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Original scientific paper

Common milkweed (*Asclepias syriaca* L.) response to sulcotrione

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SUMMARY

A laboratory bioassay was conducted to investigate common milkweed response to sulcotrione. Sulcotrione was applied in concentration series of 0.15-0.90 kg a.i./ha without a surfactant and with Dash® at 1 L/ha. Plants grew for 14 days, upon which period morphological (height and fresh weight) and physiological parameters (content of carotenoids, chlorophyll *a* and chlorophyll *b*) were measured. Visual crop injury was estimated 7 and 14 days after treatment.

Sulcotrione caused leaf bleaching and reduction in pigments content in common milkweed leaves and the degree of change depended on application rates and whether it was applied with or without the surfactant. Inhibition was slightly higher in plants which were treated with a combination of herbicide and surfactant. Based on the findings in this study, common milkweed showed moderate susceptibility to the recommended field rates of sulcotrione.

Keywords: Common milkweed; Herbicide; Morphology; Carotenoids; Chlorophyll

INTRODUCTION

Common milkweed (*Asclepias syriaca* L.) is a robust, stemmed, unbranched, perennial, dicotyledonous weed native to North America. It was brought to Europe as an ornamental plant in the 19th century, but now it has become naturalized in much of central and southern Europe, including Serbia (Tutin et al., 1972; Vrbničanin et al., 2008; Csontos et al., 2009; Hulina, 2010; Szatmari, 2012; Paukova et al., 2013). After escaping from cultivation it has become widespread over the last decades of the 20th century. Common milkweed prefers fertile, well drained soils. It is mainly found in soybean, maize, wheat, and oat and often occurs also in roadside vegetation (Yenish et al., 1997; Hartzler & Buhler, 2000).

Common milkweed spreads quickly by its windborne seed and its creeping perennial rootstocks. Plants originating from seeds flower in their second season of growth, producing a large number of seeds, up to 450 per pod. Its perennial roots form a large underground network spreading out from the original plant. In spring, root buds push through the soil to become new plants which flower and set seed. Seeds quickly germinate and seedlings become perennial within 21 days after germination. Seeds remain viable for up to seven years in the soil (Konstantinović et al., 2008; Csontos et al., 2009).

Common milkweed is difficult to get rid of and it is a strong competitor for water and nutritiens, so that its further invasion is expected. The species can cause yield losses to farmers. Mechanical control of common milkweed, such as mowing, cutting or tilling is not effective, as new plants will arise from roots, resulting in larger colonies. Once common milkweed becomes established, a post-emergence herbicide that will translocate into its roots is required for control. Various herbicides, such as glyphosate, bentazon and dicamba, are recommended for control of common milkweed (Duke & Powles, 2008; Dolmagić, 2010; Pleasants & Oberhauser, 2013; Popov, 2016). Common milkweed control efficiency with herbicides is often variable and highly dependent upon the plant growth stage and time of treatment (Bhowmik, 1982). Also, an addition of some surfactant can increase the effectiveness of herbicide applications. A surfactant (a combination of the words "surface active agent") is an organic compound that is soluble in chemical solutions or water and allows mixtures to blend, adhere and work better. The primary purpose of a surfactant is to reduce surface tension and to increase leaf wettability and cuticle penetration (Hess & Foy, 2000). Maximum reduction in surface tension occurs at surfactant concentrations ranging from 0.01 to 0.1%, while the greatest biological effectiveness occurs at concentrations exceeding 0.1%.

Sulcotrione, [2-(2-chloro-4-(methylsulfonyl)benzoyl) cyclohexane-1,3-dione], is a triketone herbicide used to control a wide range of grasses and broadleaf weeds in maize crops (Tomlin, 2009). It is mainly recommended for post-emergence treatments at application doses of 300 to 450 g a.i./ha. Sulcotrione herbicide is absorbed by leaves and also by roots. Its mode of action is inhibition of the enzyme 4-hydroxyphenylpyruvate dioxygenase, leading to disturbance in plastoquinone and α-tocopherol biosynthesis. Internal symptoms include bleaching or whitening of young, developing tissues, followed by necrosis and plant death (Monaco et al., 2002; Chaabane et al., 2008). Bleached tissues contain no pigmentation as absence of carotenoids prevents normal chloroplast and pigment development. Old leaves remain green when treated with this compound (Carlile, 2006).

The objective of this study was to determine the effectiveness of sulcotrione and a combination of sulcotrione and a surfactant for control of five-week old common milkweed seedlings.

MATERIAL AND METHODS

Common milkweed seeds were collected at Tavankut (North Serbia, W 7 382 591, E 5 098 903, altitude 102 m) in September of 2012 and stored in the laboratory at 20-22 °C temperature. The seeds were germinated in plastic cells with seed-starting soil in a growth chamber with 16 h of light (300 $\mu E\ m^{-2}s^{-1}$) at 28 °C, 8 h of darkness at 21 °C, and a relative humidity of 80 ± 5%. The 14-days-old, cotyledonary-stage seedlings were transferred to plastic pots (ø 12) filled with sandy soil (sampled at Tavankut, organic matter 0.91%, sand 91.44%, silt 1.32%, and clay 7.24%). The potted seedlings were watered daily to 50% soil moisture capacity (determined gravimetrically). Uniform, single-shoot plants, each with 2-4 leaves, were selected for use in treatments.

Sulcotrione (300 g a.i./L) was applied at 0.15, 0.30, 0.45, 0.60, 0.75 and 0.90 kg a.i./ha either without surfactant, or with Dash® (45 g/L oleic acid; 205 g/L polyoxyalkylated fatty alcohol phosphate esters; 345 g/L methyl esters of fatty acids, BASF, Germany) at 1 L/ha. The treatment solutions were prepared immediately before use and applied with a thin-layer chromatography sprayer under constant pressure of 3 bars. After spraying, the experimental pots were returned to the growth chamber conditions as described. Control plants remained untreated.

Visual crop injury was rated on a scale of 0 to 100% at intervals of 7 and 14 days after treatment (DAT). The 0% rating was defined as no visible plant injury, and 100% as total plant necrosis. Visual crop injury symptoms included leaf bleaching, leaf chlorosis and leaf necrosis. Above-ground biomass was harvested 14 DAT, weighed and measured and presented as fresh weight in g and height in cm.

Pigments contents were measured spectrophotometrically after methanol extraction. Samples (leaves) were collected from each plant and stored in deep-freeze (-20 °C) until next analysis. About 0.5 g of leaves were used to measure chlorophyll and carotenoid contents. The leaves were ground in a blender with 5 ml methanol. Chlorophyll extract was vacuum filtered and centrifuged for 10 min at 4000 rpm. Absorbance readings for the extracts were obtained at 666 nm (chlorophyll *a*) and 653 nm (chlorophyll *b*). The coefficients in equations for chlorophyll *a* and *b* concentrations were calculated using Lichtenthaler and Wellburn's (1983) formula. Absorption of total carotenoids was read at 470 nm wavelength, and their concentration calculated by Wellburn's (1994) formula. The content of pigments was converted from µg/ml to mg/g of fresh leaf weight.

The experimental design was a randomized complete block with four replications. The experiment was repeated and data combined for analyses. The effect of herbicide concentrations on growth inhibition percentage, reflected through morphological and physiological parameters, was calculated using the formula:

% growth inhibition = [(Xc-Xt)/Xc]×100, where
Xc – height, weight or pigment content in control plants and
Xt – height, weight or pigment content in treated plants

The data were subjected to standard statistical processing, using the analysis of variance (one way ANOVA) in StatSoft 8.0. The significance of differences between different treatments was determined by Fisher´s Least Significant Difference (LSD) test at the significance level of 5% (p<0.05). The data were used for a regression analysis to estimate the EC_{50} (i.e. the effective concentration of herbicide that reduced pigment content by 50%) using the software package BIOASSAY97 (Onofri, 2005).

RESULTS AND DISCUSSION

The application of different sulcotrione concentrations caused visual injuries which were observed as bright, white leaf chlorosis. In evaluations conducted 7 DAT, concentrations ≤ 0.60 kg a.i./ha resulted in less than 50% leaf bleaching. Combinations of the same rates of sulcotrione and Dash® (1 L/ha) caused bleaching symptoms in the meristematic tissue of common milkweed up to 60%. Plants exposed to two highiest concentrations (0.75-0.90 kg a.i./ha) had visual injuries 55-65%, while their combination with the surfactant caused severe injuries (75-85%). Weed injury increased over time and was also dose responsive. By the 14th DAT, sulcotrione applied at 0.15-0.60 kg a.i./ha caused 40-65% injury, compared with the untreated check. Adding the surfactant to these concentrations led to more intense bleaching (45-70%) of leaves. Common milkweed injury intensified with the increasing rates of sulcotrione (0.75-0.90 kg a.i./ha) applied alone or with Dash®, reaching up to 80-85% and 90-95%, respectively.

Inhibition of common milkweed height was negligible (8.8-14.3%) under all sulcotrione concentrations, while fresh weight was a somewhat more sensitive parameter and its observed inhibition was from 6.8 to 37.4% (Table 1). The combination of sulcotrione and Dash® caused a statistically significant decrease in the growth of common milkweed plants (Table 2 and 3). Reduction in height ranged from 14.7 to 32.7%, while the inhibition of fresh weight was 33.5-50.5%.

Table 1. Influence of sulcotrione on height, fresh weight and pigments content of common milkweed plants

Sulcotrione (kg a.i./ha)	Parameter				
	Height*	Fresh weight	Chl *a*	Chl *b*	Carot.
	% inhibition				
control	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
0.15	8.9 ab	6.8 ab	27.3 ab	30.3 ab	23.1 ab
0.30	9.7 ab	17.3 ab	39.0 b	46.9 b	33.3 b
0.45	13.6 b	24.4 ab	60.1 c	60.6 c	61.6 c
0.60	10.6 ab	34.7 b	76.4 cd	80.1 cd	77.4 cd
0.75	10.8 ab	34.7 b	87.0 d	90.2 d	87.5 d
0.90	14.3 b	37.4 b	94.6 d	95.3 d	96.8 d

*Values within a column marked by the same letters are not significantly different at the 0.05 level according to Fisher´s LSD test

Table 2. Influence of sulcotrione and Dash® on height, fresh weight and pigments content of common milkweed plants

Sulcotrione (kg a.i./ha) + Dash® (L/ha)	Parameter				
	Height	Fresh weight	Chl *a*	Chl *b*	Carot.
	% inhibition				
control	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
0.15 + 1	14.7 b	33.5 b	36.0 b	36.0 b	37.5 b
0.30 + 1	20.8 bc	37.3 b	48.5 bc	49.6 bc	49.9 bc
0.45 + 1	16.6 b	32.2 b	68.6 cd	70.8 cd	68.8 cd
0.60 + 1	30.5 c	43.8 b	83.6 de	84.7 de	83.8 de
0.75 + 1	26.9 bc	41.9 b	92.3 de	93.6 de	93.0 de
0.90 + 1	32.7 c	50.5 b	97.9 e	97.2 e	98.1 e

*Values within a column marked by the same letters are not significantly different at the 0.05 level according to Fisher´s LSD test

Table 3. One-way ANOVA for determining the effects of sulcotrione and sulcotrione with Dash® on measured parameters of common milkweed

Parameter	Sulcotrione		Sulcotrione + Dash®	
	F	*p*	F	*p*
Height	1.24	0.3035	5.59	0.0002
Fresh weight	1.55	0.1821	3.75	0.0038
Chlorophyll *a*	24.34	0.0000	17.17	0.0000
Chlorophyll *b*	22.16	0.0000	18.42	0.0000
Carotenoids	18.41	0.0000	15.29	0.0000

No significant difference (p >0.05); *(0.01< p <0.05); **(p <0.01)

Considering that sulcotrione, applied both alone and with Dash®, caused bleaching of common milkweed leaves, changes in pigment contents were expected (Tables 1 and 2). Statistically significant reduction in pigment contents (33.3-46.9%) was already registered for the second lowest sulcotrione concentration (0.30 kg a.i./ha).

The next two higher concentrations (0.45 and 0.60 kg a.i./ha) caused an inhibition of pigment contents of 60.1-61.6% and 76.4-80.1%, respectively. However, when applied at the rates of 0.75 and 0.90 kg a.i./ha, sulcotrione reduced the content of pigments by over 80%, as follows: chlorophyll *a* (87-94.6%), chlorophyll *b* (90.2-95.3%) and carotenoids (87.5-96.8%) (Table 1). The reduction in pigments contents was below 50% for the sulcotrione concentrations 0.15 and 0.30 kg a.i./ha applied with the surfactant (Tables 2 and 3). The combination of sulcotrione (0.45 kg a.i./ha) and Dash® (1 L/ha) significantly reduced pigments contents in a range of 68.6-70.8%, while it was even more prominent (83.6-84.7%) at the concentration of 0.60 kg a.i./ha. The surfactant and two highest sulcotrione concentrations (0.75 and 0.90 kg a.i./ha) reduced the content of pigments by over 90% as follows: chlorophyll *a* (92.3-97.9%), chlorophyll *b* (93.6-97.2%) and carotenoids (93-98.1%) (Table 2).

Regression analysis was used to determine the dependence of the contents of common milkweed pigments on different sulcotrione concentrations, either alone or with Dash®, and EC_{50} values were calculated from that analysis as indicators of plant sensitivity (Figure 1). Considering carotenoids, chlorophyll *a* and *b* contents, the EC_{50} values were lower when different sulcotrione concentrations were applied with the surfactant Dash®. The estimated EC_{50} values were: 0.46 kg a.i./ha for chlorophyll *a*, 0.44 kg a.i./ha for chlorophyll *b* and 0.46 kg a.i./ha for carotenoids. However, when sulcotrione was applied alone the calculated EC_{50} values for chlorophyll *a,* chlorophyll *b* and carotenoids were 0.49 kg a.i./ha, 0.50 kg a.i./ha and 0.47 kg a.i./ha, respectively.

Like many other perennials, common milkweed tolerates most herbicides, which generally kill the above-ground plant while leaving its reproductive root system intact to regenerate new plants. It is, however, easier to control it in noncropping land with nonselective herbicide (Bhowmik, 1994). Glyphosate is particularly praised for its efficacy against perennial weeds, such as common milkweed, which most other herbicides fail to kill (Franz et al., 1997). When glyphosate is sprayed on a common milkweed plant, it is absorbed by leaves and stems and then translocated inside the plant to concentrate in actively growing meristematic tissues, including the plant's roots and developing buds (Duke & Powles, 2008). By killing common milkweed at the root, regrowth in the following year is largely prevented (Bhowmik, 1994). Data reported by Bhowmik (1982) showed that glyphosate applied at 2.2 to 3.5 kg/ha in the early phase of growth proved satisfactory control (70% or better) of common milkweed plants. However, when applied to fully grown plants or at their later development stage, glyphosate applied at 3.84 kg/ha rate showed no efficiency (Konstantinović et al., 2008).

On the other hand, common milkweed is still one of difficult-to-control weeds in cropping systems. Buhler et al. (1994) found that small common milkweed populations in maize and soybean fields were not reduced by annual use of common herbicides such as atrazine, alachlor, cyanazine and metribuzin, over a 10-year period. Also, a five-year study in which soybean was grown every year in a conservation tillage system showed a slight increase in common milkweed prevalence after annual use of the herbicides bentazon and imazethapyr (Colbach et al., 2000). However, Dolmagić (2010) showed that a combination of oxasulfuron and bentazon applied in soybean fields achieved an efficiency of over 82% in common milkweed control. In field studies on older plants, Bhowmik (1982) found that neither 2,4-D nor dicamba provided much control of common milkweed within a year or two following a single application. Also, Zollinger (1998) conducted tests evaluating the effectiveness of various herbicides on common milkweed and concluded that even higher than normal rates of dicamba (1.2 kg/ha) and 2,4-D (2.4 kg/ha) provided levels of control rated at 61 and 48%, respectively. Similar results were achieved by Popov (2016) in field trial experiments with the same herbicides. Yenish et al. (1997) in their two-year study of common milkweed prevalence in maize, soybeans and wheat found that common milkweed seedlings were little affected by cyanazine (maize), diclofop (wheat), or imazethapyr (soybean), although alachlor (maize and soybean) and bromoxynil (wheat) were found to have limited effectiveness.

Seedlings are more susceptible to herbicides than mature plants. Based on laboratory trials with the recommended field rates of imazamox and oxasulfuron, Popov (2016) reported that common milkweed seedlings were killed by these herbicides 19 DAT. However, imazamox showed faster activity and its efficacy recorded 4 and 8 DAT were 60 and 80%, respectively. Conversely, oxasulfuron had no efficiency 4 DAT, while it was only 12.5% 8 DAT.

There is no available data to our knowledge about the efficacy of sulcotrione on common milkweed seedlings. In our trials, recommended field rates of sulcotrione (0.30 and 0.45 kg a.i./ha) showed moderate efficiency and caused visual injuries of 50 and 55%, respectively. Also, the inhibition of pigment contents

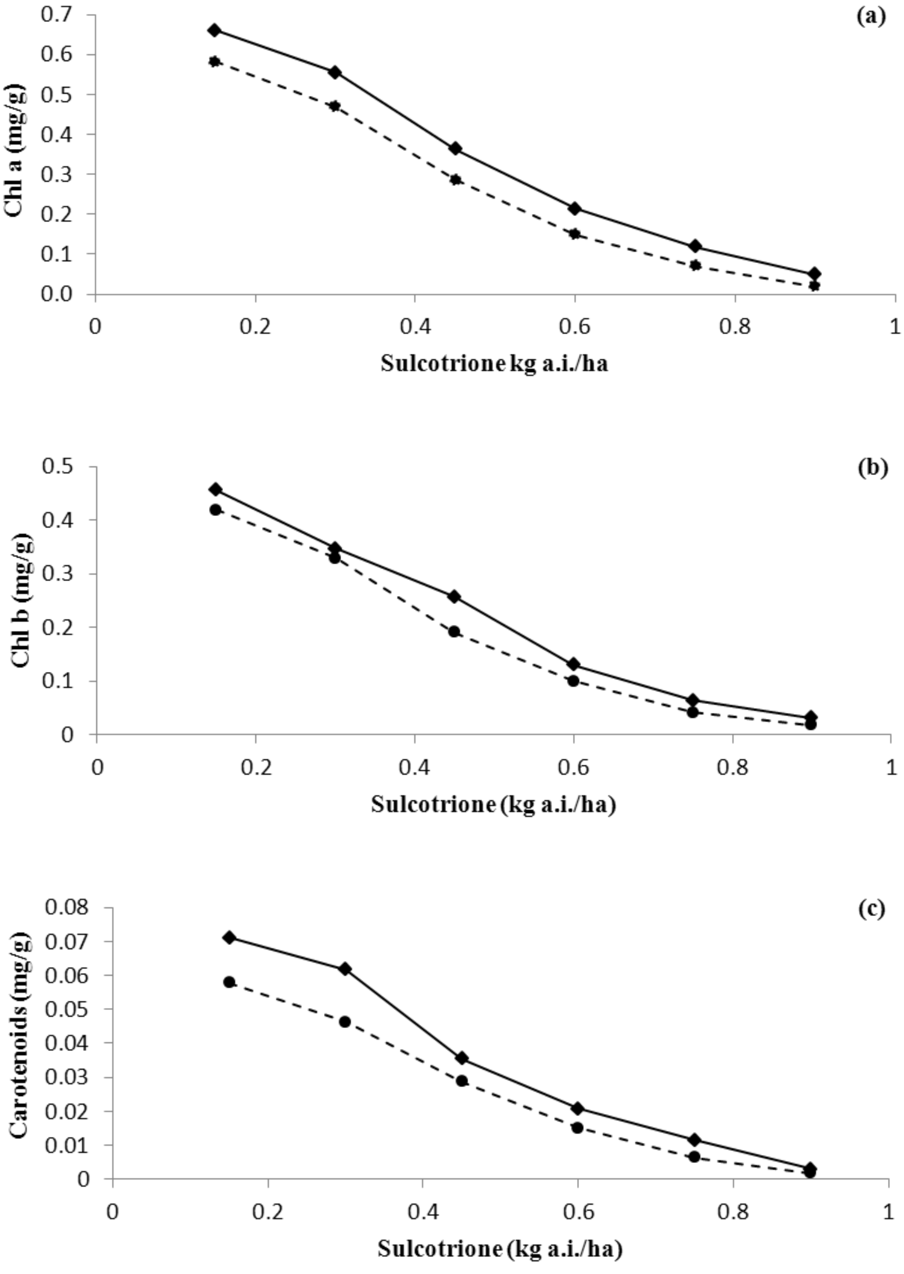

Figure 1. Response of common milkweed to increasing rates of sulcotrione (• - -) and sulcotrione with Dash® (⬩ -) based on contents of chlorophyll *a* (a), chlorophyll *b* (b) and carotenoids (c)

was bellow 50% for the lower concentration, while it was slightly higher (60%) for the other (0.45 kg a.i./ha). Combination with a surfactant can enhance herbicide toxicity, which may be attributed to reduced surface tension, and increased leaf wettability and cuticle penetration. The results obtained in this experiment showed that a combination of sulcotrione and the surfactant Dash® slightly increased the efficiency of common milkweed control. Visual injuries caused by the recommended field rates reached 50-60%, while reductions in pigment contents were 50-70%. This study was conducted under laboratory conditions but the results may serve as a guideline to help growers make a sound selection of herbicide for control of this invasive species, considering that sulcotrione showed moderate efficacy in common milkweed control.

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Osetljivost ciganskog perja (*Asclepias syriaca* L.) na sulkotrion

REZIME

Biotest metodom, u laboratorijskim uslovima, ispitivana je osetljivost ciganskog perja na sulkotrion. Sulkotrion je primenjen u seriji koncentracija od 0.15-0.90 kg a.s./ha sa ili bez okvašivača Dash® (1 L/ha). Biljke su rasle 14 dana, a nakon tog perioda mereni su morfološki (visina i sveža masa) i fiziološki parametri (sadržaj karotenoida i hlorofila *a* i *b*). Stepen vizuelnih oštećenja ocenjivan je 7. i 14. dana nakon tretmana.

Sulkotrion je izazvao izbeljivanje listova i smanjenje sadržaja pigmenata u listovima ciganskog perja, a stepen ispoljenih promena je zavisio od koncentracije herbicida, kao i od toga da li je primenjen sa ili bez okvašivača. Veći stepen osetljivosti ispoljile su biljke koje su tretirane kombinacijom herbicida i okvašivača. Na osnovu dobijenih rezultata može se zaključiti da je cigansko perje umereno osetljivo na preporučene količine primene sulkotriona u polju.

Ključne reči: Cigansko perje; Herbicid; Morfologija; Karotenoidi; Hlorofil