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FLAME-WEEDING: IMPACT ON SOYBEAN PLANTS AND SOIL MICROORGANISMS

ABSTRACT: Flame-weeding is a very useful method for weed control, especially in organic production where the use of herbicides is prohibited. With this method heat suppresses weeds in row within a second. Apart from this, heat also affects growing crop plants and surrounding soil. The aim of this paper was to determine the effect of different propane doses, on photosynthetic and polyphenolic (total flavonoids and anthocyanins) pigments in soybean leaves, as well as the number of microorganisms in the soil. Soybean plants exposed to flame showed a different reaction to high temperature stress, which was reflected in different content of analyzed biochemical parameters, but the most responsive were anthocyanins. Actinomycetes turned out to be the most sensitive group of soil microorganisms affected by weed flaming, while fungi were the most tolerant.
KEYWORDS: pigments, microorganisms, flaming, soybean, temperature stress

INTRODUCTION

In the last decade, organic production has increased considerably. The need for successful, fast and efficient weed control methods without use of herbicides became priority. Thermal suppression of weeds with flame is one of non-chemical methods with great potential for use in practice, especially by organic farmers (Rajković et al., 2015). Compared to chemical measures, flaming has its advantages, such as: quick effect on weeds, no residues in plants and soil, no limits in the crop rotation, decrease in weeds resistance, and as opposed to mechanical measures, there is no inhibition of weed germination and no prob-

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lem with application on wet soil. However, there are some challenges that need to be overcome: slow speed (low efficiency), use of fossil fuels, CO_2 emission, fire danger (in windy conditions), influence on soil organisms and selectivity of crops (heat stress to plants).

Oxidative stress is a part of drought, salt or temperature stress. It is accompanied with diffusion limitations through stomata and mesophyll and the alterations in photosynthetic metabolism (Guidi and Calatayud, 2014). The main cellular components susceptible to damage by reactive oxygen species (ROS) that are produced during oxidative stress are lipids (peroxidation of unsaturated fatty acids in membranes), proteins and enzymes (denaturation), carbohydrates and nucleic acids. However, plants possess a wide range of antioxidant defense systems to counteract oxidative damage by ROS consisting of: (i) antioxidant enzymes, i.e. superoxide dismutase (SOD), catalase and different plant peroxidases, which function as ROS scavengers; (ii) non-enzymatic compounds, i.e. ascorbic acid, α-tocopherol, carotenoids, reduced glutathione (GSH), polyphenols, etc. (Blokhina et al., 2003).

The essential part of flaming application is determination of high temperatures effects on microbiological community of the soil. Microorganisms are one of the key factors responsible for maintaining fertility of the soil, availability of nutrients (Alexander, 1967; Diaz-Ravina et al., 1993) and preservation of the soil aggregates (Brussaard and Kooistra, 1993). Soil microorganisms react strongly to any sudden change in their environment, including the temperature. Extremely high temperature (125 °C) in the surface layer of the soil up to the depth of 2 cm causes sterilization of the soil, transformation of organic matter into ash, evaporation of water and degradation of the soil structure (Attivill and Leeper, 1987). Hence, it is necessary to conduct further research on the effects of high temperatures on soil microorganisms, as well as the possibility of revitalization of the soil afterwards.

Since open flame used in flame weeding gets in contact with the cultivated plants, beside weeds, this may cause damage in plants, i.e. oxidative stress and affect soil microorganisms. Thus, the aim of this study was to examine the influence of flaming on oxidative stress level in young soybean plants by monitoring the change in content of photosynthetic (chlorophylls and carotenoids) and polyphenolic (flavonoids and anthocyanins) pigments in soybean leaves, as well as the number of microorganisms in the soil after flaming.

MATERIALS AND METHODS

Weeds suppression using open flame

Soybean plants (cv. Sava) were grown on experimental fields at the Institute of Field and Vegetable Crops on the location Rimski Šančevi, near Novi Sad (45°16’N and 19°51’E at an altitude of about 80 m). The trial was set in complete randomized block design in four replications. Seeds were sown in 10x3 m plots, with four rows, 75 cm distance between rows and at 4 cm depth.

The dominant soil type was calcareous chernozem with the following basic physical and chemical properties: coarse sand 0.80%; fine sand 39.95%; silt 35.76%; clay 27.49%; density 2.57 g/cm^3; pH in H_2O 6.96; content of humus 2.59%; total nitrogen 0.192% N; light-chain phosphorus 8.4 mg P_2O_5/100 g soil; light-chain potassium 27.3 mg K_2O/100 g soil. Flame-weeding machine (Rajković et al., 2011) is modified four row cultivator with two burners placed on both side of each row to suppress weeds in the row of the crop, while between rows, weeds are suppressed mechanically.

Young soybean plants were treated with flame in three growth stages: the first trifoliate or V1, the three trifoliate or V3 and the six trifoliate stage or V6. The treatment comprised of the control dose (without flaming) and eight different propane doses (20, 30, 40, 50, 60, 70, 80 and 100 kg/ha propane), in four replications. Measured temperatures ranged from 33 °C (18 cm distance from the nozzle; 20 kg/ha propane dose) up to 234 °C (4 cm distance and 100 kg/ha propane dose) (Rajković et al., 2020). Higher propane doses induced higher temperatures on all altitudes. Plants were sampled together with their roots (five hours after treatment) to avoid further stress and then transported to laboratory in a portable refrigerator. Soil samples for microbiological analysis were taken between the rows of soybean 1h and 24h after flaming, at the depth of 2 cm.

Plant pigments determination

Photosynthetic pigments, chlorophylls *a* and *b* (Chl*a* and Chl*b*) and carotenoids were determined by Sairam et al. (2003/2004) and the results were expressed in mg/g of fresh weight. The amount of flavonoids was determined by Marckam (1989) and expressed as mg rutin/g dry weight, while anthocyanins were determined using the pH differential method (Shen et al., 2007) and expressed as mg of cyanidin-3-glucoside/g of dried plant material.

Microbiological analysis of soil

Total number of bacteria on soil agar (10^{-6}), number of actinomycetes in synthetic agar (10^{-4}) and the number of fungi in Czapek's agar (10^{-4}) were determined with the method of forming colonies on selective nutritious substrates with three replications (Jarak and Đurić, 2006). The number of the examined microorganisms was recalculated per gram of absolutely dry soil.

Statistical analysis

Values of the biochemical parameters were expressed as means ± standard error of determinations made in triplicates and tested by ANOVA, followed by comparison of the means by Duncan's multiple range test ($P<0.05$). Data were analyzed using Statistica for Windows version 11. Data from microbiological analysis were logarithmized and processed statistically by using the software Statistica 11 and performing Tukey's test.

RESULTS AND DISSCUSION

In our study, different propane doses used in V1 stage did not show statistically significant differences in Chl*b* and carotenoid content compared to control. Yet, significant accumulation of Chl*a* was observed after the application of 80 kg/ha propane dose (Figure 1), probably due to desiccation of the plant tissue caused by high temperature. In V3 stage, similar was noticed with the Chl*b* and carotenoid contents, i.e. different propane doses did not influence the change in pigments content compared to control. Only doses of 50 (for both Chl*a* and Chl*b*) and 60 kg/ha (for Chl*b*) of propane were stimulative to their content (Figure 2). Similar trend in photosynthetic pigments accumulation was also visible in the next monitored stage of development (V6). Only dose of 60 kg/ha of propane induced accumulation of Chl*b*. Apparent accumulation of pigments in mentioned treatments could be due to desiccation of plant tissue. Higher doses of propane induced plant deterioration and decreased pigment contents (Figure 3).

The most important function of leaves is to produce assimilate through photosynthesis. The leaf chlorophyll content is one of the key factors in determining the rate of photosynthesis and dry matter production (Ghosh et al., 2004). The exposure of weeds to extremely high temperatures obtained by propane burning leads to the rupture of cell membranes structure, protein denaturation, protoplast leakage, dehydration and ultimately, the cell death and plant wilting. Some plant species differ greatly in tolerance to flaming where monocotyledon plants show higher tolerance towards heat of the flame (Ascard, 1998). Furthermore, species with protected apical meristem (at the tip of the stem) are more tolerant compared to species with no protection of meristem, erect habitus and thin leaves (Cisneros et al., 2008). Reaction of plants to open flame depends on burner position, stage of the plant development, leaf moisture, etc. (Knezevic et al., 2012). Different plant organs show different susceptibility to high temperatures which is in close correlation with the presence of protective layers such as hairs, waxes, suberized tissues or water content. All above mentioned indicates the possibility of crop and weed regeneration after flaming. Thus, it is very important to treat weeds with flaming in the most sensitive stages of their development. Weeds are more tolerant in later phenophases, and the most sensitive in the phase of cotyledons. According to Tercé-Laforgue et al. (2004), chlorophyll content in different leaf stages of tobacco was one of the main senescence biomarkers and regardless of the mode of N nutrition, a progressive decrease in chlorophyll was noticed from younger to older leaves. Ascard (1995) found the sigmoidal dose-response and speed-response curves imply that propane dose and the ground speed can be adjusted to the required control effect, the weed flora and the developmental stage of the plants. A 95% reduction in susceptible annual weed species, in phase 0–4 true leaves, was achieved at propane doses of 10–20 kg/ha, and the weeds were completely destroyed at 20–50 kg/ha. Considerably higher doses were needed at later developmental stages and for more tolerant species. According to Stepanović (2013), both corn and soybean were able to tolerate up to two flaming applications with propane dose of 45 kg/ha without any yield reduction. He suggested that for the best results soybean should be flamed at VC and V4-V5. Combination of both flaming and interrow cultivation applied twice during the season is the most effective weed control treatment in soybean (Stepanović, 2013).

As for total flavonoids content, there were no significant differences between treatments and their controls (Figure 4). Only high doses of propane (60–80 kg/ha) in the V1 and V6 phases caused a slight decrease in flavonoids content, probably due to extended cell destruction. High temperatures may cause inactivation and/or denaturation of the cell proteins (enzymes) which might contribute to decrease in flavonoid biosynthesis.

Many elicitors, both biotic and abiotic, enhance anthocyanins biosynthesis and accumulation: different carbon and nitrogen sources, osmotic pressure, plant hormones, bacteria and yeast, UV light, salicylic acid etc. (Deroles, 2008). In this experiment, all propane doses significantly affected anthocyanins accumulation, except for the lowest propane dose (20 kg/ha) (Figure 5). Intensive accumulation of these phenolic pigments with protective role especially occurred with the propane doses of 30, 40 and 50 kg/ha and they were significantly higher compared to the control. That effect was observed in all three stages of the development. Application of very high propane doses (70 and 80 kg/ha) in V1 and V6 stage resulted in thermal destruction of the cells and the content of anthocyanins was very low. Contrary to this, there was no thermal destruction in V3 stage and all seven treatments with propane showed higher or similar anthocyanins content when compared to the control (Figure 5). Obtained data showed that soybean polyphenolic pigments exhibited different metabolic reaction towards heat induced stress, with anthocyanins as the most responsive pigments to applied abiotic stress.

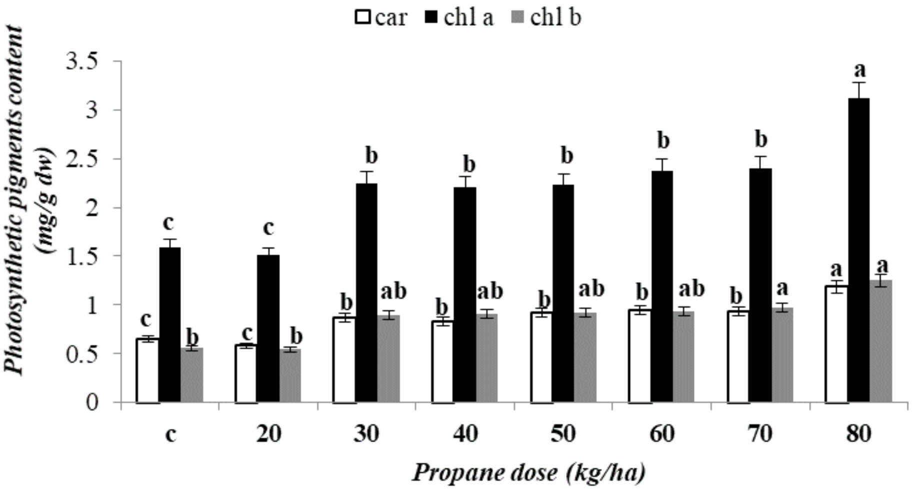

Figure 1. Changes in the photosynthetic pigments content in dependence to propane dose in the stage V1

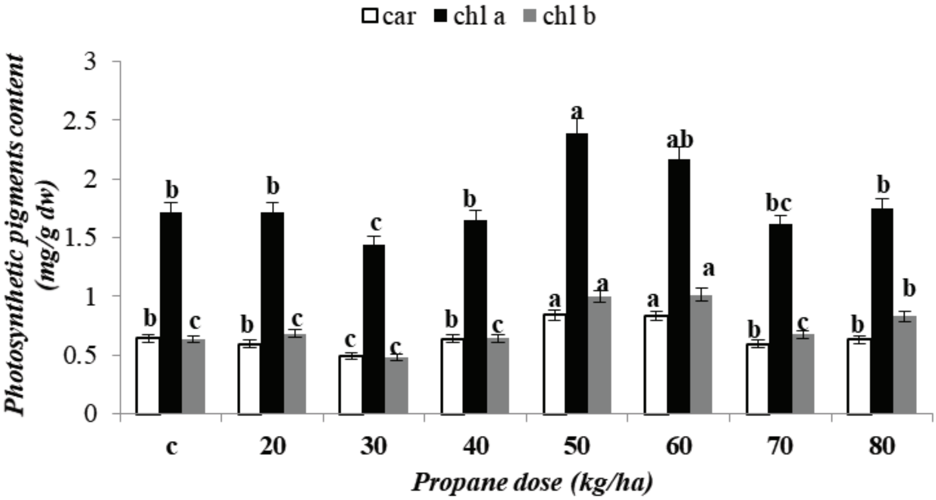

Figure 2. Changes in the photosynthetic pigments content in dependence to propane dose in the stage V3

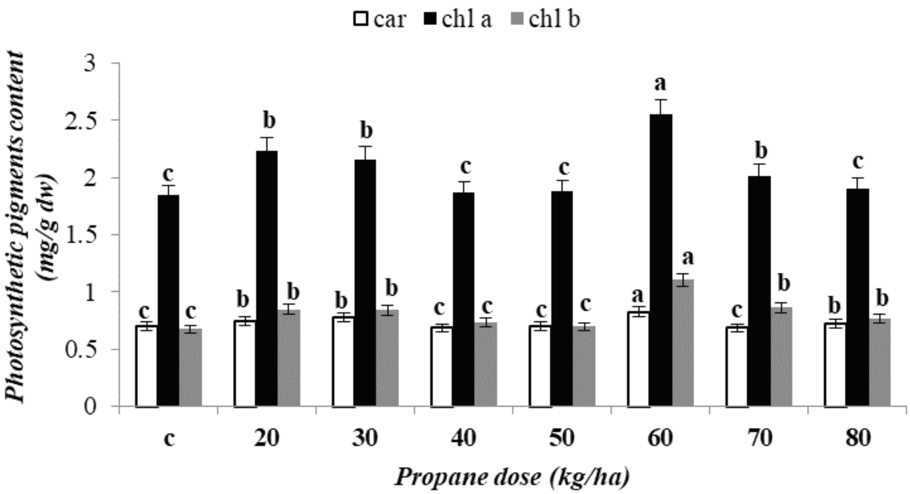

Figure 3. Changes in the photosynthetic pigments content in dependence to propane dose in the stage V6

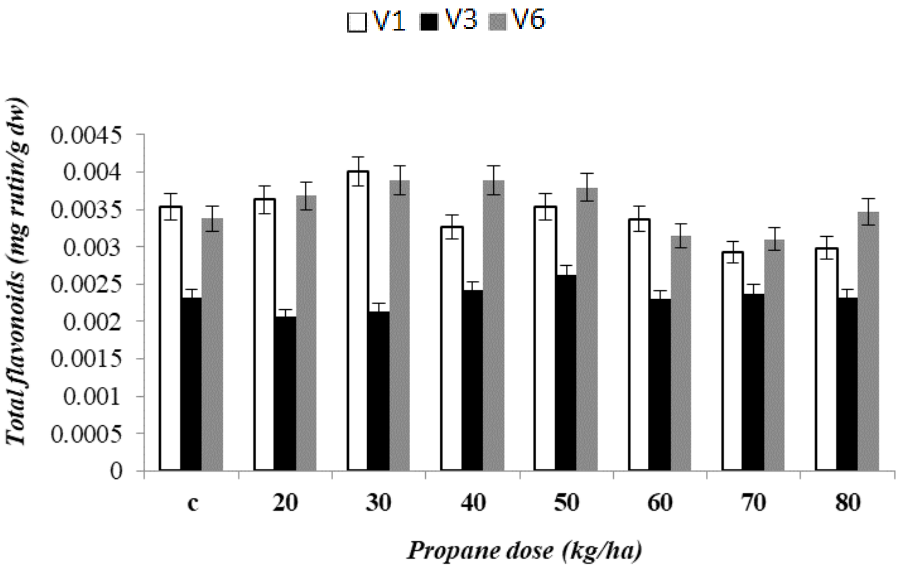

Figure 4. Changes in the total flavonoids content in dependence to propane dose in the stage V1, V3 and V6

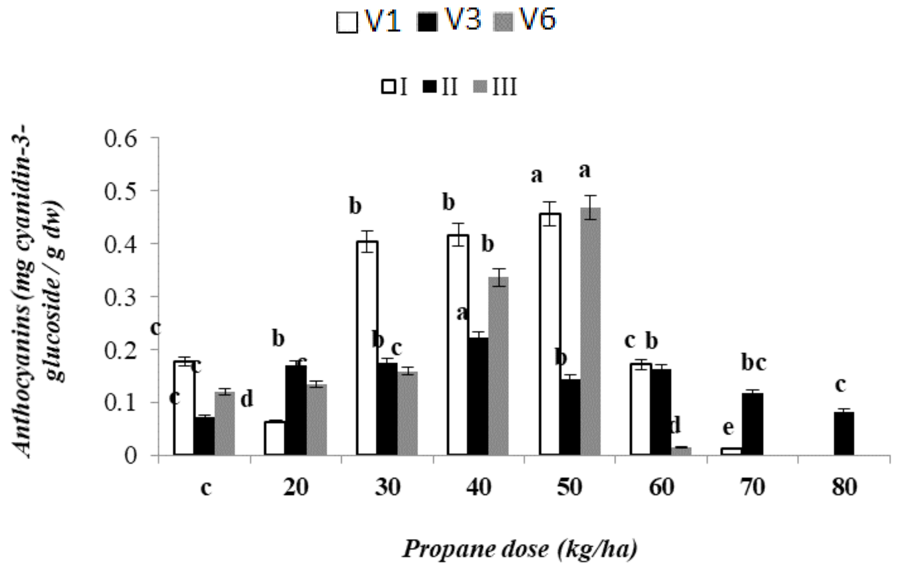

Figure 5. Changes in the anthocyanins content in dependence to propane dose in the stage V1, V3 and V6

The use of different amounts of propane in the soybean crop flaming had affected the total number of bacteria (24h after flaming) and actinomycetes in the soil (24h after flaming), whereas the amount of fungi seemed almost unaffected (Table 1). Bearing in mind the importance of all microbial groups in soil fertility, further research on the effects of high temperatures on soil microorganisms have to include integrative approach with potential soil revitalization methods.

Table 1. The effect of flaming on the total number of bacteria, total number of actinomycetes and total number of fungi (logarithmized number per gram of absolutely dry soil) in the soybean crop 1h and 24h after flame application. a,b,c – different letters indicate significant difference according to Tukey's test of homogeneity of variants.

Variants	Bacteria		Actinomycetes		Fungi	
	1 h	24h	1h	24h	1h	24h
1	6.975 **c**	6.614 **b**	5.607 **a,c**	4.546 **a**	4.915 **a**	4.138 **a**
2	7.256 **b**	7.045 **a,b**	5.333 **b,c**	4.921 **a**	4.818 **a**	4.775 **a**
3	7.203 **b,c**	7.280 **a,b**	5.531 **a,c**	4.746 **a**	5.103 **a**	4.672 **a**
4	7.846 **a**	7.561**a**	5.366 **b,c**	4.694 **a**	4.943 **a**	4.895 **a**
5	7.132 **b,c**	7.228 **a,b**	5.168 **b**	5.062 **a**	5.046 **a**	4.236 **a**
6	7.738 **a**	7.259 **a,b**	5.866 **a**	4.728 **a**	5.184 **a**	4.902 **a**
7	7.666 **a**	6.811 **b**	5.528 **c**	5.110 **a**	5.107 **a**	4.728 **a**
8	7.143 **b,c**	7.149 **a,b**	5.287 **b,c**	4.872 **a**	4.709 **a**	4.872 **a**

CONCLUSIONS

Soybean plants exposed to oxidative stress induced with high temperatures responded by accumulation of anthocyanins almost proportionally with the increase of propane concentrations. Thus, this biochemical parameter could be recommended as stress marker in the field practice due to positive effectiveness/damaging ratio. The use of weed flaming as agrotechnical method in the soybean crop had negative effect on the number of soil actinomycetes, especially 24h after the flaming treatment.

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УТИЦАЈ ПРИМЕНЕ ПЛАМЕНА НА УСЕВ СОЈЕ И ЗЕМЉИШНЕ МИКРООРГАНИЗМЕ

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РЕЗИМЕ: Сузбијање корова пламеном је корисна метода за њихову контролу, нарочито у органској производњи где је забрањена употреба синтетичких хербицида. Применом ове методе се, у делу секунде, пламеном сузбијају корови у реду усева. Контакт с пламеном утиче не само на гајену биљку, већ и на земљиште. Циљ овог рада био је утврђивање утицаја примене различитих доза пламена на биљке и земљишне микроорганизме у усеву соје. Мерен је садржај фотосинтетичких и полифенолних (укупних флавоноида и антоцијанина) пигмената у биљкама соје, као и број микроорганизама у земљишту. При сузбијању корова пламеном, биљке соје су показале различиту реакцију на температурни стрес, што се одразило на различит садржај анализираних биохемијских параметара, али највећа промена запажена је у садржају антоцијанина. Најосетљивија група микроорганизама на примену пламена у сузбијању корова су биле актиномицете, док су гљиве биле најтолерантније.

КЉУЧНЕ РЕЧИ: пигменти, микроорганизми, пламен, соја, температурни стрес