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Scientific paper

A Bioassay Technique to Study Clomazone Residues in Sandy Loam Soil

Jelena Gajić Umiljendić[1], Ljiljana Radivojević[1], Tijana Đorđević[1], Katarina Jovanović-Radovanov[2], Ljiljana Šantrić[1], Rada Đurović-Pejčev[1] and Ibrahim Elezović[2]

[1]Institute of Pesticides and Environmental Protection, Banatska 31b, 11080 Belgrade, Serbia
[2]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia
(pecikos@gmail.com)

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SUMMARY

A bioassay test was conducted to evaluate the sensitivity of maize, sunflower and barley to clomazone residues in sandy loam soil. Clomazone was applied at different rates from 0.12 to 12 mg a.i./kg of soil. The parameters measured 14 days after treatment were: shoot height, fresh and dry weight, and content of pigments (carotenoids, chlorophyll *a* and chlorophyll *b*). The results showed that the lowest clomazone concentration caused a significant reduction in all measured parameters for barley and sunflower shoots. Fresh weight of maize shoots was not sensitive to clomazone residual activity in soil while the other parameters were highly inhibited.

Nomenclature: clomazone (2-(2-chlorbenzyl)-4,4-dimethyl-1,2-oxazolidin-3-one), maize (*Zea mays* L.), sunflower (*Helianthus annuus* L.), barley (*Hordeum vulgare* L.)

Keywords: Sunflower; Bioassay; Residues; Maize; Clomazone; Barley

INTRODUCTION

Herbicides are applied to soil to manage weeds. While it is desirable for chemicals to control weeds during the season of application, it is not desirable for them to persist and affect subsequent crop growth. Soil-applied herbicides should have enough residual activity to reduce weed interference, but to pose minimal threat to nontarget species or future crops.

Herbicide dissipation is affected by many interactive factors, including soil composition, soil chemistry and microbial activity. Soil composition is a physical factor determined by relative amounts of sand, silt and clay in the soil (soil texture), as well as by organic-matter (OM) content. In general, medium- and fine-textured soils with an OM content of more than 3% have the greatest potential to bind or hold herbicides and to injure sensitive succeeding crops. Coarse- to medium-textured soils with a lower OM content (less than 3%) are less likely to retain herbicides and to cause carryover problems. Under certain circumstances, however, herbicide carryover can occur in any type of soil (Beulke et al., 2000; Cumming et al., 2002; Khoury et al., 2003).

Soil factors influence the activity of clomazone. The adsorption and availability of clomazone have been highly and positively correlated with soil OM content and less highly correlated with clay content. Clomazone activity is the highest in sandy soils where organic carbon levels and cation exchange capacity are low. Also, clomazone is more persistent in high OM than in low OM soils (Gallandt et al., 1989; Loux et al., 1989a, 1989b; Kirksey et al., 1996; Gunasekara et al., 2009). The persistence of clomazone is significantly affected by environmental factors, such as moisture content, and the degradation process is therefore slower when precipitation, as well as temperature, are lower. The influence of tillage on persistence of this herbicide is not consistant, while an application method may reduce the loss of clomazone fumes from soil surface and plant remains (Mills and Witt, 1989; Ahrens and Fuerst, 1990; Curran et al., 1991, 1992). Leaching studies indicate a low mobility of clomazone in sandy loam, silt loam and clay loam soils and intermediate mobility in fine sand. The half-life (DT_{50}) of clomazone ranges from 5 to 117 days, depending on soil type (Loux et al., 1989a; Mills et al., 1989; Gallaher and Mueller, 1996; Kirksey et al., 1996; Cumming et al., 2002).

In Serbia, clomazone is used to control broad-leaved and some grass weeds in soybean, tobacco, oil seed rape and some vegetable crops (Janjić and Elezović, 2010). It is a selective systemic herbicide from the i*soxazolidinone group,* absorbed by root and shoot, transported ascendingly through the xylem to the top of a plant and reaching the leaves by diffusion. Clomazone inhibits the biosynthesis of chlorophyll and carotenoid pigments in susceptible plant species, resulting in white, yellow or light-green plants (Duke et al., 1985; Young, 1991; Böger and Sandmann, 1993). While this herbicide has found significant use in soybean weed control programmes, there has also been concern about its persistence in soil. Former studies of damage caused by clomazone residues to succeeding crops in rotation involved several plant species, but their results varied and were often inconsistent even for the same species (Gallandt et al., 1989; Loux et al. 1989a; Ahrens and Fuerst, 1990; Monks and Banks, 1991; Krausz et al., 1992). Gunsolus et al. (1986) detected carryover and subsequent injury of corn, wheat, alfalfa and oat in the year following application of clomazone.

The studies of mobility and persistence of herbicides in soil usually tend to determine the fate of herbicides and effects of their residues to the following crops in rotation. Bioassay techniques, in general, have been used extensively as a rapid and inexpensive method for quantitative determination of various herbicide residues in soil (Günther et al., 1993; Streibig and Kudsk, 1993; Mitrić, 2011). Unlike chemical analyses, bioassay techniques provide a low-cost method of monitoring actual phytotoxic amounts of herbicides available to sensitive crops. In contrast, chemical analysis determines the amounts of herbicides present in soil, which may or may not be directly available to a crop (Groves and Foster, 1985).

This study was conducted to assess the phytotoxic potential of clomazone residues in sandy soil, using bioassay, with particular reference to carryover effects on maize, sunflower and barley as potential succeeding crops in rotation.

MATERIAL AND METHODS

Technical-grade clomazone (Shenzhen, China) of 95% purity was obtained from Galenika Fitofarmacija. Seeds of maize (PR35F38, Pioneer), sunflower (Krajišnik, Novi Sad Institute of Field and Vegetable Crops) and barley (525 Novi Sad Institute of Field and Vegetable Crops) were used in the assay. Sandy loam soil was collected from 10 cm depth at the locality Tavankut, an area that had not been treated previously with herbicides, and was cleaned from above- and underground plant remains and sifted through a 3 mm sieve. The soil was medium calcareous, medium alkaline, very weakly humic and with a moderate supply of total nitrogen and good supply of available phorphorus and potassium (Table 1).

Table 1. Physical and chemical properties of sandy loam soil samples

Chemical properties							
$CaCO_3$	pH		C	Humus	N	P_2O_5	K_2O
%	H_2O	KCl	%	%	%	mg/100g	mg/100g
5.77	8.04	7.63	0.53	0.91	0.061	24.50	22.0

Soil texture				
Sand			Silt	Clay
Coarse (mm) 2-0.2	Fine (mm) 0.2-0.02	Total (mm) 2-0.02	0.02-0.002 mm	<0.002mm
20.59	70.85	91.44	1.32	7.24

Bioassay experiments were carried out by using 250 g of air-dried soil treated with different clomazone concentrations (0.12, 0.25, 0.50, 1, 2, 4, 6, 8, 10 and 12 mg a.i./kg of soil). The concentration of 6 mg a.i./kg corresponded to the label rate of 0.75 l/ha (480 clomazone g/l) for field application. The soil was treated uniformly over the surface and hand-stirred immediately after application, then transferred to pots, planted with seeds of the tested plant species and watered up to 50% of field water capacity. Simultaneously, control variants were prepared without herbicide application and all of the three plant species were grown. The experiment was set in four replicates for each plant species and performed twice. The soil samples were placed in a growth chamber at 26^{0}C/day and 21^{0}C/night with a photoperiod of 14 h of light. During the experiment, soil moisture was maintained at constant 50% field water capacity.

The vegetative parameters of shoot height, and fresh and dry weight of shoots were measured as indicators of phytotoxicity, while the physiological parameters were the contents of carotenoids, chlorophyll *a* and chlorophyll *b*.

The intensity of bleaching was assessed by measuring the content of pigments and inhibition percentage in relation to pigments content in plants on the control soil (no clomazone added). Pigments content was determined by cutting fresh shoots (leaf slices until total weight of 0.1 g) and extraction with 3 mL of *N,N*-dimethylformamide (DMF). The solutions were made in the dark at 4°C over 24 hours. After that period, extract absorption was measured by visible spectroscopy at 480 nm wavelength for carotenoids, 664 nm for chlorophyll *a* and 647 nm for chlorophyll *b* (spectrophotometer LKB Biochrom Novaspec II 4040, Great Britain). A formula according to Wellburn (1994) was used to calculate the concentration of pigments (mg/ml), and afterwards a conversion of pigment content (mg/g fresh leaf weight) was conducted.

The effect of clomazone concentrations on the parameters assessed was evaluated using F-test at significance level of 5%. Statistical analysis was done and graphics constructed using StatSoft 6.0. The data obtained were used for regression analysis to estimate the ED_{50} (effective doses of clomazone that reduced pigment content by 50 %) using the software package BIOASSAY97 (Onofri, 1995).

RESULTS AND DISSCUSION

The results showed a significant influence of clomazone concentrations in sandy loam soil on the development of all of three succeeding crops.

Clomazone concentrations of up to 12 mg a.i./kg had no measurable effects on shoot fresh weight of maize plants. Statistically significant reductions in shoot height (8.69-44.41%) and dry weight (13.64-50.00%) were detected at concentrations ≥ 2 mg a.i./kg (Figure 1). This unexpected disproportion between fresh and dry shoot weight could be a consequence of water accumulation in shoots (Boyer, 1969). Significant differences between treatments and the control were detected by analysis of variance, and for shoot height and dry weight they were F = 23.70 and F = 3.66, respectively.

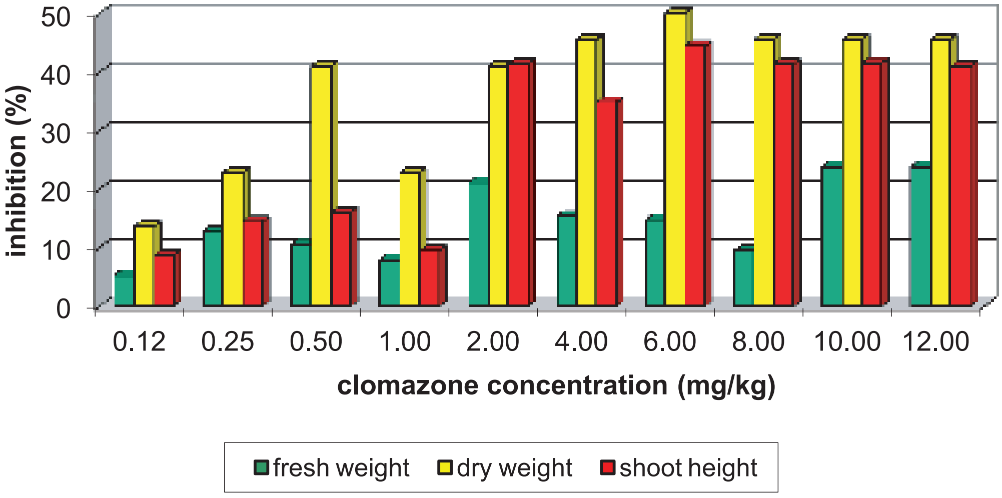

Figure 1. Changes in vegetative parameters of maize influenced by residual activity of clomazone

Fresh (12.67-51.58%) and dry weight (31.82-63.64%) and height (7.76-46.55%) of sunflower shoots were equally inhibited by clomazone concentrations ≥ 0.25 mg a.i./kg (Figure 2). The analysis of variance showed that differences between treatments and the control were significant for all three parameters (F = 111.10 for fresh weight; F = 9.67 for dry weight; F = 34.01 for shoot height).

The measured vegetative parameters of barley plants (Figure 3) were sensitive enough to detect as low as 0.12 mg a.i./kg of clomazone in sandy loam soil. The lowest clomazone rate applied caused significant reductions in shoot fresh weight (50.00-70.00%), dry weight (36.97-74.79%) and shoot height (25.36-49.28%). The analysis of variance confirmed that differences between treatments and control were statistically significant for all vegetative parameters (fresh weight F = 13.16; dry weight F = 9.02; shoot height F = 68.11).

A significant reduction in chlorophyll *a* and chlorophyll *b* contents in maize plants was registered at ≥ 0.25 mg a.i./kg of clomazone concentration (92.80-99.84%). A statistically significant reduction in carotenoid content was caused by the same concentration (0.25 mg a.i./kg), while a more significant reduction (73.83-97.14%) was detected at clomazone rates of ≥ 4 mg a.i./kg (Figure 4). Significant differences between treatments and the control for all three measured parameters were detected by the analysis of variance (Table 2).

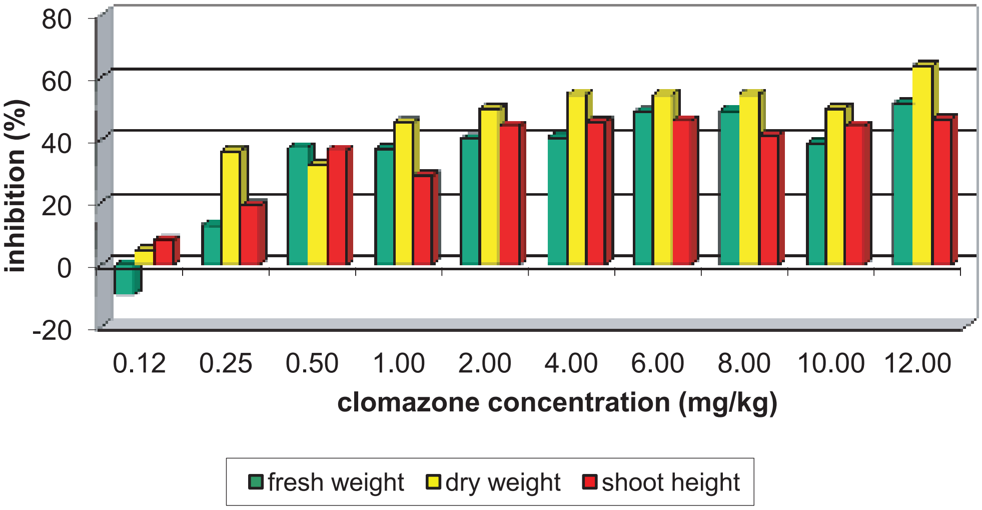

Figure 2. Changes in vegetative parameters of sunflower influenced by residual activity of clomazone

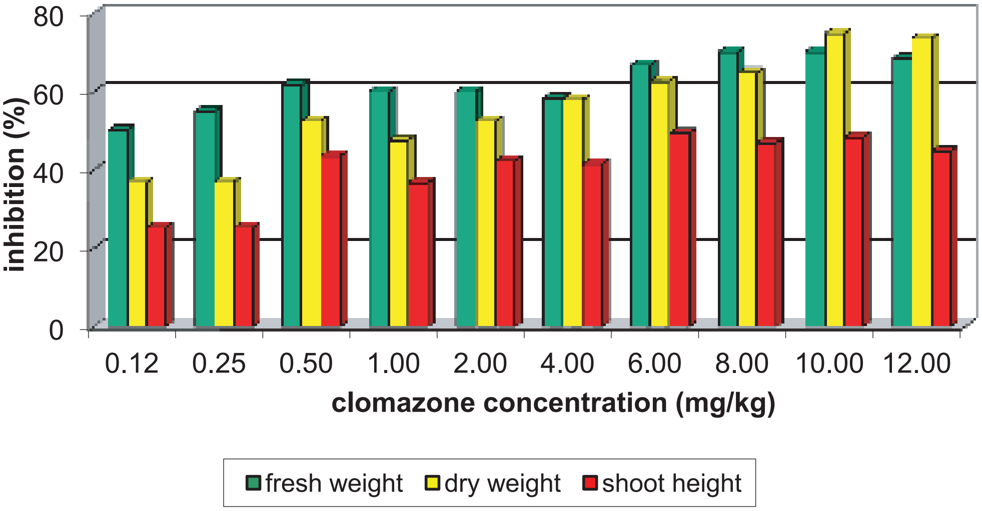

Figure 3. Changes in vegetative parameters of barley influenced by residual activity of clomazone

The lowest clomazone concentration (0.12 mg a.i./kg) reduced chlorophyll *a* and chlorophyll *b* contents in sunflower plants. The next higher concentration (0.25 mg a.i./kg) caused a reduction in carotenoid content (Figure 5). However, when applied at the rates ≥ 1 mg a.i./kg, clomazone reduced the content of chlorophylls by over 80%, as follows: chlorophyll *a* (85.92-98.15%) and chlorophyll *b* (89.68-97.96%). A greater reduction in carotenoids (72.39-78.42%) was found under the higher application rates (≥ 4 mg a.i./kg). The analysis of variance confirmed that differences between treatments and the control were statistically significant for all three parameters (Table 2).

The most sensitive were barley plants (Figure 6), which responded by pigment content reduction at the lowest clomazone concentrations (0.12 mg a.i./kg). When applied at the rates ≥ 1 mg a.i./kg, clomazone caused reductions in chlorophyll *a* (93.51-99.17%), chlorophyll *b* (93.73-99.45%) and carotenoids (89.50-95.03%) contents. The analysis of variance showed that differences between treatments and the control were significant for all three parameters (Table 2).

The ED_{50} values for each measured parameter and all three plant species were calculated by regression analysis of the data obtained for plant pigment content depending on clomazone concentration. Barley showed high sensitivity at the lowest ED_{50} values (0.07 mg a.i./kg for chlorophyll *a*, 0.04 mg a.i./kg for chlorophyll *b* and 0.16 mg a.i./kg for carotenoids). Sunflower and maize displayed somewhat lower sensitivity to clomazone. The calculated ED_{50} values for chlorophyll *a*, chlorophyll *b* and carotenoids in sunflower were 0.32 mg a.i./kg, 0.26 mg a.i./kg and 1.13 mg a.i./kg, respectively. The estimated ED_{50} values for maize were: 0.21 mg a.i./kg for chlorophyll *a*, 0.20 mg a.i./kg for chlorophyll *b* and 1.71 mg a.i./kg for carotenoids. These results highlight the differential sensitivities of plant species to clomazone. In developing a bioassay procedure, when there is an unknown concentration of a herbicide in soil, it may be best to use plant species with a range of sensitivities to accurately determine the herbicide concentration and reduce the risk of false positives or negatives.

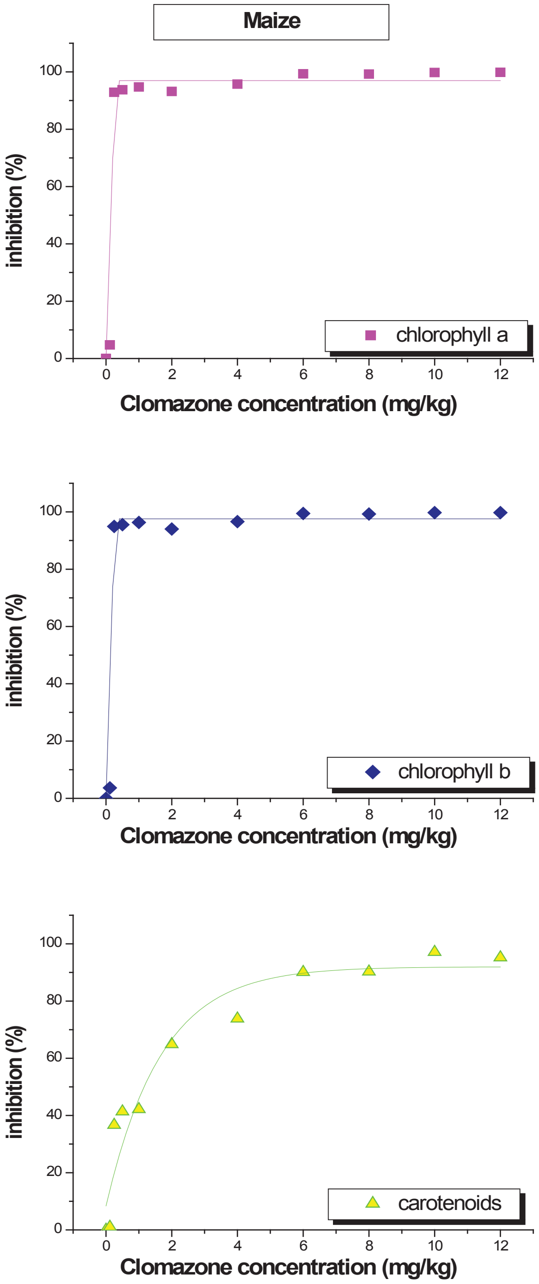

Figure 4. The residual effect of clomazone on chlorophyll *a*, chlorophyll *b* and carotenoids in leaves of maize

Table 2. Influence of different concentrations of clomazone in soil on physiological parameters (ANOVA)

Plant pigments	Plant species					
	Maize		Sunflower		Barley	
	$F_{(10,33)}$	p	$F_{(10,33)}$	p	$F_{(10,33)}$	p
chlorophyll *a*	248.34	<0.05	474.17	<0.05	642.04	<0.05
chlorophyll *b*	224.16	<0.05	417.42	<0.05	484.80	<0.05
carotenoids	90.09	<0.05	19.32	<0.05	91.44	<0.05

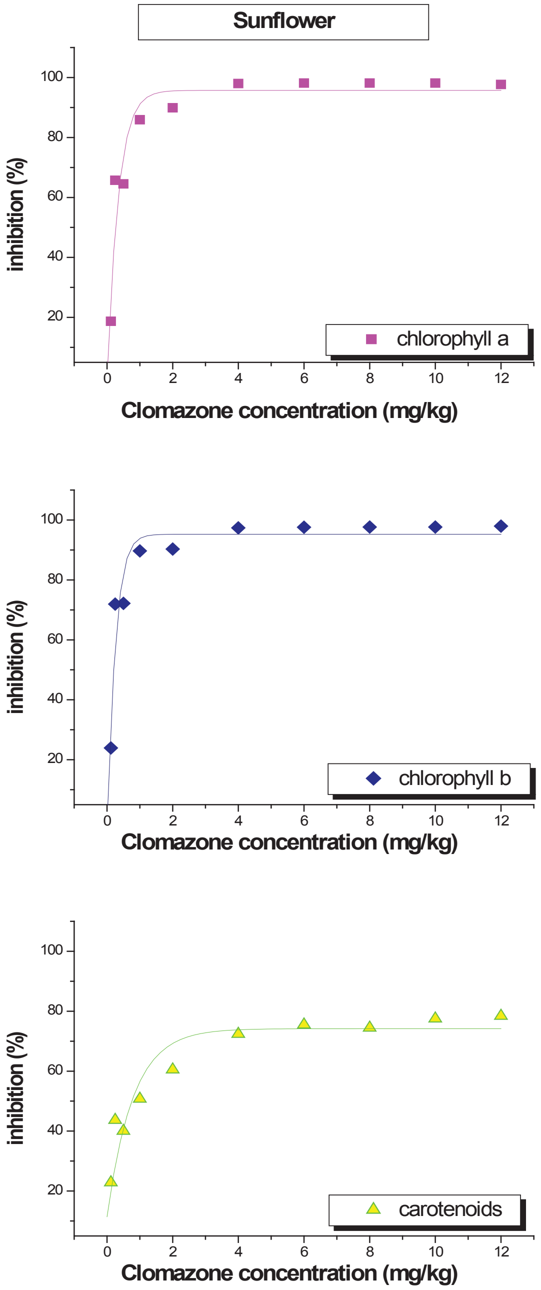

Figure 5. The residual effect of clomazone on chlorophyll *a*, chlorophyll *b* and carotenoids in leaves of unflower

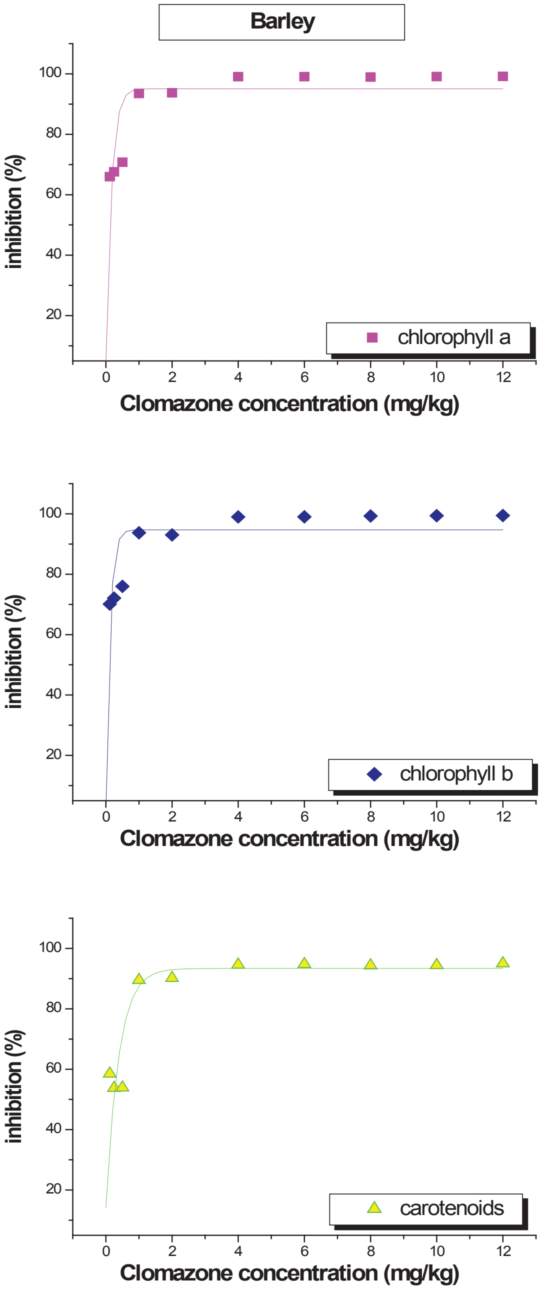

Figure 6. The residual effect of clomazone on chlorophyll *a*, chlorophyll *b* and carotenoids in leaves of barley

The application of different clomazone concentrations caused leaf bleaching and high reduction in pigment contents in all three tested plant species. These results showed that barley, sunflower and maize could be considered as suitable indicator species for bioassay test of clomazone persistence in sandy loam soil.

In a previous bioassay study (Gajić Umiljendić et al., 2012), we had shown that sunflower plants displayed a reduction of over 70% in all contents of pigments when clomazone was applied at the rate of 1 mg a.i./kg in loamy soil (3.96% OM). Maize and barley showed somewhat lower sensitivity. A significant decrease in the content of all pigments was found at 6 and 8 mg a.i./kg clomazone, respectively. Also, the measured vegetative parameters of these bioassay species in loamy soil were significantly inhibited only by clomazone concentrations greater than 6 mg a.i./kg.

The bioassay experiments conducted by Jovanović-Radovanov (2011) showed no differences in the sensitivity of several crops (maize, sunflower, sugar beat, wheat and mustard) to clomazone residues in silty clay soil (2.46% OM) regarding shoot fresh and dry weight. Based on the EC_{50} values for carotenoids, chlorophyll *a* and chlorophyll *b* contents reduction, the most sensitive crop was mustard, followed by sugar beat and wheat. The least sensitive were sunflower and maize.

The effects of soil type on clomazone bioactivity were similar to those found in experiments conducted by Loux et al. (1989a). Availability of clomazone in a wheat bioassay was greater in silt loam soil that contained 1.3% OM than in silty clay loam soil having 5.8% OM. As a contrast, in a field experiment, clomazone was more persistent in silty clay loam (the EC_{50} value was up to 3.5 times higher) than in silt loam soil. Similar results were reported by Gallandt et al. (1989) using the same bioassay plant in experiments with soils which contained 1.6 and 2.3% OM.

Differences in bioactivity could be due to different levels of herbicide adsorption between the two soils. The reduction in herbicidal activity by soil OM results from the bonding of herbicide molecules by lipophilic OM surfaces. OM content has been the soil property most often shown to influence preemergence herbicide activity andalmost all soil-applied herbicide recommendations are therefore based in part on the percent of OM in soil.

Clomazone has weak mobility in soil, depending on the content of OM and clay, but also on the presence of plant cover on soil surface (Mills et al., 1989; Mills and Witt, 1989; Curran et al., 1992; Mervosh et al., 1995). Its persistence depends on availability, which is determined by the intensity and degree of adsorption which strongly correlates with the content of OM in soil.

Inhibition of the measured parameters of maize, sunflower and barley in the present experiment was followed by injury of shoots, expressed as foliar chlorosis („bleaching") and necrosis of tissue, symptoms characteristic of clomazone injury. In many trials with different herbicide rates and soil types, injuries from clomazone residues on sensitive rotational crops such as wheat and oat were noticed (Gallandt et al., 1989; Loux et al., 1989a; Walsh et al., 1993; Mervosh et al., 1995). The injuries were observed as bright, white leaf chlorosis.

Clomazone applied to soybean crops on silty clay soil (5.0% OM) injured wheat planted 11 to 12 months later, causing 20-50% leaf chlorosis, 5% or less injury on loam soil (3.0% OM) and no significant injury on fine sandy loam (2.6% OM) (Ahrens and Fuerst, 1990). Apparently, low availability to wheat plants may counteract longer persistence in fine-textured, high OM soils. Higher OM content results in decreased phytotoxicity of clomazone, but dissipation is slower perhaps because more clomazone is adsorbed to OM. Also, one of the conditions enabling greatest clomazone carryover injury of sensitive crops is drought during the year of clomazone application, followed by normal or above average rainfall in the following year. Drought occurring after clomazone application enhances its persistence. The potential for herbicide injury to succeeding crops may change from season to season, according to changes in environmental conditions, such as soil moisture and temperature (Monks and Banks, 1991).

Bioassay provides an indication of potential herbicide residues in soil, especially when related herbicide residues have persisting potential. Our bioassay study shows that clomazone can injure maize, sunflower and barley in sandy loam soil. However, taking into consideration the low adsorptive capacity of that soil, it would be safe to predict that there will be no residues remaining one season following the application. Moreover, it is recommendable to conduct the experiment in field conditions.

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Mogućnosti korišćenja biotesta za praćenje ostataka klomazona u zemljištu tipa peskuša

REZIME

U radu je ispitivana osetljivost kukuruza, suncokreta i ječma na rezidualno delovanje klomazona u zemljištu tipa peskuša, metodom biotesta. Klomazon je primenjen u seriji koncentracija 0.12-12 mg a.s./kg zemljišta. Nakon 14 dana rasta biljaka mereni su vegetativni (visina, sveža i suva masa izdanka) i fiziološki parametri (sadržaj karotenoida hlorofila *a* i hlorofila *b*). Dobijeni rezultati pokazuju da su i najmanje koncentracije klomazona izazvale značajno smanjenje merenih vegetativnih i fizioloških parametara kod ječma i suncokreta. Izdanci kukuruza nisu pokazali istu osetljivost prema ostacima klomazona u zemljištu tipa peskuša pa je sveža masa izdanka ostala nepromenjena u varijantama sa različitim koncentracijama herbicida, a ostali mereni parametri su bili značajno inhibirani.

Ključne reči: Suncokret; biotest; ostaci; kukuruz; klomazon; ječam