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ANTIOXIDANT POTENTIAL OF RAGWEEDS: *Ambrosia artemisiifolia, A. trifida* AND *Iva xanthifolia*

ABSTRACT: The purpose of this study was to analyze antioxidant systems among three invasive ragweed species, *Ambrosia artemisiifolia* L., *A. trifida* L. and *Iva xanthifolia* Nutt. Antioxidant capacity could be a possible marker of adaptation to variable environmental conditions, since change in amount of antioxidants represents one of the first responses to various environmental stimuli. Among investigated ragweeds, *I. xanthifolia* leaves had more pronounced guaiacol peroxidase activity (87.5 and 62.5%) and reduced glutathione content (2.3 and 28.8%) than *A. artemisiifolia and A. trifida*, respectively. However, superoxide dismutase activity was invariable in all investigated plants (234.1–247.5 U g^{-1} fresh weight). The highest content of total phenolics, tannins, flavonoids and proanthocyanidins were detected in *A. trifida* leaves (up to 3.7 – fold the amount of the others). According to antioxidant activity tests, investigated ragweed species could be presented in a scale: *A. trifida* > *I. xanthifolia* > *A. artemisiifolia*. Accumulation of non-enzymatic antioxidants and lower content of reduced glutathione point to different oxidative stress avoidance strategies of *A. trifida* when compared to *A. artemisiifolia* and *I. xanthifolia* within the same environmental conditions.

KEYWORDS: antioxidants, phenolics, ragweed, secondary metabolism

INTRODUCTION

Among *Ambrosia* species, only *A. maritima* L. is native to Europe, while others, such as: *A. artemisiifolia* L. (*Ambrosia elatior* L.), *A. trifida* L., *A. tenuifolia* Spreng. and *A. psilostachya* DC. (*A. coronopifolia* Torr. & Gray) are native to North America and were introduced to Europe (Smith et al., 2013). In the recent years, relatively reduced crop rotation, shallow tillage, inadequate

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pre-sowing cultivation and use of the same or identical herbicide groups enabled spread and domination of *A. artemissifolia* and *Iva xanthifolia* Nutt. in the Northern Serbia (Konstantinovic et al., 2006). *Ambrosia artemisiifolia* L. (Common ragweed), *A. trifida* L. (Giant ragweed) and *Iva xanthifolia* Nutt. (False ragweed) represent serious allergenic and agricultural weed in Vojvodina province. Higher prevalence of competitively advantageous traits (Matzek, 2012) and greater phenotypic plasticity that permits the species to survive the colonization period and to spread within a broad range of environments (Davidson et al., 2011) are main characteristics of functional traits which ensures the process of alien species becoming invasive. During invasive processes, native and alien species compete for the same resources within the ecosystem by means of the twists and turns in fortune that result from different environmental stresses (Pintó-Marijuan and Munné-Bosch, 2013). The same authors stated that the combination of reproductive success with high stress tolerance (through osmotic adjustment and antioxidants) is essential for invasion success, particularly in stressful environments in the frame of global change. Photosynthesis, photorespiration, and respiration processes are sources of reactive oxygen species (ROS) (Queval and Foyer, 2012). Although early research involving ROS metabolism focused on the potential toxicity of ROS and the different ROS-scavenging mechanisms, more recent studies have focused on the role ROS play as signaling molecules. To utilize ROS as signaling molecules, non-toxic levels must be maintained in a delicate balancing act between ROS production, involving ROS-producing enzymes and the unavoidable production of ROS during basic cellular processes, and the metabolic counter-process involving ROS-scavenging pathways (Mittler et al., 2004). The toxic effect of ROS is suppressed by a strong antioxidant system consisting of antioxidant enzymes (superoxide dismutase, catalase, peroxidases, glutathione reductase, etc.) and non-enzymatic components (proteins and peptides, phenolics, carotenoids, etc.) (Halliwell and Gutteridge, 2007). Some authors (Davis and Swanson, 2001; Lu et al., 2007) proposed that monitoring antioxidant system of weeds could provide an innovative approach to control their spread by overcoming its inherent resistence to environmental stresses. Identifying the genes that regulate some of the enzymes involved in regulating oxidative stress and finding ways to interfere with gene regulation may prove to be an economically viable way to control persistent perennial weeds in inaccessible locations or along waterways where the use of herbicides is undesirable (Davis and Swanson, 2001).

The purpose of this study was to determine possible differences in antioxidant contents and antioxidant ability among populations of ragweed species in Vojvodina province. Also, this would be the first report on comparison of antioxidant capacity of these important weeds and allergenic plant species. Information about these biochemical traits could provide useful information for models that predict establishment and spread of ragweed species in the same environmental conditions.

MATERIAL AND METHODS

Plant material

Plant material for this research represented plants from population of *Ambrosia artemisiifolia* L., *Ambrosia trifida* L. and *Iva xanthifolia* Nutt. (number of plants per species, n=40). Plants were collected in June/July (2007 and 2017), in the area of Despotovo, South Backa District in the autonomous province of Vojvodina, Serbia (coordinates: N45°26.9', E19°31'). Semiarid conditions with dry, hot spring and summer, neutral autumn and moderately cold winter characterize this location (Figure 1). Plants were harvested by hand. A part of fresh collected leaves were immediately frozen in liquid nitrogen and the others were dried in a shaded and well-ventilated place.

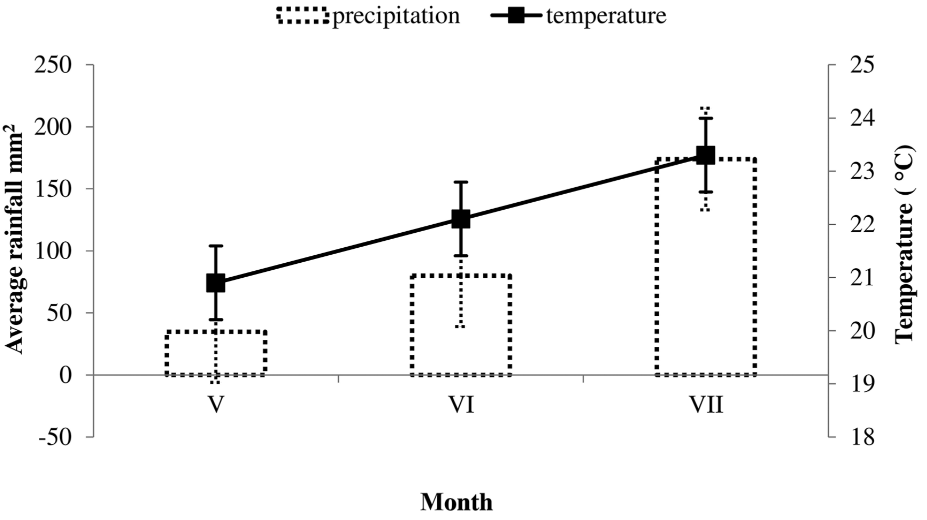

Figure 1. Average monthly air temperatures [AT (°C)] and precipitation [AP (mm)] sampling site in May, June, and July (2007–2017)

Preparation of extracts for biochemical analyses

One g of fresh leaves was ground in liquid nitrogen with cooled mortar and pestle and then homogenized with 10 ml of phosphate buffer solution (0.1M K_2HPO_4, pH 7.0). After centrifugation of 15,000 g for 10 min at 4 °C aliquots of the supernatant were used for measurements of antioxidant enzymes, reduced glutathione (GSH), superoxide anion ($O_2^{\cdot -}$) and hydroxyl radical ($^{\cdot}OH$) scavenging tests. Dried leaves were ground to a fine powder in cooled mortar and pestle. Total phenolics, tannins, proanthocyanidins and DPPH-radical scavenging activity were determined in 70% aqueous acetone extracts, and total flavonoids in MeOH : H_2O : CH_3COOH (140:50:10) extracts (1/50, w/V). The extracts were rapidly vacuum-filtered through a sintered glass funnel and kept refrigerated until assayed.

Enzymatic antioxidant system and reduced glutathione (GSH) analyses

Biochemical analyses, quantification of investigated compounds and antioxidant ability of ragweed leaves extracts were performed using spectrophotometer UV/Visible Evolution 220 (Thermo Scientific, San Jose, USA). Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured by monitoring the inhibition of nitroblue tetrazolioum (NBT) reduction at 560 nm (Mandal et al., 2008). Peroxidase (EC 1.11.1.7) activity was measured using guaiacol (guaiacol peroxidase; GPX) as substrate according to Morkunas and Gmerek (2007). SOD and GPX activities were expressed as U g^{-1} fresh weight (U g^{-1} fw). Reduced glutathione (GSH) was determined according to Sedlak & Lindsay (1968) and expressed as μmol GSH g^{-1} fw.

Determination of total contents of phenolic compounds

Total phenolic content was determined by Folin-Ciocalteu method (Makkar, 2003). Total tannin content was determined by the Folin-Ciocalteu procedure, after removal of tannins by adsorption on an insoluble matrix (polyvinylpolypyrrolidone, PVPP). Calculated values were subtracted from total phenolic contents and both contents are expressed as gallic acid equivalents (GAE) in mg g^{-1} dry weight (dw) of leaves. Determination of total flavonoids content was performed according to Pękal and Pyrzynska (2014) with slight modifications. The amount of flavonoids was calculated as a rutin equivalent from the calibration curve of rutin standard solutions, and expressed as mg rutin g^{-1} dry weight (dw) of leaves. Proanthocyanidins were determined by a butanol-HCl assay (Makkar, 2003). Proanthocyanidins contents were expressed as mg leucoanthocyanidin g^{-1} dry weight (dw) of leaves.

Antioxidant activity tests

Superoxide anion ($O_2^{\cdot -}$) and hydroxyl radical ($^{\cdot}OH$) scavenging tests represent assessment of total antioxidant activity (enzymatic and non-enzymatic) of fresh plant material extracts, i.e. ability of plant extracts to efficiently remove these ROS. The assay for superoxide anion ($O_2^{\cdot -}$) scavenging activity was based on a riboflavin-light-NBT system (Ahmed et al., 2013). Ascorbic acid was used as standard. Hydroxyl radical ($^{\cdot}OH$) scavenging activity of extracts was assayed by the method of Sánchez-Moreno (2002). Total potential non-enzymatic antioxidant activity of investigated acetone extracts was assessed based on their scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals (Panda, 2012). All antioxidant activity tests were given as % of neutralized radicals.

Statistical analysis

All extraction procedures were performed in five replicas and measurements of the biochemical parameters were performed in triplicates and expressed as means ± standard error. Differences in analyzed biochemical parameters among investigated ragweed species were tested by ANOVA followed by comparisons of means by the Duncan test ($P<0.05$). To discover natural groupings of ragweed species, cluster analysis was done with Unweighted pair-group average analysis using Euclidean distance. All statistical analyses were performed using STATISTICA for Windows version 13.0.

RESULTS

Activity of SOD was invariable in all three ragweed species (76.1 U g^{-1} fw, on average). However, activity of GPx was markedly higher in *I. xanthifolia* leaves (2.5– to 9.7–fold) in comparison to *Ambrosia* species (Figure 2A). Also, *A. trifida* leaves had significantly higher GPx activity than *A. artemisiifolia* (26%). *A. artemisiifolia* and *I. xanthifolia* leaves had similar GSH content (13.05 μmol g^{-1} fw), while *A. trifida* had 1.3-fold lower GSH concentrations (Figure 2B).

Total phenolic compounds ranged from 30.0 to 111.1 mg GAE g^{-1} dw. *A. trifida* had pronounced contents of all measured phenolic compounds: total phenolics, tannins, flavonoids and proanthocyanidins in comparison to other two ragweed species, which did not differ only for total flavonoids and proanthocyanidins contents (Figure 2C). As for antioxidant activities, there is no difference in superoxide anion scavenging capacity between ragweed species, however, *A. trifida* leaves extracts had markedly higher hydroxyl and DPPH radical scavenging abilities than *A. artemisiifolia* and *I. xanthifolia* (on average, 1.0 – fold and 1.6 – fold, respectively) (Figure 2D). According to antioxidant activity tests, investigated ragweed species could be presented in a scale: *A. trifida* > *I. xanthifolia* > *A. artemisiifolia*. Despite the difference in genus, it can be clearly distinguished that *A. trifida* and *I. xanthifolia* can be separated from *A. aretmisiifolia* according to scavenging capacity.

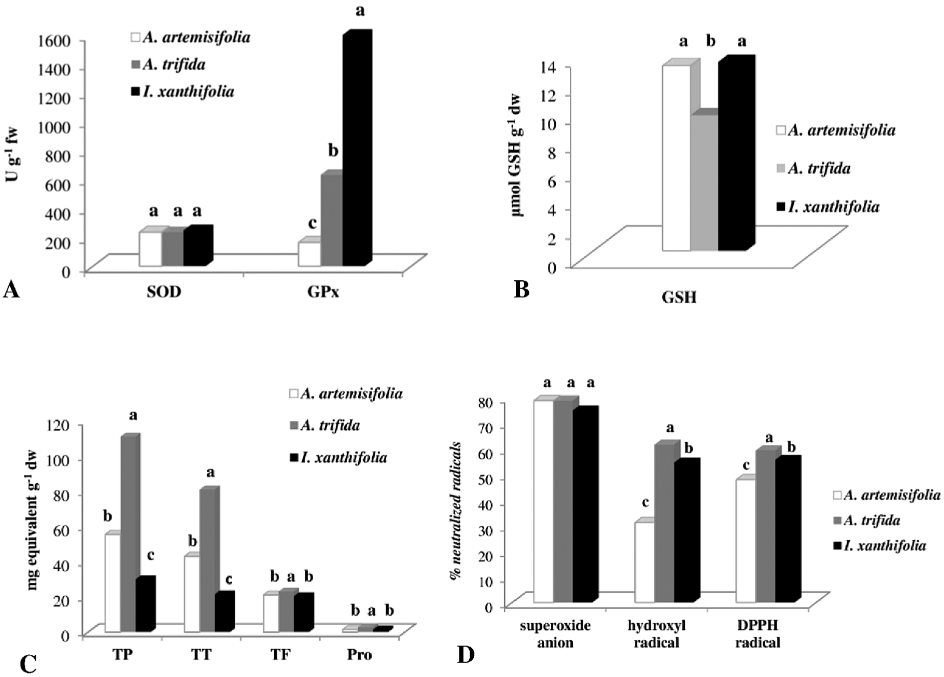

Figure 2. A – activity of antioxidant enzymes (A: SOD-superoxide dismutase, GPx – guaiacol peroxidase), B – reduced glutathione content (GSH), C – polyphenolics content (TP – total phenolics, TT – total tannin, TF – total flavonoids, Pro – proanthocyanidins) and D – antioxidant activities of ragweed leaves. Results marked with different letters differ significantly at P<0.05 (Duncan's test).

DISCUSSION

Antioxidant enzymes, total phenolics and total antioxidant capacity are some of the parameters that were employed in the research on the ecophysyological traits that are considered when it comes to invasive species (Pintó-Marijuan and Munné-Bosch, 2013). Deng et al. (2010) showed that during various environmental stresses including heat, cold, drought and flooding, activities of superoxide dismutase and guaiacol peroxidase increased in response to all stresses, while glutathione contents and anti-superoxide anion activities decreased in Common ragweed. However, Qin et al. (2013) showed that under high irradiance levels, *A. artemisiifolia* was able to scavenge oxygen radicals more efficiently by enhancing catalase and glutathione reductase activity and accumulating polyphenolics content, while superoxide dismutase activity was not greatly enhanced. Our results showed that *I. xanthifolia* had more enhanced enzymatic antioxidant system in comparison to *Ambrosia* species, which points to a different response of their antioxidant systems when exposed to the same environmental conditions.

Environmental stresses selectively induce primary, as well as secondary, metabolic activities that are directly and indirectly involved in the accumulation of phenolic compounds (Cheynier et al., 2013). Phenylalanine ammonia lyase (PAL, EC 4.3.1.5) is the entry-point enzyme into the phenylpropanoid pathway responsible for synthesis of plant phenylpropanoids or phenolics, many of which play important roles in plant defense and present important non-enzymatic antioxidants (Gerasimova et al., 2005). Polyphenols and flavonoids suppress ROS formation by inhibiting enzymes and chelating trace elements involved in free-radical production, scavenging reactive species and up-regulating or protecting antioxidant defenses (Grassmann et al., 2002). Mueller et al. (2008) proved that *A. trifida* had significantly higher total phenolics content in comparison to *A. artemisiifolia* when treated with herbicide, however, our results showed that *A. trifida* had markedly high phenolics content constitutively.

Analyzed ragweed species occur primarily in ruderal habitats; whereas *I. xanthiifolia* and *A. artemisiifolia* had also increasingly invaded arable fields (Konstantinovic et al., 2006; Follak et al., 2013; Smith et al., 2013). When comparing these three species, Pajevic et al. (2010) concluded that *A. artemisiifolia* has the highest physiological potential (photosynthetic potential) which gives it significant advantage in colonization to new localities.

Results of performed antioxidant tests showed that *A. trifida* excelled in scavenging hydroxyl- and DPPH-radicals, which can be in correlation to high phenolics content. Accumulation of phenolic compounds is in significant correlation with high antioxidant capacity (Jacobo-Velázquez and Cisneros-Zevallos, 2009). The key for wide and rapid dispersal of ragweed species is due to the diversified resistant mechanism to various environmental stresses and ability to acclimatize itself to different habitats (Deng et al, 2010). However, *A. trifida* has a relatively low fecundity, a transient seed-bank and a high percentage of non-viable or low-survivorship seeds (Harrison et al., 2007), features which may have constrained its establishment and spread. Giant ragweed has remained largely restricted to ruderal habitats, which might be one of the reasons for its slow spread in Central and Eastern Europe (Follak et al., 2013). Outside of this region, *A. trifida* occurs in several (semi-) natural habitats (Lee et al., 2010). As for *A. artemisiifolia* and *I. xanthifolia*, they have both undergone a habitat shift and expansion during their invasion, which may have contributed to their more extensive colonization of Central and Eastern Europe (Follak et al., 2013; Essl et al., 2009).

We could conclude that *A. trifida* had the highest antioxidant ability of all investigated ragweed species. Accumulation of non-enzymatic antioxidants in leaves of these plants could be the mechanism of adaptation to ruderal habitat and compensation of slow spread with higher ability to endure in unfavorable conditions. Furthermore, activation of enzymatic antioxidant system and higher content of reduced glutathione in leaves of *A. artemisiifolia* and *I. xanthifoilia*, point to different oxidative stress avoidance strategies of these ragweed species when compared to *A. trifida*. Further research on correlation among antioxidants contents and antioxidant ability of these weed species and their distribution could be of great importance to modeling experts to design accurate models for the prediction of future invasions of ragweed in similar environmental conditions.

REFERENCES

Ahmed D, Zara S, Baig H (2013): *In vitro* analysis of antioxidant activities of *Oxalis corniculata* Linn. fractions in various solvents. *Afr. J. Tradit. Complement. Altern. Med.* 10: 158–165.

Cheynier V, Comte G, Davies K, Lattanzio V, Martens S (2013): Plant phenolics: Recent advances on their biosynthesis, genetics, and ecophysiology. *Plant Physiol. Biochem*. 72: 1–20.

Davidson AM, Jennions M, Nicotra AB (2011): Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecol. Lett*. 14: 419–443.

Davis DG, Swanson HR (2001): Activity of stress-related enzymes in the perennial weed leafy spurge (*Euphorbia esula* L.). *Environ. Exp. Botany* 46: 95–108.

Deng X, Wang J, Tan JC (2010): Physiological response of invasive ragweed (*Ambrosia artemisiifolia*) to various environmental stresses. *Plant Physiol. Commun*. 46: 1013–1019.

Essl F, Dullinger S, Kleinbauer I (2009): Changes in the spatio-temporal patterns and habitat preferences of *Ambrosia artemisiifolia* during its invasion of Austria. *Preslia* 81: 119–133.

Follak S, Dullinger S, Kleinbauer I, Moser D, Essl F (2013): Invasion dynamics of three allergenic invasive *Asteraceae* (*Ambrosia trifida*, *Artemisia annua*, *Iva xanthiifolia*) in central and eastern Europe. *Preslia* 85: 41–61.

Gerasimova NG, Pridvorova SM, Ozeretskovskaya OL (2005): Role of L-phenylalanine ammonia lyase in the induced resistance and susceptibility of potato plants. *Appl. Biochem. Microbiol.* 41: 103–105.

Grassmann J, Hippeli S, Elstner EF (2002): Plant's defence and its benefits for animals and medicine: role of phenolics and terpenoids in avoiding oxygen stress. *Plant Physiol. Biochem*. 40: 471–478.

Halliwell B, Gutteridge JMC (2007): *Free radicals in biology and medicine*, New York Oxford University Press Inc.

Harrison SK, Regnier EE, Schmoll JT, Harrison JM (2007): Seed size and burial effects on giant ragweed (*Ambrosia trifida*) emergence and seed demise. *Weed Sci.* 55: 16–22.

Jacobo-Velázquez DA, Cisneros-Zevallos L (2009): Correlations of antioxidant activity against phenolic content revisited: a new approach in data analysis for food and medicinal plants. *J. Food Sci.* 74: R107–R113.

Konstantinovic B, Meseldzija M, Konstantinovic B (2006): *Ambrosia artemisiifolia* and *Iva xanthifolia* spread and distribution in Vojvodina region, In: Abstract Book, *5th International Plant Protection Symposium (5th IPPS) at Debrecen University*, Hungary, 281–287.

Lee CS, Choy C, Shin HC, Kim GS, Pi JP (2010): Control of an invasive alien species, *Ambrosia trifida* with restoration by introducing willows as a typical riparian vegetation. *J. Ecol. Field Biol.* 33: 157–164.

Lu P, Wei-Guo S, Ke-Ping M (2007): Activity of stress-related antioxidative enzymes in the invasive plant Crofton weed (*Eupatorium adenophorum*). *J. Integr. Plant Biol.* 49: 1555–1564.

Makkar HPS (2003): *Quantification of tannins in tree and shrub foliage: a laboratory manual*. Springer Kluwer Academic Publishers, The Netherlands, 49–53.

Mandal S, Mitra A, Mallick N (2008): Biochemical characterization of oxidative burst during interaction between *Solanum lycopersicum* and *Fusarium oxysporum* f. *sp. lycopersici*. *Physiol. Mol. Plant Pathol*. 72: 56–61.

Matzek V (2012): Trait values, not trait plasticity, best explain invasive species' performance in a changing environment. *PLoS ONE* 7: e48821.

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004): Reactive oxygen gene network of plants. *Trends Plant Sci*. 9: 490–498.

Morkunas I, Gmerek J (2007): The possible involvement of peroxidase in defense of yellow lupine embryo axes against *Fusarium oxysporum*. *J. Plant Physiol.* 164: 185–194.

Mueller TC, Ellis AT, Beeler JE, Sharma SD, Singh M (2008): Shikimate accumulation in nine weedy species following glyphosate application. *Weed Res*. 48: 455–460.

Pajević S, Borišev M, Orčić D, Boža P, Nikolić N (2010): Photosynthetic and biochemical characteristics of invasive species (*Ambrosia artemisiifolia* L., *Ambrosia trifida* L. and *Iva xanthifolia* Nutt.) depending on soil humidity and phenological phase. *Russ. J. Ecol*. 41: 498–505.

Panda SK (2012): Assay guided comparison for enzymatic and non-enzymatic antioxidant activities with special reference to medicinal plant, In: El-Missiry, M.A. (Ed.), *Biochemistry, Genetics and Molecular Biology 'Antioxidant Enzyme'*, InTech.

Pękal A, Pyrzynska K (2014): Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Anal. Method*. 7: 1776–1782.

Pintó-Marijuan M, Munné-Bosch S (2013): Ecophysiology of invasive plants: osmotic adjustment and antioxidants. *Trends Plant Sci*. 18: 660–666.

Qin Z, Mao D, Quan G, Zhang JE, Xie J, Ditommaso A (2013): Antioxidant response of the invasive herb *Ambrosia artemisiifolia* L. to different irradiance levels. *Phytoprotection* 93: 8–15.

Queval G, Foyer CH (2012): Redox regulation of photosynthetic gene expression. *Philos. T. Roy. Soc. B*. 367: 3475–3485.

Saint-Louis S, Ditommaso A, Watson AK (2005): A common ragweed (*Ambrosia artemisiifolia*) biotype in Southwestern Québec resistant to Linuron1. *Weed Tech*. 19: 737–743.

Sánchez-Moreno C (2002): Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Sci. Technol. Int.* 8: 121–137.

Sedlak J, Lindsay H (1968) Estimation of total protein bound and non-protein sulphydryl groups in tissue with Ellman's reagent. *Anal. Biochem*. 25: 192–205.

Smith M, Cecchi L, Skjøth CA, Karrer G, Šikoparija B (2013): Common ragweed: A threat to environmental health in Europe. *Environ. Int*. 61: 115–126.

АНТИОКСИДАНТНИ ПОТЕНЦИЈАЛ КОРОВА: *Ambrosia artemisiifolia, A. trifida* И *Iva xanthifolia*

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РЕЗИМЕ: Сврха ове студије била је анализа антиоксидантих система три инвазивне врсте: *Ambrosia artemisiifolia* L., *A. trifida* L. и *Iva xanthifolia* Nutt. Антиоксидантни капацитет може бити потенцијални маркер адаптације на променљиве услове околине, јер промена у количини антиоксиданата представља један од првих одговора на различите еколошке факторе. Међу испитиваним коровима, листови *I. xanthifolia* имали су израженију активност гвајакол пероксидазе (87,5 и 62,5%) и садржај редукованог глутатиона (2,3 и 28,8%) у односу на *A. artemisiifolia* и *A. trifida*, респективно. Међутим, активност супероксид дисмутазе била је непроменљива у свим испитиваним биљкама (234,1–247,5 U g^{-1} свеже масе). Највећи садржај укупних фенола, танина, флавоноида и проантоцианидина детектован је у листовима *A. trifida* (до 3,7 пута више од осталих). Према испитиваним антиоксидантним активностима, дате врсте могу бити представљене скалом: *A. trifida* > *I. xanthifolia* > *A. artemisiifolia*. Акумулација неензимских антиоксиданата и мањи садржај редукованог глутатиона указују на различите стратегије избегавања оксидативног стреса код *A. trifida* у поређењу са осталим испитиваним коровима у истим условима животне средине.

КЉУЧНЕ РЕЧИ: антиоксиданти, феноли, амброзија, секундарни метаболизам