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Original scientific paper

Impact of field dodder (*Cuscuta campestris* Yunk.) on physiological and anatomical changes in untreated and herbicide-treated alfalfa plants

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SUMMARY

The effects of field dodder on physiological processes and the anatomy of alfalfa plants were examined under controlled conditions. The experiment included the following variants: N - noninfested alfalfa plants (control); I - infested alfalfa plants (untreated); T - infested plants treated with imazethapyr. Imazethapyr application rate was 100 g a.i. ha^{-1}. The following parameters were checked: physiological - pigment content (chlorophyll *a*, chlorophyll *b*, total carotenoids); anatomical - stem parameters: thickness of epidermis and cortex, and diameter of stem and central cylinder; leaf parameters: thickness of epidermis, parenchyma and spongy tissue, mesophyll and underside leaf epidermis, and diameter of bundle sheath cells in alfalfa plants. Pigment contents and anatomical parameters were measured: prior to herbicide treatment (0 assessment), then 7 (I assessment), 14 (II assessment), 21 (III assessment), 28 (IV assessment) and 35 (V assessment) days after application (DAA).

Field dodder was found to affect the contents of chlorophyll *a*, chlorophyll *a* and carotenoids in untreated alfalfa plants, causing significant reductions in pigment content. Conversely, percent reduction in the treated plants decreased 22-5% for chlorophyll *a*, 25-1%, for chlorophyll *b*, and 21-11% for carotenoids, while a stimulating effect of 1-6% was observed for the contents of chlorophyll *b* and carotenoids 35 DAA. Plants infested (untreated) by field dodder had lower values of most anatomical parameters, compared to noninfested plants. The measured anatomical parameters of alfalfa stems and leaves had significantly higher values in noninfested plants and plants treated with imazethapyr than in untreated plants.

Keywords: Field dodder; Alfalfa; Herbicide; Pigments; Anatomical parameters

INTRODUCTION

Flowering plants include a group of approximately 3,900 known parasitic plant species classified into more than 20 plant families (Westwood et al., 2010). Well-known and agriculturally important genera include *Striga*, *Orobanche* and *Cuscuta*. *Cuscuta* spp. thrive in regions with a warm and humid climate where the highest *Cuscuta*-related crop yield losses also occur (Dawson et al., 1994). All members of this genus are

vines with twining, slender, pale stems, with reduced, scalelike leaves, and no roots. Parasitic plants of the genus *Cuscuta* either have no chlorophyll at all, or merely low amounts of it, and usually do not have a photosynhetic activity (Hibberd at al., 1998; García et al., 2014). Only a few *Cuscuta* species still show residual photosynthesis (Dawson et al., 1994; Hibberd at al., 1998) and have thus been designated as cryptically photosynthetic (Funk et al., 2007; McNeal et al., 2007a,b). However, all *Cuscuta* species fully depend on host plants to complete their life cycle, and are therefore considered obligate holoparasites. For agricultural purposes, *C. campestris* and *C. pentagona* are the most important *Cuscuta* species due to their almost worldwide distribution and a wide spectrum of hosts.

Field dodders parasitize many different plants, inducing negative impact on the growth and yield of infested hosts and having significant effect on the structure and functioning of plant communities infested by these holoparasites (Koskela et al., 2001; Fathoulla & Duhoky, 2008). Damage can ultimately lead to total destruction and death of the host. Field dodder causes most damage during massive infestation of newly-established leguminous crops (alfalfa or clover), challenging the very feasibility of crop production (Dawson et al., 1994). Damage caused to these crops consists mainly of reduced fresh biomass yield, which may be upwards of 50%, and significantly reduced seed production (Cudney et al., 1992). Mishra (2009) reported some 60% yield reduction in alfalfa infested with *C. campestris*. Stojanović and Mijatović (1973) reported a yield reduction of around 80% in alfalfa crops infested with *C. campestris*, and some 20% in red clover crops.

There is no single technology to control this parasitic weed (Joel et al., 2007; Parker, 2009). Large areas of new fields are at a risk of invasion unless introduction of its seeds is carefully prevented, and farmers and others are educated on how to be on alert for new infestations (Panetta & Lawes, 2005). The only way to cope with parasitic weeds is through an integrated approach, employing a variety of measures in a concerted manner, starting with containment and sanitation, direct and indirect measures to prevent parasites-caused damage, and finally eradication of the parasite seedbank in soil. To establish strategies for controlling parasite growth and restrict the spread of field dodder in crop fields, it is important to learn more about the pest, studying its life-cycle and development.

Therefore, the objectives of this research were to: (1) examine the effects of field dodder on physiological and anatomical processes in untreated alfalfa plants and (2) test the effects of imazethapyr on field dodder infested alfalfa plants.

MATERIAL AND METHODS

Pot trial

Alfalfa plants were grown in plastic pots (Ø 17 cm) in a mixture of a commercial substrate (Flora Gard TKS1, Germany) and soil collected from a field without herbicide treatment history. After thinning, each pot contained 20 plants and they were watered daily. The herbicide was applied by a thin-layer chromatography sprayer under 1-2 bars pressure when alfalfa plants were 10-12 cm high and dodder plants fixed to the host. The trial included two controls: alfalfa plants infested (I) and noninfested (N) with field dodder, which were not treated with the herbicide, and infested plants treated with imazethapyr (T). Imazethapyr application rate was 100 g a.i. ha^{-1}(T). The parameters were checked: prior to herbicide treatment (0 assessment), then 7 (I assessment), 14 (II assessment), 21 (III assessment), 28 (IV assessment) and 35 (V assessment) days after aplication (DAA). All trial variants had five replicates and the trial was repeated twice.

Physiological analysis
Pigment contents

Chlorophyll content was measured spectrophotometrically after methanol extraction. Measurements were taken: 0, 7, 14, 21, 28 and 35 DAA. Samples (leaves) were collected from each plant and stored in deep-freeze (-20˚C) until next analysis. About 0.5 g of leaves was used to measure chlorophyll and carotenoid contents by methanol extraction. The leaves were ground in a blender with 5 ml methanol. Chlorophyll extract was vacuum filtered and centrifuged for 10 min at 1500 g. Supernatant was stored in the dark at +4˚C until spectrophotometric analysis. Absorbance readings for the extracts were obtained at 666 nm (chlorophyll *a*) and 653 nm (chlorophyll *b*), and 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:

chlorophyll a: ca= 15.65xA666-7.34A653 [1]
chlorophyll b: cb=27.05xA653-11.21A666 [2]
total carotenoids : ck= (1000A470-3.27 chla-104 chlb)/198 [3]

Chlorophyll content was converted from µg/ml to mg/g of fresh leaf weight using the formula:

$$C = c \cdot V \cdot R / m \cdot 1000 \quad [4]$$

where: C - chlorophyll content (mg/g), c - chlorophyll content (µg/ml), V - total extract volume (ml), R - dilute factor, m - sample fresh leaf weight (g), 1000 - convert factor for µg to mg.

Anatomical analysis

Samples for light microscopy (stems, leaves) were collected six times: 0, 7, 14, 21, 28 and 35 DAA. Alfalfa plants were sampled by cutting out 10 stem parts, 1 cm long, at the points of field dodder contact, and 10 samples of each of the first three true leaves for each treatment. The samples were fixed in 50% ethanol solution. A conventional paraffin wax method (Ruzin, 1999) was used to prepare the samples for microscopy. Embedded samples were then sliced on a LEICA SM 2000 R microtome, making 5-15 μm thick sections, and stained with toluidine blue, safranin and alcian blue. The following anatomical parameters were measured: **alfalfa stem -** thickness of epidermis and cortex, diameter of stem and central cylinder (pith); **leaf parameters -** thickness of upper epidermis and underside epidermis, thickness of palisade, spongy and mesophyll tissues, and diameter of vascular bundle cells.

Visualization was made on a light microscope LEICA DMLS, photographs were made by digital camera LEICA DC 300, and measurements were processed in the LEICA IM 1000 software package. All parameters were measured in 30 replicates.

Data analysis

The data were processed in Statistika 8.0. software using a two-way (for stem) and three-way (for leaf) factorial analyses of variance (ANOVA), and the differences between treatments were tested by Fisher's Least Significant Difference (LSD) test ($p<0.05$).

RESULTS AND DISCUSSION

Impact of field dodder on chlorophyll and carotenoids contents in untreated and treated alfalfa plants

Obligate parasites are not able to develop without assimilates supplied from their hosts because they are either unable to perform any photosynthetic activity on their own or such photosynthetic capacity is very low (Hibberd & Jeschke, 2001). It explains the differences in reported chlorophyll contents in various *Cuscuta* species, while most of them either do not contain any (Salimi, 2000) or only in traces (Berg et al., 2003). Their dependence on hosts is therefore stronger, as well as their negative impact on the host plant in terms of reducing chlorophyll and accessory pigments (van der Kooij et al., 2005). Our data revealed a highly significant impact of field dodder on chlorophyll *a*, chlorophyll *b* and carotenoids contents ($F = 23.040$, $p = 0.000000$) in untreated and treated alfalfa plants. Untreated alfalfa plants had reduced contents of chlorophyll *a*, chlorophyll *b* and carotenoids. The percent reduction in chlorophyll *a* ranged from 15% to 64%, and from 16% to 68 % in chlorophyll *b,* while carotenoids reduction ranged from 8% to 60% (Figure 1).

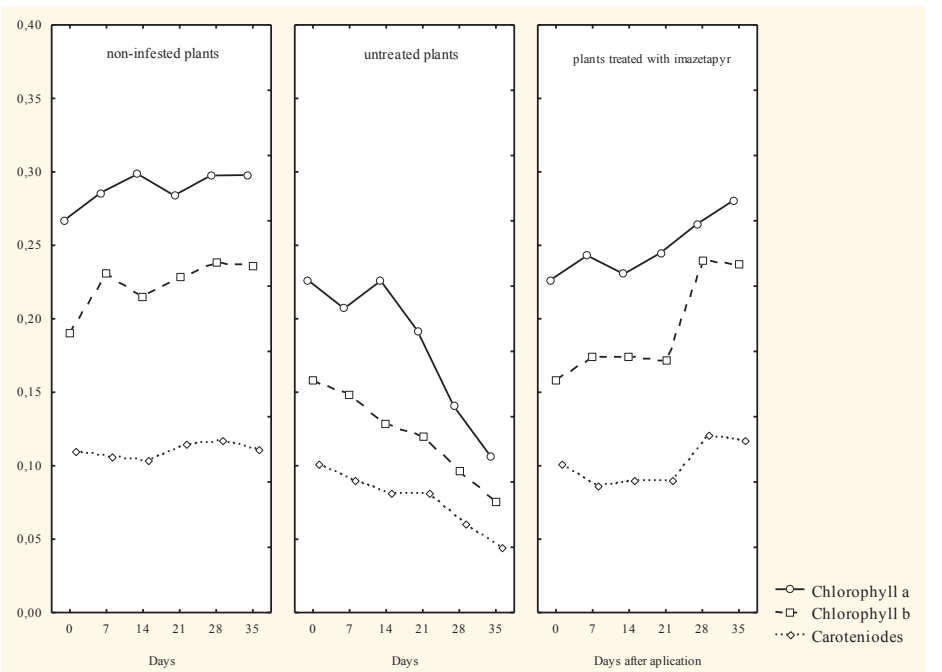

Figure 1. Chlorophyll *a*, chlorophyll *b* and carotenoids contents (mg/g) in alfalfa plants noninfested, untreated and treated with imazetapyr

The highest percent reduction in all pigment data were found 35 days after the beginning of the experiment. Conversely, percent reduction decreased in infested and treated plants from 22% to 5% for chlorophyll *a*, from 25% to 1% for chlorophyll *b,* and a stimulating effect of 1% was observed for the pigment 35 DAA. Reduction in carotenoids also took a decreasing trend from 21% to 11%, and stimulation of 4% and 6% was observed 28 and 35 DAA, respectively.

Impact of field dodder on anatomical parameters in untreated and treated alfalfa plants

Our data showed that the measured parameters of alfalfa stems are sensitive to dodder parasitism (F=18.063, p=0.000000). Statistically significant reductions in untreated plants ($p<0.05$) were found 28 DAI for all parameters except epidermis thickness, which was significantly reduced only later, 35 DAI. The highest percent reduction at the end of our experiment was detected in cortex thickness (51%), and the lowest in the diameter of central cylinder (33%). Conversely, significantly lower pecent reduction data were detected in treated plants (Table 1). The % reduction was highest in the thickness of epidermis (10%), and lowest in stem diameter (0.2%). Data show a recovery of the infested alfalfa plants and satisfactory efficacy of the herbicide.

The measured alfalfa leaf parameters displayed a sensitivity to field dodder parasitism (F = 6.71, p=0.000001)). All parameters of leaf anatomy had lower values in plants infested with dodder (and untreated) than in noninfested alfalfa plants (Table 1). However, their reduction was insignificant before the assessment 28 DAI,

Table 1. Impact of field dodder on anatomical parameters (stem and leaf) of untreated and herbicide-treated alfalfa plants

		Days					
		0	7	14	21	28	35
Parameter (μm)	Treatment	Alfalfa stem (point of field dodder contact)					
Thickness of epidermis	N	16.1±4.0	17.5±5.3	17.5±2.9	17.8±5.8	18.6±4.2	20.3±4.2 a
	I	15.5±3.7	14.5±4.2	14.3±2.8	13.9±2.2	13.5±3.1	13.2±4.2 b
	T	–	15.0±3.4	15.5±2.7	16.1±2.6	16.9±3.3	18.2± 4.5 c
Thickness of cortex	N	62.6±18.7	66.1±9.2	70.5±19.7	74.4±24.2 a	73.9±13.7 a	81.9±15.4 a
	I	59.1±14.6	55.3±13.7	53.0±15.4	52.4±12.6 b	52.1±12.2 b	39.9±9.4 b
	T	–	56.6±14.3	60.9±16.1	66.9±10.7 c	68.5±21.6 c	76.7±19.7 c
Diameter of central cylinder	N	947.8±83.418	1043.3±237.8	1074.4±312.1	1138.6±209.7 a	1213.6±191.7 a	1227.5±244.4 a
	I	1017.2±182.4	977.9±172.2	952.3±166.4	950.4±90.8 b	948.1±81.1 b	814.9±92.7 b
	T	–	1005.1±174.2	1050.7±155.5	1082.3±112.1 c	1143.7±275.1 c	1198.1±161.5 c
Diameter of stem	N	1154.1±104.7	1252.5±353.3	1240.2±236.4	1285.8±209.8 a	1343.1±195.6 a	1414.6±261.6 a
	I	1211.4±254.6	1159.2±150.2	1121.2±110.9	1085.7±171.9 b	1040.4±72.5 b	911.6±114.5 b
	T	–	1101.1±198.7	1133.4±133.1	1203.9±117.8 c	1340.8±257.1 a	1411.6±91.6 a
		Alfalfa leaf					
Thickness of upper epidermis	N	15.5±5.28	15.6±2.8	17.0±3.8	17.7±4.6	19.0±3.6 a	19.2±2.8 a
	I	14.4±4.57	15.3±3.8	14.2±4.1	14.1±2.4	14.1±2.7 b	14.0±4.3 b
	T	–	15.4±3.5	15.7±3.3	16.8±4.0	17.1±2.9 c	17.9±1.7 c
Thickness of palisade tissue	N	45.5±7.9	54.7±13.7	54.8±9.2	54.4±15.5	55.4±21.8	66.7±14.1 a
	I	45.1±10.1	44.5±9.8	44.1±7.1	43.1±7.9	42.7±9.4	35.1±10.7 b
	T	–	46.2±8.9	48.1±8.7	50.0±8.4	52.1±9.9	58.6±8.8 c
Thickness of spongy tissue	N	47.5±10.6	50.1±15.6	52.5±8.9	52.2±16.9	54.5±11.2 a	66.6±15.0 a
	I	46.5±10.8	45.8±10.7	44.6±10.3	43.0±8.1	41.4±16.8 b	32.8±9.1 b
	T	–	47.3±12.0	48.6±10.3	49.5±10.5	52.7±6.3 a	64.4±6.6 a
Thickness of mesophyll tissue	N	14.0±4.50	14.1±3.7	14.8±3.4	15.3±4.1	15.7±3.6	16.7±6.2 a
	I	13.6±3.87	13.5±3.1	13.5±4.8	13.3±4.3	13.2±3.5	8.2±3.5 b
	T	–	13.7±2.5	13.9±4.1	14.1±4.7	14.5±3.4	16.1±2.3 a
Thickness of underside epidermis	N	93.5±22.5	106.1±28.4	106.7±18.8	105.5±34.4	111.0±22.6 a	112.1±28.7 a
	I	91.5±15.6	90.6±17.4	88.8±13.8	87.5±14.6	85.8±24.8 b	69.2±21.1 b
	T	–	93.1±18.7	93.7±18.1	95.9±15.4	99.2±13.5 c	105.6±24.0 c
Diameter of vascular bundles cells	N	12.3±4.8	12.6±2.8	13.1±4.4	13.3±2.6	13.6±4.6 a	13.9±4.1 a
	I	11.6±3.1	11.8±3.3	10.1±2.7	10.0±2.6	9.3±2.7 b	9.8±2.3 b
	T	–	11.9±3.9	12.1±4.0	12.6±4.0	13.0±4.3 a	13.3±3.8 a

N - noninfested alfalfa plants ; I- untreated alfalfa plants ; T- herbicide-treated alfalfa plants; a, b - LSD test, $P<0.05$

when statistically significant ($p<0.05$) reductions were revealed for some of the parameters (i.e. thickness of upper epidermis, thickness of spongy tissue, thickness of underside epidermis, diameter of vascular bundles cells). Reductions in the last assessment ranged from 26% to 51%, and the lowest was in the thickness of upper epidermis (26%), while the greatest reduction was revealed for the thickness of mesophyll tissue (51%) (Table 1). As in stem parameters, leaf parameters of the treated plants also showed lower percent reductions, the lowest being in the thickness of spongy tissue (3%), then mesophyll thickness, diameter of vascular bundles cells (4%), thickness of underside epidermis (6%) and thickness of upper epidermis (7%), while the greatest reduction was detected in the thickness of palisade tissue (12%).

The most successful control of field dodder requires a systematic integrated pest management approach, beginning with dodder monitoring in crops and ruderal areas, and integrating adequate crop rotation, adequate choice of crops that are not suitable hosts to field dodder, a variety of preventive measures and physical removal, the use of tolerant cultivars and biological agents, as well as treatments with herbicides when it is not possible to solve the problem any other way. The results of our study showed that imazethapyr is an adequate herbicide for control of field dodder at the stage of early infestation.

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REFERENCES

Berg, S., Krupinska, K., & Krause, K. (2003). Plastids of three *Cuscuta* species differing in plastid coding capacity have a common parasite-specific RNA composition. *Planta*, *218*(1), 135-142. pmid:12898255. doi:10.1007/s00425-003-1082-8

Cudney, D.W., Orloff, S.B., & Reints, J.S. (1992). An integrated weed management procedure for the control of dodder (*Cuscuta indecora*) in alfalfa (*Medicago sativa*). *Weed Technology, 6*(3), 603-606.

Dawson, J.H., Musselman, L.J., Wolswinkel, P., & Dörr, I. (1994). Biology and control of *Cuscuta*. *Reviews of Weed Science*, *6*, 265-317.

Fathoulla, C.N., & Duhoky, M.M.S. (2008). Biological and anatomical study of different *Cuscuta* species (Kurdistan 1st Conference on Biological Sciences). *Journal of Dohuk University, 11*(1), 22-39.

Funk, H.T., Berg, S., Krupinska, K., Maier, U.G., & Krause, K. (2007). Complete DNA sequences of the plastid genomes of two parasitic flowering plant species, *Cuscuta reflexa* and *Cuscuta gronovii*. *BMC Plant Biology*, *7*(1), 45. doi: 10.1186/1471-2229-7-45

García, M.A., Costea, M., Kuzmina, M. & Stefanović, S. (2014). Phylogeny, character evolution, and biogeography of *Cuscuta* (dodders; Convolvulaceae) inferred from coding plastid and nuclear sequences. *American Journal of Botany*, *101*(4), 670-690. doi: 10.3732/ajb.1300449

Hibberd, J.M., Bungard, R.A., Press, M.C., Jeschke, W.D., Scholes, J.D., & Quick, W.P. (1998). Localization of photosynthetic metabolism in the parasitic angiosperm *Cuscuta reflexa*. *Planta, 205*(4), 506-513. doi: 10.1007/s004250050349

Hibberd, J.M., & Jeschke, W.D. (2001). Solute flux into parasitic plants. J*ournal of Experimental Botany, 52*, 2043-2049. doi: 10.1093/jexbot/52.363.2043

Joel, D.M., Hershenhorn, J., Eizenberg, H., Aly, R., Ejeta, G., Rich, P.J., ... Rubiales, D. (2007). Biology and management of weedy root parasites. *Horticultural reviews*, *33*, 267-349.

Koskela, T., Salonen, V., & Mutikainen, P. (2001). Interaction of a host plant and its holoparasite: Effects of previous selection by the parasite. *Journal of Evolutionary Biology*, *14*(6), 910-917.

Lichtenthaler, H.K. & Wellburn, A.R. (1983). Determinations of total carotenoides and chlorophylls *a* and *b* of leaf extracts in different solvents. *Biochemical Society Transactions*, *11*(5), 591-592. doi: 10.1042/bst0110591

McNeal, J.R., Arumugunathan, K., Kuehl, J.V., Boore, J.L., & de Pamphilis, C.W. (2007a). Systematics and plastid genome evolution of the cryptically photosynthetic parasitic plant genus *Cuscuta* (*Convolvulaceae*). *BMC Biology*, 5, 55. doi: 10.1186/1741-7007-5-55

McNeal, J.R., Kuehl, J.V., Boore, J.L., & de Pamphilis, C.W. (2007b). Complete plastid genome sequences suggest strong selection for retention of photosynthetic genes in the parasitic plant genus *Cuscuta*. *BMC Plant Biology*, *7*, 57. doi: 10.1186/1471-2229-7-57

Mishra, J.S. (2009): Biology and management of *Cuscuta* species. *Indian Journal of Weed Science, 41*(1-2), 1-11.

Panetta, F.D., & Lawes, R. (2005). Evaluation of weed eradication programs: The delimitation of extent. *Diversity and Distribution*, 11(5), 435-442. doi: 10.1111/j.1366-9516.2005.00179.x

Parker, C. (2009). Observations on the current status of *Orobanche* and *Striga* problems worldwide. *Pest Management Science*, 65(5), 453-459.

Ruzin, S.E. (1999). Plant Microtechnique and Microscopy (p 321). New York, NY: Oxford University Press.

Salimi, H. (2000). A study on comparison of seed dormancy and germination in three species of dodder. *Rostaniha*, *1*(4), 33-35.

Stojanović, D., & Mijatović, K. (1973): Distribution, biology and control of *Cuscuta* spp. in Yugoslavia. In *EWRS Symposium on Parasitic Weeds*, Malta (pp 269-279). Doorwerth, NL: EWRS.

Van der Kooij, T.A., Krupinska, K., & Krause, K. (2005). Tocochromanol content and composition in different species of the parasitic flowering plant genus *Cuscuta*. *Journal of Plant Physiology*, 162, 777-781.

Wellburn, A.R. (1994). The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology*, 144(3), 307-313.

Westwood, J.H., Yoder, J.I., Timko, M.P. & de Pamphilis, C. W. (2010). The evolution of parasitism in plants. *Trends Plant Science*, *15*(4), 227-235. doi: 10.1016/j.tplants.2010.01.004

Uticaj viline kosice (*Cuscuta campestris* Yunk.) na fiziološke i anatomske parametre lucerke u uslovima sa i bez primene herbicida

REZIME

Uticaj viline kosice na anatomske i fiziološke promene kod biljaka lucerke ispitivan je u kontrolisanim uslovima. Praćene su sledeće varijante: N - nezaražene biljke lucerke (kontrola); I- zaražene biljke lucerke i zaražene biljke lucerke tretirane sa imazetapirom (T). Imazetapir je primenjen u količini od 100 g a.i. ha^{-1}. Mereni su sledeći parametri: fiziološki - sadržaj pigmenata (hlorofil *a*, hlorofil *b* i ukupni karotenoidi); anatomski - stablo: debljina epidermisa i primarne kore stabla, prečnik centralnog cilindra i prečnik stabla; list: debljina epidermalnih ćelija lica i naličja lista, debljina parenhimskog i sunđerastog tkiva, debljina mezofila i prečnik ćelija omotača provodnih snopića. Sadržaj pigmenata i anatomski parametri su mereni: pre primene herbicida (0 ocena), potom 7, 14, 21, 35 dana nakon primene herbicida.

Vilina kosica je prouzrokovala značajnu redukciju sadržaja pigmenata kod netretiranih biljaka lucerke. Nasuprot ovome, kod tretiranim biljaka lucerke procenat redukcije se smanjivao od 22-5% za hlorofil *a*, 25-1%, za hlorofil *b* i 21-11% za karotenoide, dok je za hlorofil *a* i karotenoide 35 dana nakon primene herbicida zabeležen stimulativni efekat od 1-6%. Biljke zaražene vilinom kosicom (netretirane) su imale značajno manje vrednosti za sve merene anatomske parametare u odnosu na tretirane. Naime, mereni anatomski parametri stabla i lista lucerke su značajno veći kod kontrolnih biljaka i tretitanih sa imazetapirom u odnosu na netretirane.

Ključne reči: Vilina kosica, Lucerka, Herbicidi, Pigmenti, Anatomski parametri