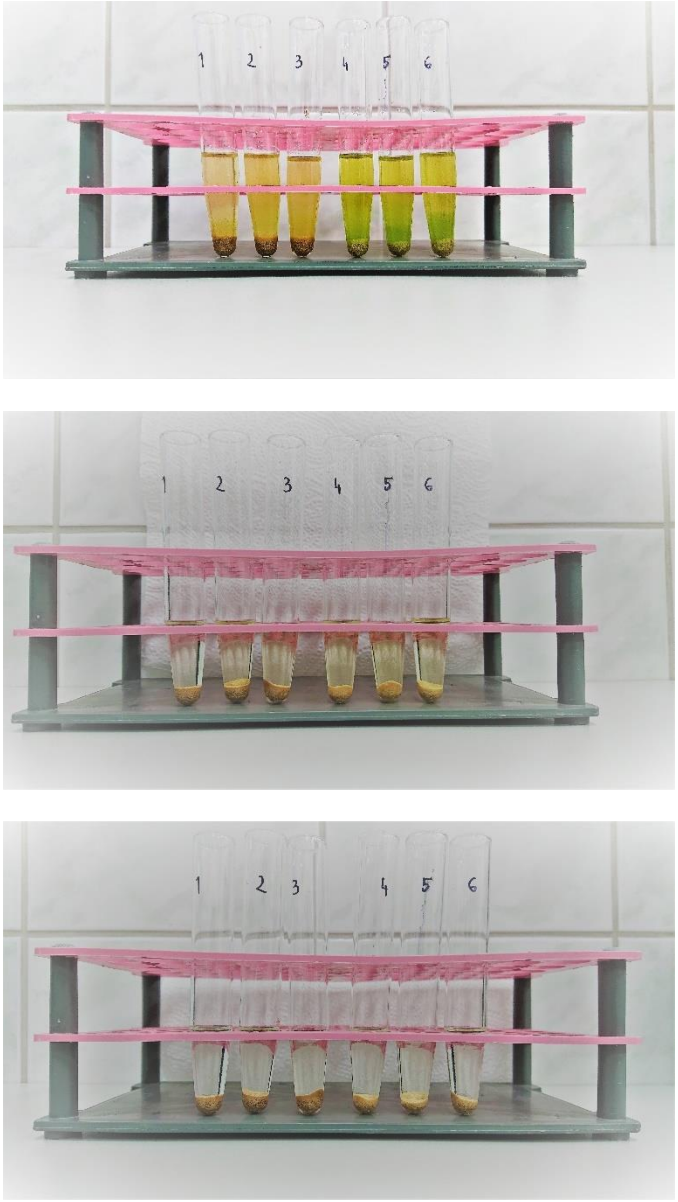


Figure 4.5. Photosintethic pigments extraction with acetone

4.4. Statistical analysis

The obtained data were processed and visualized in R software (R version 4.0.0 (2020-04-24)). Two-way ANOVA was used to analyse the obtained data, followed by Fisher's LSD test for P=0.05 to compare the means.

5. Results

5.1. Visual injury assessment 7 days after treatment

The results of statistical analysis (ANOVA) of visual injury assessment 7 DAT on bean are shown in Table 5.1. A highly significant difference (p=0.001) in the visual injury of bean treated with different simulated mesotrione residues and grown on different soil pH evaluated 7 DAT was observed. Also, a significant interaction (p=0.001) of simulated mesotrione residues x pH was observed.

Table 5.1. Two-way analysis of variance for visual injury evaluation of bean at 7 days after treatment with simulated mesotrione residues applied to the soil at pH 4.5, 5.5, 6.5 and 7.5

Source of variability	n-1	SS	s^2	F value	F test
Simulated mesotrione residues (SMR)	7	35165	5024	8573.45	***
pH	3	2156	719	1226.61	***
SMR x pH	21	1207	57	98.08	***
Error	96	56	1		

***Significant F-test for P=0.001

The average mean values of visual injuries gradually increased from the lowest to the highest simulated mesotrione residues, i.e., bean injuries were exponentially related to simulated mesotrione residues (Figure 5.6).

The highest bean sensitivity was observed in plants treated with the highest (72 g a.i.h^{-1}) simulated mesotrione residues at soil pH7.5 (pH_1) with 61.25% injury. Same simulated mesotrione residues caused 60% injury on bean grown at a soil pH6.5 (pH_2) and 55% injury at a soil pH5.5 (pH_3). Bean grown on soil pH4.5 (pH_4) treated with the highest simulated mesotrione residues was injured by 45%. Bean treated with 9 g a.i. h^{-1} and 24 g a.i. h^{-1} has shown the highest sensitivity at soil pH_2, causing 30 and 45% injury, respectively.

There were no significant differences in injuries related to soil pH when the lowest simulated mesotrione residues were applied, except for the weaker effect of 4.5 g a.i. h^{-1} at soil pH_4 with average injury of 15 and 12%, respectively.

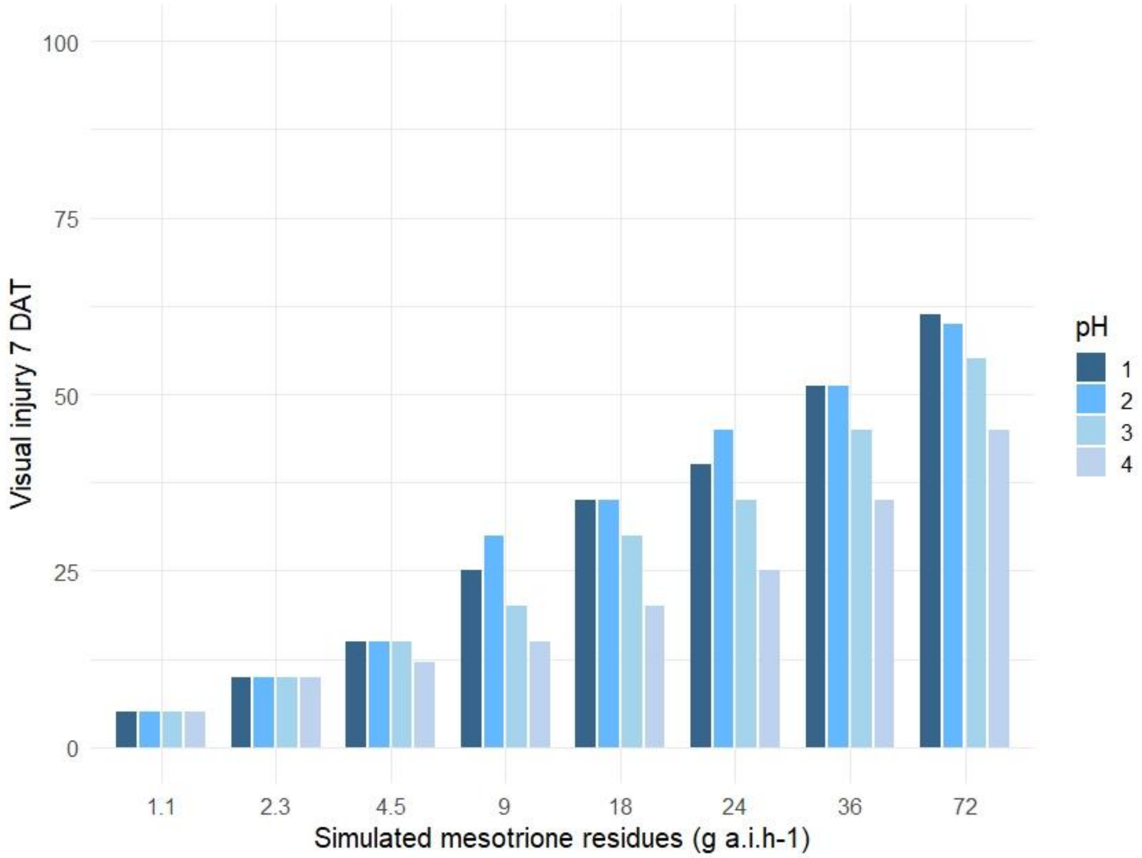


Figure 5.6. Visual injury evaluation of bean at 7 days after treatment with simulated mesotrione residues applied to the soil at pH 4.5 (pH_4), 5.5 (pH_3), 6.5 (pH_2) and 7.5. (pH_1), LSD = 1.07%

5. 2. Visual injury assessment 14 days after treatment

The results of statistical analysis of visual injury assessment 14 DAT on bean are shown in Table 5.2. A highly significant difference (p=0.001) in visual injury of bean treated with different simulated mesotrione residues and grown on different soil pH evaluated 14 DAT was observed and significant interaction (p=0.001) of simulated mesotrione residues x pH was also observed.

Table 5.2. Two-way analysis of variance for visual injury evaluation of bean at 14 days after treatment with simulated mesotrione residues applied to soil at pH 4.5, 5.5, 6.5 and 7.5

Source of variability	n-1	SS	s^2	F value	F test
Simulated mesotrione residues (SMR)	7	48226	6889	1181.578	***
pH	3	1854	618	105.999	***
SMR x pH	21	827	39	6.753	***
Error	96	560	6		

***Significant F-test for P=0.001

Bean injury was exponentially related to the simulated mesotrione residues (Figure 5.7), with plants grown in soil pH_1 showing the highest injuries at all applied simulated mesotrione residues. The difference in visual injury of bean grown in different soil pH can be seen at the lowest simulated mesotrione residues (1.1, 2.3 g a.i. ha^{-1}) in contrast to the results 7 DAT where the first difference is assessed at 4.5 g a.i. ha^{-1}. Specifically, bean treated with 1/6 (24 g a.i. ha^{-1}) of the recommended mesotrione rate caused 56.25% injury while the highest (72 g a.i. ha^{-1}) simulated mesotrione residues injury was 70%. Plants grown at a soil pH_2 and treated with all simulated mesotrione residues (except 2.3 and 36 g a.i. ha^{-1}) showed the second highest sensitivity with 65% of injuries following simulated mesotrione residues of 72 g a.i. ha^{-1}. Furthermore, this result was not significantly different from 63.75% injury in plants grown in soil pH_1 and treated with 36 g a.i. ha^{-1} of mesotrione (Figure 5.7).

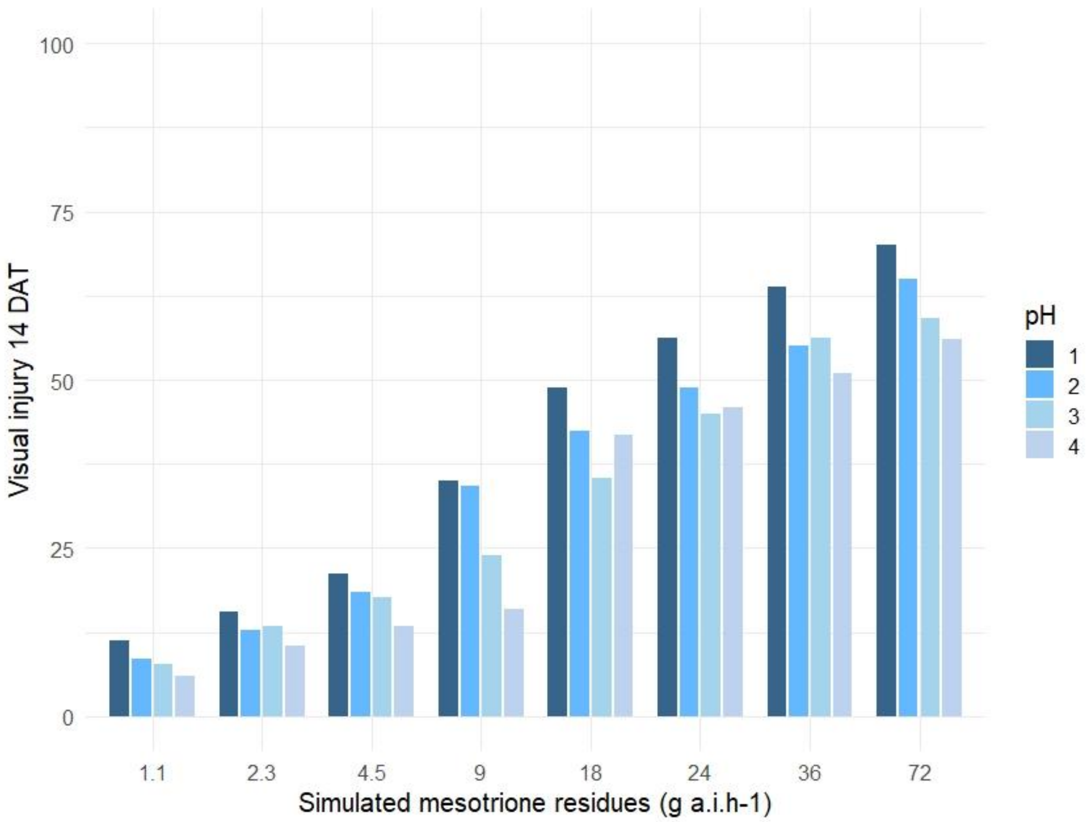


Figure 5.7. Visual injury evaluation of bean at 14 days after treatment with simulated mesotrione residues applied to the soil at pH 4.5 (pH_4), 5.5 (pH_3), 6.5 (pH_2) and 7.5. (pH_1), LSD = 3.39%

5.1.1. Visual injury assessment 21 days after treatment

The results of statistical analysis of visual injury assessment 21 DAT on bean are shown in Table 5.3. A highly significant difference (p=0.001) in the visual injury of bean treated with different simulated mesotrione residues and grown on different soil pH evaluated 21 DAT was observed and significant interaction (p=0.001) of simulated mesotrione residues x pH was also observed.

Table 5.3. Two-way analysis of variance for visual injury evaluation of bean at 21 days after treatment with simulated mesotrione residues applied to soil at pH 4.5, 5.5, 6.5 and 7.5

Source of variability	n-1	SS	s^2	F value	F test
Simulated mesotrione residues (SMR)	7	62753	8965	911.42	***
pH	3	2355	785	79.80	***
SMR x pH	21	2606	124	12.62	***
Error	96	944	10		

***Significant F-test for P=0.001.

Bean injury continued to be exponentially related to mesotrione rate (Figure 5.8) 21 DAT. In contrast to the previous two measurements (7 and 14 DAT), the highest injuries 21 DAT were observed on plants grown at soil pH_2 and pH_3 at simulated mesotrione residues of 24 (83.75%, 85%), 36 (92.75%, 88.75%) and 72 g a.i. ha^{-1} (95%). However, bean sensitivity was highest at lower simulated mesotrione residues (2.3. – 9 g a.i. ha^{-1}) and at soil pH_1, while bean grown in soil pH_3 generally exhibited the least visual injuries at simulated mesotrione residues ranging from 1.1. to 18 g a.i. ha^{-1}. The effect of soil pH_4 varied from least effective at higher simulated mesotrione residues to second least effective at lower simulated mesotrione residues.

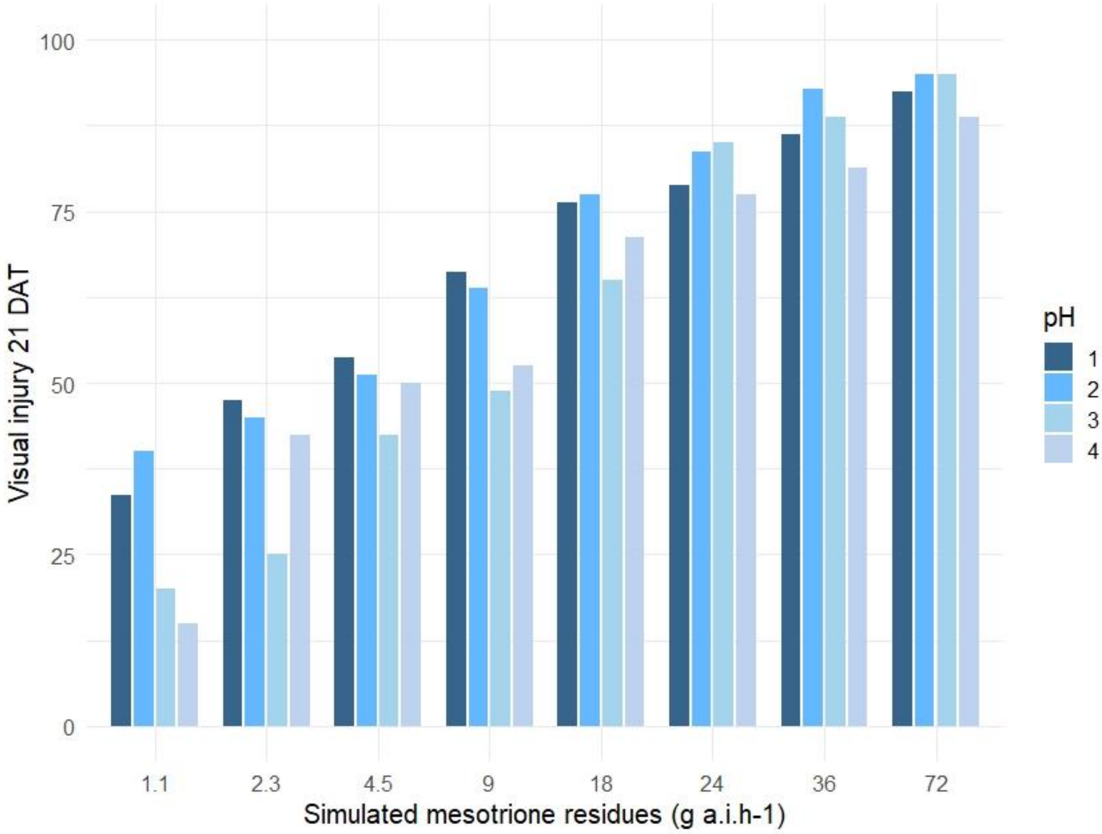


Figure 5.8. Visual injury evaluation of bean at 21 days after treatment with simulated mesotrione residues applied to the soil at pH 4.5 (pH_4), 5.5 (pH_3), 6.5 (pH_2) and 7.5. (pH_1), LSD = 4.4%

5.1.2. Visual injury assessment 28 days after treatment

The results of statistical analysis of visual injury assessment 28 DAT on bean are shown in Table 5.4. A highly significant difference (p=0.001) in the visual injury of bean treated with different simulated mesotrione residues and grown on different soil pH evaluated 28 DAT was observed and significant interaction (p=0.001) of simulated mesotrione residues x pH was also observed.

Table 5.4. Two-way analysis of variance for visual injury evaluation of bean at 28 days after treatment with simulated mesotrione residues applied to soil at pH 4.5, 5.5, 6.5 and 7.5

Source of variability	n-1	SS	s^2	F value	F test
Simulated mesotrione residues (SMR)	7	64594	9228	925.91	***
pH	3	4176	1392	139.66	***
SMR x pH	21	3632	173	17.35	***
Error	96	957	10		

***Significant F-test for P=0.001.

At 28 DAT, the lower sensitivity of bean to simulated mesotrione residues ranging from 2.3. to 18 g a.i. ha^{-1} was observed on soil pH_3 (Figure 5.9). Simulated mesotrione residues from 1.1. to 18 g a.i. ha^{-1} caused the highest injury on soil pH_1 and soil pH_2, which is from around 10 – 25% higher than injuries on soil pH_3 and soil pH_4. There were no determined statistical differences between group means on soil pH_{1-3} at simulated mesotrione residues of 36 and 72 g a.i. ha^{-1}, where the highest phytotoxic injuries were observed (96.5 – 100%) with typical symptoms of mesotrione herbicidal action, bleaching and necrosis (Figure 5.10). The least sensitive was bean grown in soil pH_4 with the lowest injury 28 DAT, 20%, on 1.1 g a.i. ha^{-1} of simulated mesotrione residues. In contrast to lower simulated mesotrione residues where the differences in the visual injury of bean grown in different soil pH intensify and differentiate with time, the differences in visual injury at higher mesotrione residues were observed at first assessment (7 DAT) and were equalized with time.

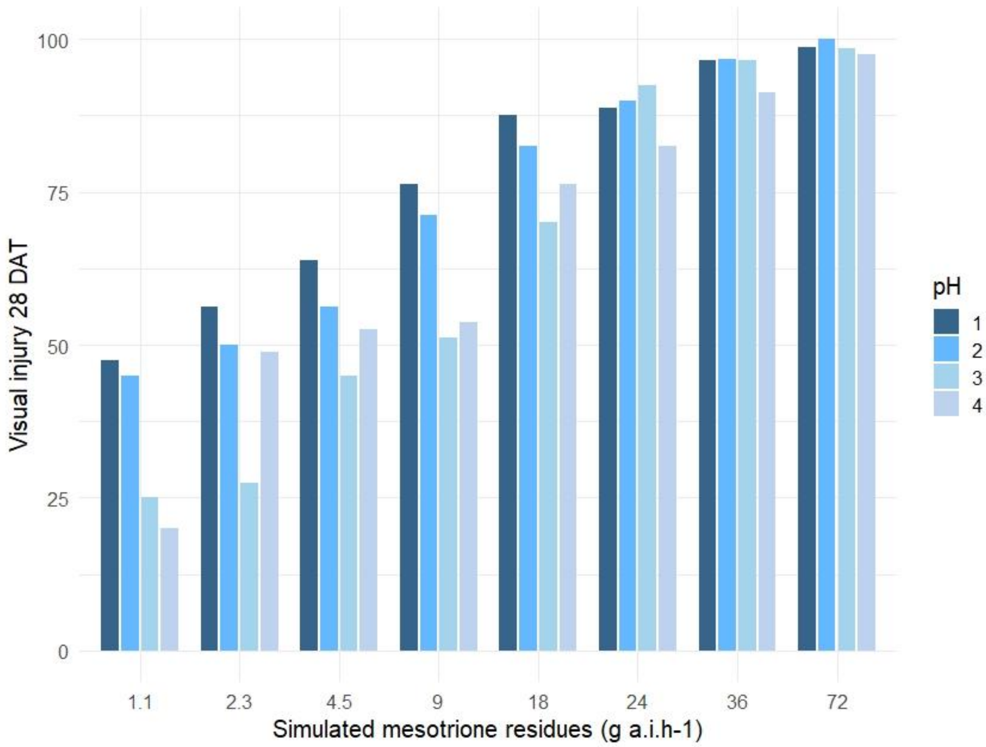


Figure 5.9. Visual injury evaluation of bean at 28 days after treatment with simulated mesotrione residues applied to the soil at pH 4.5 (pH_4), 5.5 (pH_3), 6.5 (pH_2) and 7.5. (pH_1), LSD = 4.43%

pH_1

pH_2

pH_3

pH_4

Figure 5.10. Phytotoxic damage by simulated mesotrione residues on the soil pH 7.5. (pH_1), 6.5 (pH_2), 5.5 (pH_3), and 4.5 (pH_4) on bean plants 28 DAT.

5.2. Reduction of fresh weight, chlorophyll, and carotenoid content

5.2.1. Aboveground fresh weight reduction at 28 DAT

The ANOVA table shows the significant effect of simulated mesotrione residues (SMR) and the significant effect of pH on fresh weight reduction (Table 5.5). The interaction between SMR and pH has not been determined, and therefore, the results of fresh aboveground weight reduction were averaged over the investigated simulated residues (1.1. – 72 g a.i. h^{-1}) and pH values (4.5, 5.5, 6.5, 7.5).

Table 5.5. Two-way analysis of variance for the reduction in aboveground fresh weight of bean treated with simulated mesotrione residues applied to soil at pH 4.5, 5.5, 6.5 and 7.5.

Source of variability	**n-1**	**SS**	**s^2**	**F value**	**F test**
Simulated mesotrione residues (SMR)	7	52864	7552	50.938	***
pH	3	4333	1444	9.741	***
SMR x pH	21	2455	117	0.789	ns
Error	96	14233	148		

***Significant F-test for P=0.001 ns - not significant.

The reduction in fresh weight increased exponentially with simulated mesotrione residues (Figure 5.11), which was already assessed in subchapter 5.1. The highest reduction in bean fresh weight was 86.47% at 72 g a.i. ha^{-1} of simulated mesotrione residue. The reduction of fresh weight at simulated mesotrione residues of 36 and 24 g a.i. ha^{-1} were 71.93 and 64.62%, respectively, and were not statistically different. The minimal reduction of fresh weight, 19.82%, was determined at the lowest simulated mesotrione residues (1.1 g a.i. ha^{-1}).

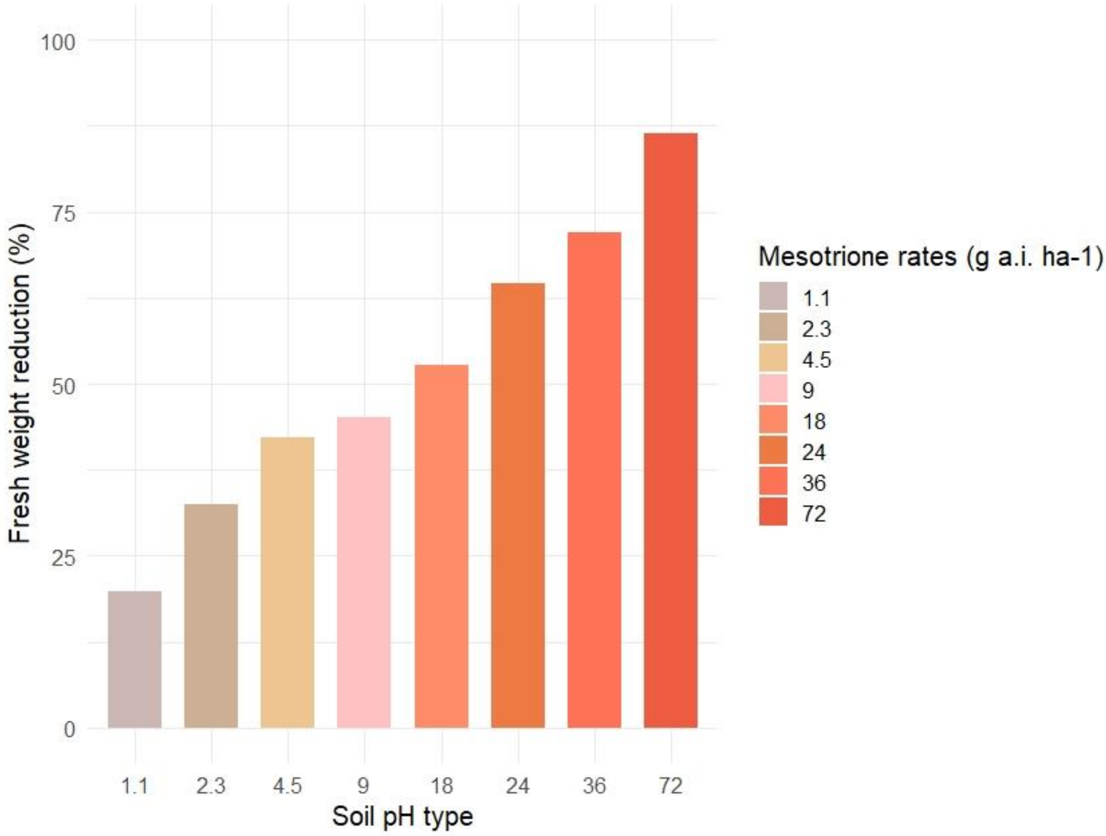


Figure 5.11. Effect of simulated mesotrione residues on reduction in fresh aboveground weight of bean plants, LSD=9.26%

The highest reduction in fresh weight of 56.34% and 56.64% was determined on soil pH_1 and pH_2, respectively (Figure 5.12) and was significantly higher compared to bean fresh weight grown in soil pH_4 where 42.27% reduction was observed.

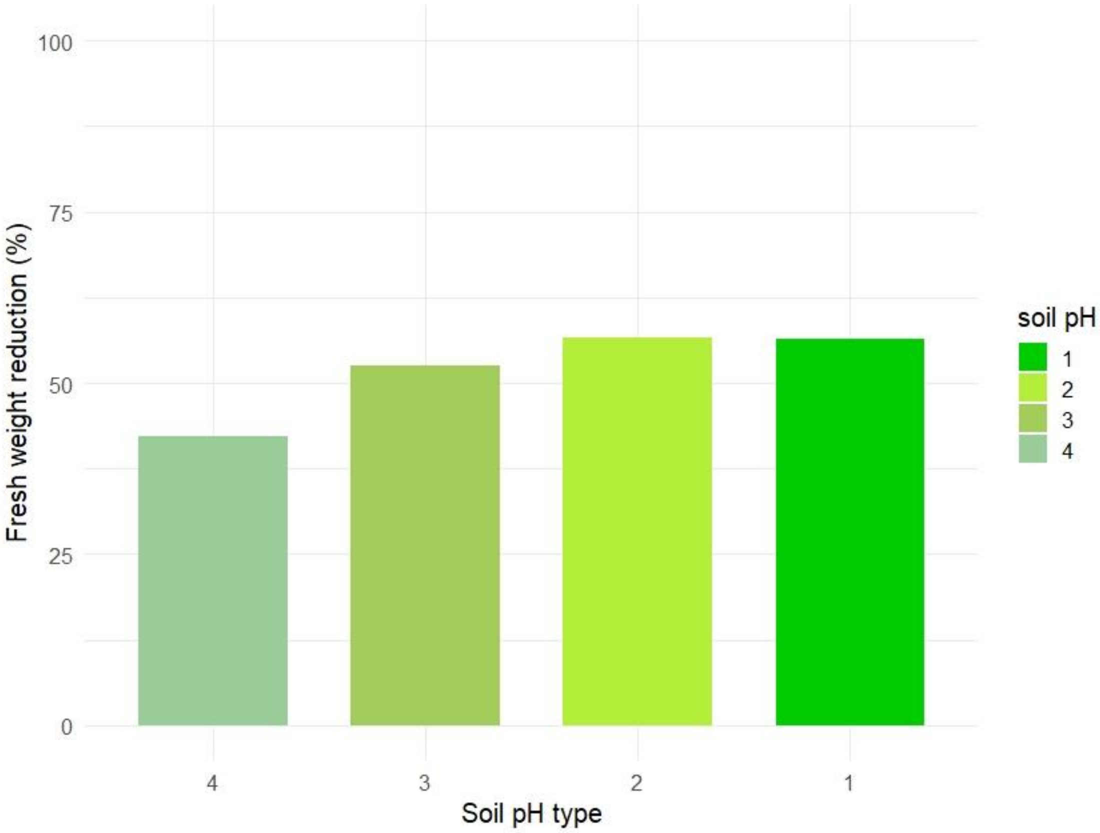


Figure 5.12. Effect of soil pH (7.5=pH_1, 6.5=pH_2, 5.5=pH_3, 4.5=pH_4) on reduction in fresh aboveground weight of bean plants, LSD=11.72%

5.2.2. Reduction of chlorophyll content, 21 DAT

The ANOVA table shows the significant effect of simulated mesotrione residues and the significant effect of pH on reduction of chlorophyll content, and the interaction between treatment and pH has not been d (Table 5.6).

Table 5.6. Two-way analysis of variance for the reduction of chlorophyll content of bean treated with simulated mesotrione residues applied to the soil at pH 4.5, 5.5, 6.5 and 7.5.

Source of variability	n-1	SS	s^2	F value	F test
Simulated mesotrione residues (SMR)	7	76898	10985	596.687	***
pH	3	3065	1022	55.487	***
SMR x pH	21	485	23	1.253	ns
Error	96	1767	18		

Significance code for P=0.001 - ***, ns - not significant.

Chlorophyll reduction values increased exponentially with simulated mesotrione residues (Figure 5.13) but there is a clear difference between lower and higher rates. For example, at 9 g a.i. h^{-1} chlorophyll was reduced by 46.12 to 59% while at 18 g a.i. h^{-1}, reduction was by 66.77 to 81.78%. The highest reduction of chlorophyll was 93.4% at simulated mesotrione residues of 72 g a.i. h^{-1} while the lowest reduction of 23.67% was at 1.1 g a.i. ha^{-1} simulated mesotrione residues.The reduction values at simulated mesotrione residues of 36 and 72 g a.i. ha^{-1}, and 18 and 24 g a.i. h^{-1} were not statistically different.

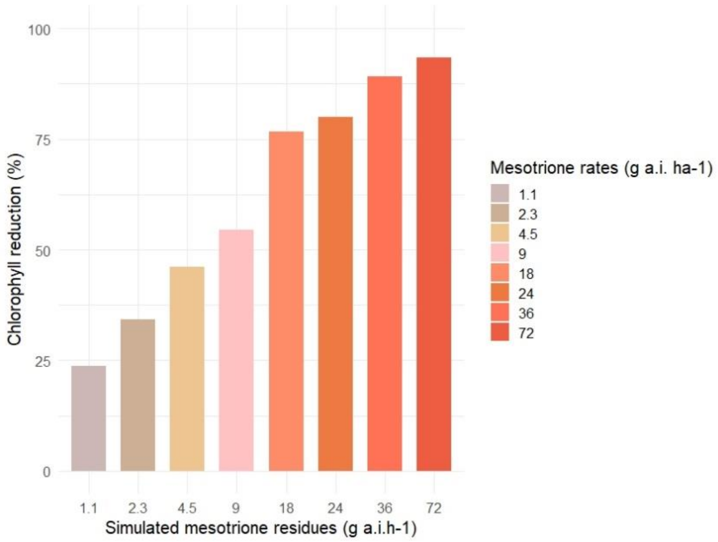


Figure 5.13. Effect of simulated mesotrione residues on chlorophyll reduction of bean plants, LSD=4.66%

In the Figure 5.14, chlorophyll reduction of bean plants depending on soil pH is shown. Bean grown in soil pH_4 was least sensitive to simulated mesotrione residues with lowest chlorophyll reduction (53.98%) compared to chlorophyl reduction in bean grown in soil pH_1 and pH_2 were 66.73% and 64.81% reduction was observed, respectively.

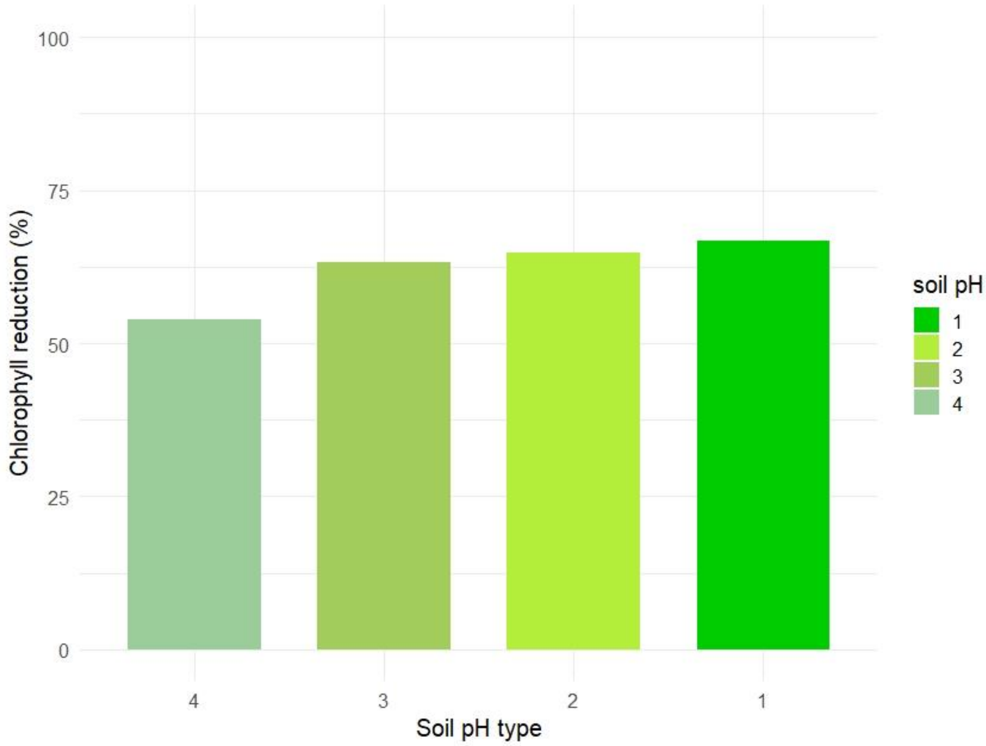


Figure 5.14. Effect of soil pH (7.5=pH_1, 6.5=pH_2, 5.5=pH_3, 4.5=pH_4) on chlorophyll reduction of bean plants, LSD=12.5%

5.2.3. Reduction of carotenoid content

The ANOVA table shows the significant effect of simulated mesotrione residues and the significant effect of pH on reduction of carotenoid content, and the interaction between treatment and pH has not been detected (Table 5.7).

Table 5.7. Two-way analysis of variance for the reduction of carotenoid content of bean treated with simulated mesotrione residues applied to soil at pH 4.5, 5.5, 6.5 and 7.5.

Source of variability	n-1	SS	s^2	F value	F test
Simulated mesotrione residues (SMR)	5	28896	5779	13.175	***
pH	3	18371	6124	13.960	***
SMR x pH	15	3431	229	0.521	ns
Error	72	31584	439		

Significance code for P=0.001 - ***, ns - not significant.

The reduction of carotenoid content increased exponentially with simulated mesotrione residues (Figure 5.15) and there is noticable difference between lower and higher rates. At 9 g a.i. h^{-1} carotenoid content reduction average was 34.48% while at 18 g a.i. h^{-1}, reduction was by 56.93% and 59.90%, at 24 g a.i. ha^{-1}. The lowest carotenoid reduction was at the lowest simulated mesotrione residue of 1.1 g a.i. ha^{-1}, and it was 16.63%.

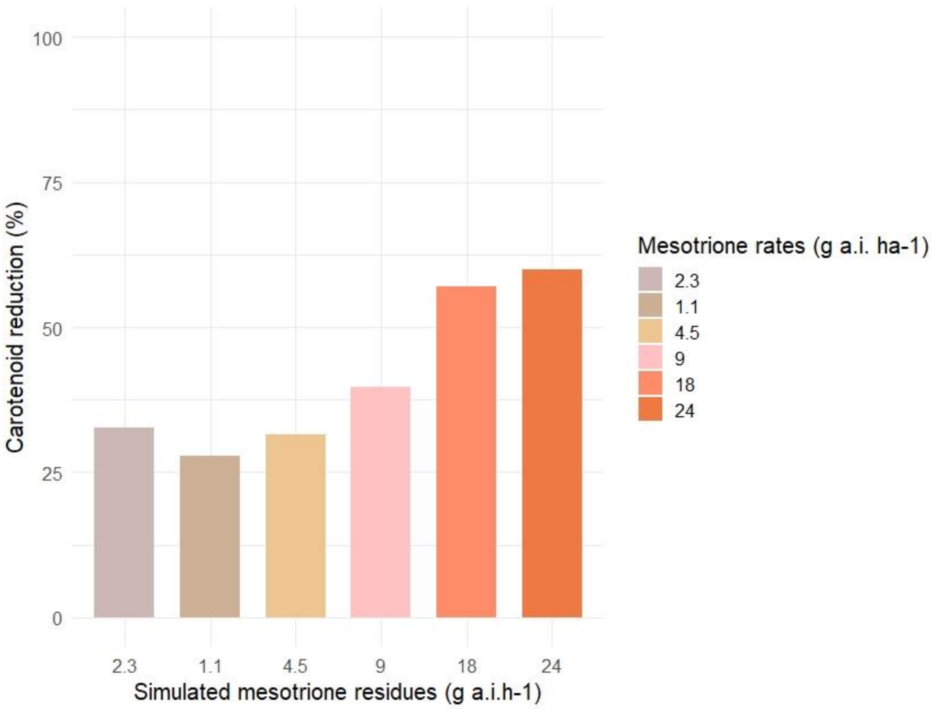


Figure 5.15. Effect of simulated mesotrione residues on reduction of carotenoid content of bean plants, LSD=17.11%

Figure 5.16 shows averaged reduction values of carotenoid content of bean depending on soil pH. Bean grown in soil pH_4 and soil pH_3 was less sensitive to simulated mesotrione residues with not statistically different carotenoid reduction of 21.77 and 21.6%, respectively. The significantly higher averaged reduction of carotenoid content was observed on bean grown in the soil pH_1 and pH_2, where 52.13% and 45.84% carotenoid reduction was detected, respectively.

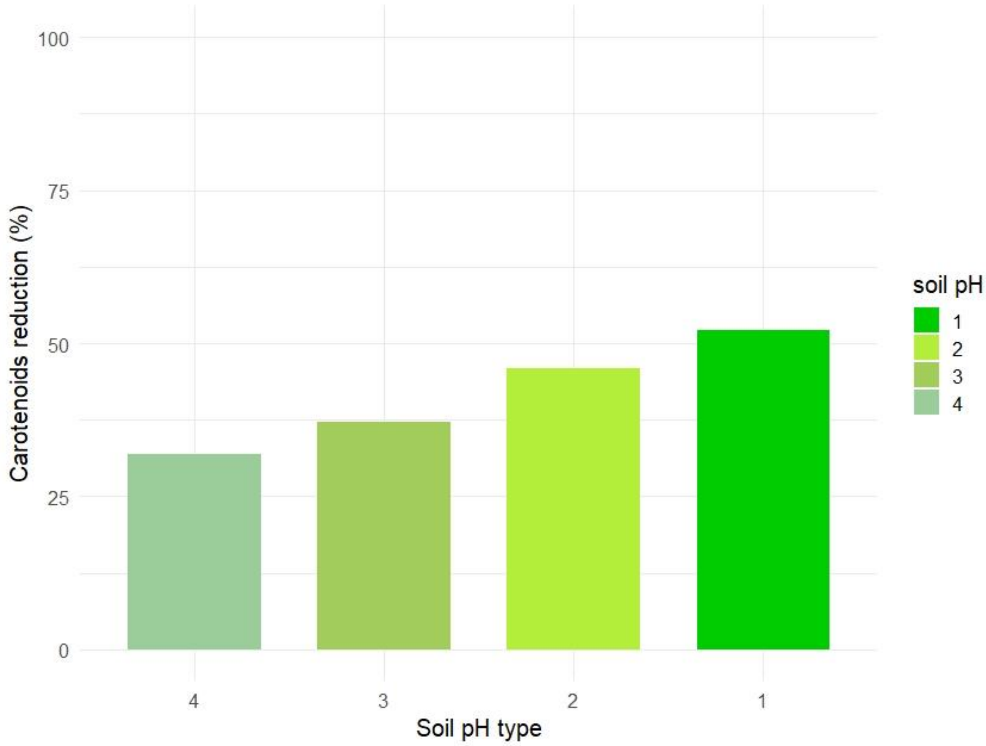


Figure 5.16. Effect of soil pH (7.5=pH_1, 6.5=pH_2, 5.5=pH_3, 4.5=pH_4) on reduction of carotenoid content of bean plants, LSD=15.11%

6. Discussion

The aim of this research was to determine the effect of mesotrione on common bean at rates lower than recommended by the producer, to simulate potential mesotrione residues in the soil after application. This is important because the Croatian Ministry of Agriculture imposes restrictions on pesticide applications that agricultural producers adhere to. The restrictions are based on the persistence of herbicides in the soil, which can cause injury to crops in the rotation due to potential residues and result in considerable economic losses to agricultural producers. Some authors suggest different restriction periods (Allemann and Molomo, 2016; Robinson, 2008; Felix et al., 2007) between the application of herbicides and the planting of certain crops, since the sensitivity of crops to herbicides depends on species affiliation (Riddle et al., 2013), soil properties (Pintar et al., 2020; Dyson et al., 2002; Rouchaud et al., 2001) and rate of decomposition (Barchanska et al., 2015).

The results confirm the **first hypothesis** of this thesis, i.e., the effect of mesotrione residues varies with mesotrione rates. As it was expected based on the literature review, common bean injuries (all measured parameters) increased with increasing simulated mesotrione residues (Figures 6, 7, 8, 10). The same result was reported by many authors (Pintar et al., 2020, 2021; Riddle et al., 2013; Felix et al., 2007; Soltani et al., 2007; Abendroth et al., 2006). Although damage was linearly dependent on the increase in simulated mesotrione residues, the sensitivity of beans grown on different soil pH levels was not linearly dependent, i.e. differences in bean sensitivity to simulated mesotrione rates were generally only found between the most acidic (pH_1) and most alkaline soils (pH_4). However, the extent of bean injuries was also dependent on the parameter measured.

Visual injury on the bean linearly decreased with the decrease in simulated mesotrione residues and was observed in all measurements (7, 14, 21 and 28 DAT). Furthermore, visual injury decreased with the decrease in pH of the soil. For example, visual injury 7 DAT was assessed highest in soil pH_1 at 72 g a.i. ha^{-1} (61.25%), followed by a decrease in injury in soil pH_3, pH_2, and pH_1 being 60, 55, and 45%, respectively. Even though there was no difference between visual injury at 28 DAT within soil pH at the highest simulated mesotrione residues (72 g a.i. ha^{-1}) because of strong herbicidal effect, at lower simulated mesotrione residues, the difference was obvious. At more alkaline soils, pH_1 and pH_2, visual injury at 1.1 g a.i. ha^{-1} was assessed at 47.5 and 45%, while at more acidic soils (pH_3 and pH_4), the injuries were half less than that, 25 and 20%, respectively.

Felix et al. (2007) tested mesotrione residues 12 months after applying 210, 420 and 840 g a.i. ha^{-1} mesotrione, which is 1, 2 and 4 times the recommended rate, on bean, cabbage, bell pepper, processing tomato, pickling cucumber, and red clover on two different soil types. Bean grown on more acidic soil, pH=5.5, treated with mesotrione one year before the experiment with 420 and 840 g a.i. ha^{-1} mesotrione rates, had a higher percentage of injury (10, 15%) than bean grown on less acidic soil (treated with mesotrione one year before) of pH=6.2 (5, 11%) 7 DAT. In the assessment of 28 DAT in the same research, bean injury on more acidic soil went up to 64 and 88% for 420 and 840 g a.i. ha^{-1} while on the less acidic soil, it was assessed as minor (0 and 11 % injury for 420 and 840 g a.i. ha^{-1} respectively). This is in contrast to results obtained in research by Pintar et al. (2021; 2020). The same can be noticed in study by Riddle et al. (2013), where the effect of mesotrione soil residues on injury to greenhouse-grown bean plants in 2006 (pH=6) and 2007 (pH=6.7), one year after mesotrione application in rates 70, 140, 280 g a.i. ha^{-1}, were 0, 8, 18 and 0, 0, 4% respectively.

In the present experiment, on 28[th] DAT, higher sensitivity of bean grown in soil $pH_{1,}$ pH_2 and pH_4 than on soil pH_3 (Figure 5.9) at lower to middle simulated mesotrione residues (1.1. – 18 g a.i. ha^{-1}) was observed. There were no statistical differences between group means per treatment at simulated mesotrione residues of 36 and 72 g a.i. ha^{-1} for soil pH_{1-3}. The highest injuries were caused by all four-soil pH at 72 g a.i. ha^{-1} (97.5 – 100%) while the lowest injuries were observed on bean grown in soil pH_4 (20%).

No matter the variabilities of the effect of different soil pH, it can be observed that there was a general tendency for greater injury to be on plants grown in more alkaline (pH=7.5) than acidic (pH=4.5) soils. The variabilities of bean plants injuries treated with the same amount of mesotrione but grown in different soil pH (pH=7.5, pH=6.5, pH=5.5, pH=4.5) confirms **second hypothesis** of this thesis.

The second part of this research focused on a laboratory analytical method, classical extraction and quantification of photosynthetic pigments (Žlabur et al., 2016), which is more objective than the visual assessment of phytotoxicity, but also more sensitive. Indeed, our results suggest that bean growth reduction at different soil pH levels is better predicted by classical chlorophyll and carotenoid analyses than by subjective (visual injury) or objective (weight reduction or direct chlorophyll measurement). Carotenoid content was significantly higher at pH 1 and 2 than at pH 3 and 4 (Figure). This was not the case for other parameters, as it was not possible to detect a reduction in bean fresh weight or chlorophyll content between pH 2 and 3. For example, carotenoids were inhibited by 21.6% in beans grown at pH3 and treated with simulated mesotrione rates and by 45% at soil pH. In comparison, the reduction in fresh weight was similar (52.5 and 56.6%, respectively). Therefore, visual assessment of phytotoxicity should not be the only parameter for determining herbicidal effect on crops in rotation, as also suggested by Pintar et al. (2020). In particular, the authors concluded that the reduction of carotenoid content in sugar beet is a better indicator of the effect of mesotrione residues on crops than the reduction of fresh weight, although this is not common in bioassay trials (Sekutowski, 2011).

Our results show high reduction in been fresh weight especially when higher simulated mesotrione residues were applied. Furthermore, bean fresh weight reduction was more pronounced on been plant grown on more alkaline soils (pH_1, pH_2) where 56% reduction was observed (Figure 5.12).

Total chlorophyll reduction was calculated based on direct measurements of chlorophyll 21 DAT. Already then, the highest reduction of chlorophyll was calculated at 72 g a.i. ha^{-1} simulated mesotrione residues of 93.4%. The lowest reduction for chlorophyll content in bean plants, 23.67%, was calculated for 1.1 g a.i. ha^{-1}. The carotenoid content was measured only for simulated mesotrione residues from 1.1 to 24 g a.i. ha^{-1} because the symptoms of herbicide action were too severe for normal preparation and homogenization of samples for spectrophotometric analysis. The highest reduction was at the highest simulated mesotrione residues, 24 g a.i. ha^{-1} (59.9%), while the lowest reduction was 16.63% at 1.1 g a.i. ha^{-1}.

Regardless of the differences in time of the measurements and analysis, it is inevitable that even 1.1 g a.i. ha^{-1} of simulated mesotrione residue, which is only 1/128 of the recommended rate of the herbicide, affects the plants. Considering the effect of soil pH on bean plants reaction to simulated mesotrione residues, reduction of chlorophyll in soil pH_{1-4} was 66.73, 64, 63, and 53.98%, respectively, while reduction of carotenoid in soil $pH_{1,2}$ was 52.13 and 45.84%, and in soil $pH_{3,4}$ 21%, respectively. Nonetheless, large differences in results relate to the mechanism of herbicide action given by Barchanska et al. (2015), who explained how inhibition of carotenoid biosynthesis starts in a series of cause-and-effect reactions, which in turn end up with the destruction of total chlorophyll.

It is a general conclusion of this experiment that common bean is less sensitive on simulated mesotrione residues in more acidic than in more alkaline soils, i.e., simulated mesotrione residues are more bioavailable in the soil solution of alkaline than of acidic media. This agrees with observations by Wangcang et al. (2017) and Dyson et al. (2002), who have proved the decrease of half-life and decrease in adsorption of mesotrione with the increase in alkalinity of the soil. However, regarding the half-life of mesotrione, if it decreases with the soil alkalinity, the results of this experiment could have been different if the experiment lasted for more than 28 days. If mesotrione is better absorbed in acidic soils, its persistence could be better observed and the effects on bean plants with longer periods of time.

To summarize, it has been concluded that for investigation of potential mesotrione residues, the common bean can be used as a test plant because it has shown good sensitivity to the herbicide. Furthermore, even 1/128 of the recommended rate of herbicide can affect the crop, most probably influence the yield, and consequently, bring financial damage to the producers. At last, soil pH has proven to be very important factor in the range of inflicted damage on plants, so agricultural producers should consider properties of the soil they use for production and not only depend on given instruction on the label of the herbicide they are planning to use.

7. Conclusions

Based upon the results of statistical analysis on data gained by visual injury assessment (7, 14, 21, 28 DAT), reduction of fresh weight of the bean and reduction of chlorophyll and carotenoid content, the following conclusions were made:

- Common bean sensitivity increased exponentially with the increase of simulated mesotrione residues.
- Common bean sensitivity variated within the same simulated mesotrione residue depending on soil pH.
- The highest phytotoxic injuries to common bean in all soils were determined 28 DAT at the highest rate of simulated mesotrione residues (72 g a.i. ha^{-1}) and ranged from 96.5 to 100%, with the lowest injuries assessed in soil pH_4 with 20 % phytotoxic injury in common bean.
- Reduction of common bean fresh weight increased from 19.82 % at 1.1 g a.i. ha^{-1} to 86.47 % at 72 g a.i. ha^{-1} simulated mesotrione residues with common bean grown in soil pH_1 and pH_2 being the most sensitive (reduction of 56%), in contrast to least sensitive common bean grown in soil pH_4 (reduction of 42.27%).
- Reduction of chlorophyll content in common bean increased from 23.67 % at 1.1 g a.i. ha^{-1} to 93.4 % at 72 g a.i. ha^{-1} simulated mesotrione residues with common bean grown in soil pH_1 and pH_2 being the most sensitive with reduction of 66.73 % and 64.81 %, respectively. The least sensitive was common bean grown in soil pH_4 with 53.98 % reduction.
- Reduction of carotenoid content in common bean increased from 16.63 % at 1.1 g a.i. ha^{-1} to 52 % at 24 g a.i. ha^{-1}. The least sensitive was common bean grown in soil pH_4 and soil pH_3 with 21.77 % and 21.6 % reduction, respectively. At 24 g a.i. ha^{-1} simulated mesotrione residues common bean grown in soil pH_1 and pH_2 were the most sensitive with reduction of 52.13 % and 45.84 %, respectively.

Considering the variabilities in obtained results and results observed in reviewed studies, further research should be carried out in both the field and the controlled environment. In the controlled environment, a study for longer than 28 DAT could be useful to observe whether the behaviour of mesotrione would change with time depending on the soil pH. The field study would broaden the insight into mesotrione behaviour under actual pedoclimatic conditions.

8. References

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Biography

Laura Pismarović was born on 6 January 1989 in Zagreb. After graduating from the XVI Gymnasium in Zagreb (2005-2008), she enrolled in undergraduate studies in geology at the Department of Geology, Faculty of Science Zagreb and obtained her Bachelor's degree in geology in 2013 with the thesis "The problem of identifying metamict minerals". Before starting her graduate studies, she completed training as an expert in environmental protection and occupational safety in the Laboratory for Analytics and Toxicology - ANT d.o.o. During the training, she passed the exam to become an expert in occupational safety of II. degree in the Ministry of Labour, Pension System, Family and Social Policy and acquires the qualification of Head of Preparation and Implementation of EU Rural Development Projects at Algebra d.o.o. Until September 2018 she was employed at ANT d.o.o. and then enrols in interdisciplinary studies in English (B2 level according to CEFR) Environment, Agriculture and Resource Management at the Faculty of Agriculture in Zagreb. During her studies, she researched the potential of phenolic acids with reduced rates of herbicide in the control of ragweed (*Ambrosia artemisiifolia*) and voluntarily participated in an Erasmus traineeship in Italy at the University of Padua, where she investigated the effect of temperature and aqueous solutions of different acids on the germination of jimsonweed (*Datura stramonium*). For her work she regularly uses Windows OS, Microsoft Office and R and is familiar with working in Hydrus and QGIS programmes. In her spare time she enjoys hiking, cooking and making her own organic natural cosmetics.