

## RESIDUES OF FORAGE SPECIES AFFECT PHOTOSYNTHETIC CHARACTERISTICS OF THE PEQUIZEIRO<sup>1</sup>

ALANA CRISTINA FERREIRA ARAÚJO<sup>2</sup>, JOÃO CARLOS MADALÃO<sup>3\*</sup>, ADRIANO JAKELAITIS<sup>2</sup>, ALAN CARLOS COSTA<sup>2</sup>, GABRIEL MARTINS ALMEIDA<sup>2</sup>

**ABSTRACT** - Some forages release allelopathic substances into the environment, and may prevent consortium with arboreal species in pastures. The objective of this work was to evaluate photosynthetic characteristics of pequi plants (hereafter pequi) influenced by concentrations of residues of the forage species *Urochloa decumbens*, *Melinis minutiflora* and *Paspalum notatum*. The treatments consisted of pequi cultivation under aerial residues of the three forage species mixed to the substrate in four concentrations (1, 2, 3 and 4% mass/mass), plus an additional treatment (comparative control) with pequi cultivated on the substrate without waste. The following parameters were evaluated: photosynthetic rate, stomatal conductance, transpiration rate, relationship between internal and external CO<sub>2</sub> concentration (*C<sub>i</sub>/C<sub>a</sub>*), maximum quantum yield, effective quantum yield of FS II, electron transport rate, nonchemical quenching and chlorophyll index, concerning the content of chlorophyll *a*, *b* and total in pequi plants at 50 and 100 days after transplanting (DAT), and the relative production of dry matter at 100 DAT. At 50 DAT, the following photosynthetic variables were affected in pequi plants: *A*, *g<sub>s</sub>*, chlorophyll *a*, chlorophyll *b* and total chlorophyll when cultivated in the presence of forage residues. At 100 DAT, the following photosynthetic variables were affected in pequi plants: *A*, Fv/Fm, ETR, NPQ, chlorophyll *a*, chlorophyll *b* and total chlorophyll, when cultivated in the presence of forage residues. Pequi plants had reduced relative dry matter yield when grown in the presence of *U. decumbens*. This variable was also affected when pequi was grown in increasing concentrations of residues of the species *U. decumbens*, *M. minutiflora* and *P. notatum*.

**Keywords:** Allelopathy. *Melinis minutiflora*. *Paspalum notatum*. Photosynthetic rate. *Urochloa decumbens*.

## RESÍDUOS DE ESPÉCIES FORRAGEIRAS AFETAM CARACTERÍSTICAS FOTOSSINTÉTICAS DO PEQUIZEIRO

**RESUMO** – Algumas forrageiras liberam no ambiente substâncias alelopáticas, podendo inviabilizar o consórcio com espécies arbóreas em pastagens. O objetivo deste trabalho foi avaliar características fotossintéticas do pequi influenciadas por concentrações de resíduos das espécies forrageiras *Urochloa decumbens*, *Melinis minutiflora* e *Paspalum notatum*. Os tratamentos consistiram do cultivo de pequi sob resíduos da parte aérea das três espécies forrageiras misturadas ao substrato em quatro concentrações (1, 2, 3 e 4% massa/massa), mais um tratamento adicional (testemunha comparativa) com pequi cultivado no substrato sem resíduos. Foi avaliada a taxa fotossintética, condutância estomática, taxa transpiratória, relação entre a concentração interna e externa de CO<sub>2</sub> (*C<sub>i</sub>/C<sub>a</sub>*), rendimento quântico máximo, rendimento quântico efetivo do FS II, taxa de transporte de elétrons, quenching não fotoquímico e índice clorofílog referente ao conteúdo das clorofilas *a*, *b* e total em plantas de pequi aos 50 e 100 dias após o transplante (DAT) e a produção relativa referente à matéria seca aos 100 DAT. Aos 50 DAT, as plantas de pequi foram afetadas quanto as variáveis fotossintéticas *A*, *g<sub>s</sub>*, clorofila *a*, clorofila *b* e clorofila total, quando cultivadas na presença de resíduos das forrageiras. Aos 100 DAT, as plantas de pequi foram afetadas quanto as variáveis fotossintéticas *A*, Fv/Fm, ETR, NPQ, clorofila *a*, clorofila *b* e clorofila total, quando cultivadas na presença de resíduos das forrageiras. O pequi tem produção de matéria seca relativa reduzida quando cultivado na presença de resíduos da espécie *U. decumbens*. Essa variável também é afetada quando o pequi é cultivado em concentrações crescentes de resíduos das espécies *U. decumbens*, *M. minutiflora* e *P. notatum*.

**Palavras-chave:** Alelopatia. *Melinis minutiflora*. *Paspalum notatum*. Taxa fotossintética. *Urochloa decumbens*.

\*Corresponding author

<sup>1</sup>Received for publication in 05/10/2016; accepted in 05/11/2017.

Paper extracted from the scientific initiation work of the first author.

<sup>2</sup>Department of Phytotechnology, Instituto Federal Goiano, Rio Verde, GO, Brazil; alanashego@hotmail.com, adriano.jakelaitis@ifgoiano.edu.br, alcarcos@gmail.com, gabrielrvmartins@gmail.com.

<sup>3</sup>Biological and Agricultural Science Department, Universidade Federal do Espírito Santo, São Mateus, ES, Brazil; joacmad@gmail.com.

## INTRODUCTION

Allelopathy can be defined as any direct or indirect, beneficial or detrimental effect of a plant or microorganism on another plant or microorganism by the release of secondary compounds into the environment (RICE, 1984). Allelopathy differs from competition, which involves the reduction or withdrawal of an environmental factor, necessary for other plants in the ecosystem, such as water, light and nutrients (RICE, 1979).

Chemicals involved in allelopathic interactions are secondary compounds and are present in all parts of the plant, such as leaves, roots, stems, flowers, fruit and pollen grains (RICE, 1984). These products are released in the medium by leaf leaching, residue decomposition, root exudation and volatilization (RICE, 1984). In addition, the allelochemical substances, when liberated into the soil, can interfere with superior plants, affecting germination, and provoking injuries during the growth of roots and meristems, thus damaging the development of the plant (GOMES et al., 2014).

The forage species *Urochloa decumbens* (brachiaria-grass), *Melinis minutiflora* (fat-grass) and *Paspalum notatum* (grass-batatais) are among the plants of interest to the national cattle ranch (VALLE; JANK; RESENDE, 2009). However, these species may release allelopathic substances causing inhibitory effects in other species, such as corn, rice, wheat, soybean, beans, cotton, lettuce, and carrot (SOUZA et al., 1999; SOUZA et al., 2006; BARBOSA; PIVELLO; MEIRELLES, 2008). These may also interfere in the physiological processes and consequently in the growth of the pequi tree when living with these forages and arboreal species.

There are no reports in the literature of the allelopathic effect on species caused by *P. notatum* and *M. minutiflora*. However, Rodrigues et al. (2012) reported a negative allelopathic effect of *U. decumbens* on *Stylosanthes guianensis* and *S. capitata* seeds. Barbosa, Pivello and Meirelles (2008) verified an inhibitory effect of *U. decumbens* on the germination of the species *Phalaris canariensis*, *Lactuca sativa* and *M. minutiflora*.

Among the species of extractivism of great importance for Brazil, the pequi (hereafter pequi) is a tree species, native to the Brazilian Cerrado, which is considered of significant economic, social and environmental importance for the regions where this species is found (SILVA

NETO; COSTA 2010). The high nutritional value of fruit pulp and the large number of applications of its by-products put pequi among the most important species among the native species of Cerrado (SANTOS et al., 2010). In addition to the importance of human feeding, the pequi is used in the reconstitution of native vegetation, favouring biodiversity by the presence of endangered animals in their natural habitat (ALVES JR. et al., 2015).

Photosynthetic activity is an essential process in the assimilation of products by plants. The interference caused by allelopathic effects between plants may impair photosynthetic activity, interfering in processes such as photosynthetic rate ( $A$ ), stomatal conductance ( $g_s$ ), transpiratory rate ( $E$ ), relationships between internal and external CO<sub>2</sub> concentration ( $C_i/C_a$ ), quantum yield ( $F_v/F_m$ ), effective quantum yield of FS II ( $\Delta F/F_m'$ ), electron transport rate (ETR), non-photochemical quenching (NPQ) and chlorophyll content index (QIAN et al., 2009; ZHANG et al., 2016). When these characteristics are affected, there may be less accumulation of dry matter, resulting in smaller plants with the ability to compete for essential developmental resources.

There are reports on the modes of action of allelochemicals in suppressing the growth and development of host plants. For example, phytotoxicity could be due to the decrease of photosynthesis and respiratory capacity by the constituents of monoterpenes and polyphenols (SINGH et al., 2005).

Given the above, the objective of this study was to evaluate the photosynthetic characteristics of the pequi influenced by concentrations of residues of the forage species *U. decumbens*, *M. minutiflora* and *P. notatum*.

## MATERIAL AND METHODS

The research was conducted in a heated greenhouse from March to July 2014. The forage species *Urochloa decumbens* (brachiaria-grass), *Melinis minutiflora* (fat-grass) and *Paspalum notatum* (grass-batatais) were planted in field plots, and shoots were collected 75 days after sowing. The aerial parts of these were dried in a forced air ventilation oven at 45°C until it reached a constant mass, and then they were ground in a mill with a 1-mm sieve and stored. Table 1 shows the results of the chemical analysis of the leaf tissue of these species.

**Table 1.** Chemical characteristics of leaf tissue of *Urochloa decumbens* (URODE), *Melinis minutiflora* (MELMI) and *Paspalum notatum* (PASNO).

Nutrients	Forage species		
	URODE	MELMI	PASNO
N (g kg <sup>-1</sup> )	5.60	5.60	14.00
P (g kg <sup>-1</sup> )	2.50	2.70	2.70
K (g kg <sup>-1</sup> )	20.00	12.00	13.50
Ca (g kg <sup>-1</sup> )	2.60	2.70	2.90
Mg (g kg <sup>-1</sup> )	2.50	1.70	2.00
S (g kg <sup>-1</sup> )	0.80	1.00	1.30
B (mg kg <sup>-1</sup> )	27.34	35.08	45.41
Cu (mg kg <sup>-1</sup> )	1.78	2.77	5.99
Fe (mg kg <sup>-1</sup> )	460.01	759.35	1461.48
Mn (mg kg <sup>-1</sup> )	55.92	92.93	40.23
Zn (mg kg <sup>-1</sup> )	19.87	25.19	14.66

The soil used in the test came from the arable layer of a dystroferic red latosol, with the following physicochemical attributes: pH in CaCl<sub>2</sub> of 5.6; 22.84 mg dm<sup>-3</sup> of P; 190 mg dm<sup>-3</sup> of K; 5.98 cmol<sub>c</sub> dm<sup>-3</sup> Ca; 1.80 cmol<sub>c</sub> dm<sup>-3</sup> Mg; 2.80 cmol<sub>c</sub> dm<sup>-3</sup> H + Al; 2.45 dag kg<sup>-1</sup> organic matter and 74.7% base saturation. After being collected, the soil was dried in the shade, sifted and fertilized. Then the dry matter of the forage species was placed in a rotary mixer for each pot, in the proportion of 50 g of single superphosphate and 10 g of potassium chloride for each 8.0 kg of soil, which corresponds to the size of the vessel used.

The pequi seedlings were obtained from a nursery from the city of Guapó, GO, and contained, at the time of transplantation, a pair of leaves. The treatments consisted of residues of the aerial part of the three forage species (*Urochloa decumbens*, *Melinis minutiflora* and *Paspalum notatum*) mixed with the substrate (soil + fertilizer) in four concentrations (1, 2, 3 and 4% w/w), constituting a 3 x 4 + 1 factorial design. In this case, a seedling of pequi (comparative control) was cultivated as an additional treatment, and transplanted to the soil + fertilizer mixture. The experimental design was a randomized block design, with four replicates, in which each experimental unit was composed of a vessel containing 8.0 kg of substrate.

After transplanting the seedlings, every 30 days, 1 g pot<sup>-1</sup> of fertilizer was used, containing the following nutrients (percentage): N: 13; P<sub>2</sub>O<sub>5</sub>: 5; K<sub>2</sub>O: 13; B: 0.04; C: 1; Cu: 0.05; S: 5; Fe: 0.2; Mg: 1; Mn: 0.08; Mo: 0.005; and Zn: 0.15. The vessels were irrigated whenever necessary. The temperature inside the greenhouse throughout the experimental period varied between 22 and 29°C and the relative humidity between 60 and 75%.

The gaseous exchanges of pequi plants were recorded for photosynthetic ( $A$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and transpiratory ( $E$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ) rates of stomatal conductance ( $g_s$ ,  $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) and the relationship between internal and external CO<sub>2</sub> concentration ( $C_i/C_a$ ). These evaluations were performed using an automated photosynthetic analyser, model LI-6400XTR (Licor®, Nebraska,

USA), with a block temperature of 24°C and photon flux density of 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The evaluations were carried out at 50 and 100 days after transplanting (DAT) of the seedlings between 08:30 and 10:30 in the morning. The evaluations were conducted on the penultimate pair of fully expanded leaves, without replicates, and in each leaf, the same area was always used. At 50 and 100 DAT, pequi plants had on average 5.2 and 6.7 fully expanded leaves at 10.8 and 13.4 cm high, respectively.

The fluorescence of chlorophyll *a* was also evaluated in the penultimate pair of fully expanded leaves, without replicates, in parallel with the gas exchange measurements, using a portable fluorometer MINI-PAM model (Walz, Effeltrich, Germany), with model 2030-B (RASCHER; LIEBIG; LÜTTGE, 2000). Initially, the leaves were adapted to the dark for 30 minutes, the foliar tissues were exposed to red modulated light ( $0.03 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and the initial fluorescence ( $F_0$ ) was determined. Subsequently, a pulse of 0.8 s of saturating actinic light ( $> 6000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) was applied, and the maximum fluorescence ( $F_m$ ) was determined. With these results, the potential quantum yield of photosystem II was estimated by superimposing a saturation pulse on leaves previously adapted to ambient light, according to Genty, Briantais and Baker (1989), using the equation:

$$F_v/F_m = (F_m - F_0)/F_m$$

Where  $F$  is the maximum fluorescence yield during the saturation pulse. The  $\Delta F/F_m'$  was used to estimate the apparent rate of electron transport (ETR), using the equation, where DFF is the photon flux density ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) incident on the sheet; 0.5 is the value corresponding to the fraction of excitation energy distributed to FSII; and 0.84 is the value corresponding to the fraction of incident light absorbed by the leaves.

The non-photochemical extinction coefficient of Stern-Volmer was calculated as  $\text{NPQ} = (F_m - F_m')/F_m$ , (BILGER; BJÖRKMAN, 1990). In the same way as described for the gas exchanges, the evaluations with the portable

fluorometer were performed between 08:30 and 10:30, always on the same area of each leaf at 50 and 100 DAT.

Chlorophyll content was evaluated using a portable meter, ClorofiLOG1030® (Falker®, Porto Alegre, Brazil), with chlorophyll *a*, chlorophyll *b* and total chlorophyll content, expressed in the chlorophyll index. At 100 DAT, the aerial part of the pequi plants and a certain relative production of the shoot dry matter (RPDM) were collected.

The results were submitted to analysis of variance (ANOVA) by the F test, and when significant, the means related to the residues were compared by the Tukey test and the comparative control by the Dunnett test, adopting a level of significance of 5%. Regarding the effects related to the concentrations of the residues, regression analysis was adopted and the models were chosen for their simplicity, biological meaning and coefficient of determination. Statistical analyses were performed using Assistat statistical software (version 7.7 beta 2014), and visualized using Sigmaplot v.12 software (SPSS Inc., USA).

## RESULTS AND DISCUSSION

For all evaluated variables, in the two seasons, no effects were observed for the interaction forage species x concentration, only isolated effects were observed. At 50 days after transplanting (DAT), pequi plants grown in soil with residues of the species *Urochloa decumbens* and *Melinis minutiflora* had a lower photosynthetic rate (*A*) and stomatal conductance (*gs*) with lower values than the control, but the respiratory rate (*E*) and the *Ci/Ca* ratio and were not affected by the residues of these two species (Table 2). The pequi plants cultivated in soils with *Paspalum notatum* residues did not differ from the control for these variables.

At 100 DAT the pequi plants grown on soils with *U. decumbens*, *M. minutiflora* and *P. notatum* were negatively affected for *A*, but not for *gs* or *E* affected. The *Ci/Ca* ratio in pequi plants grown under *M. minutiflora* residues was higher than the control and treatments with *U. decumbens* and *P. notatum* residues.

Changes in pequi plants in the presence of grass residues resulted in changes in stomatal development, which may have resulted in changes in quantity, size and opening and closing. Photosynthesis is dependent on the continuous flow of CO<sub>2</sub>, and the stomatal opening and closing is the main factor regulating its assimilation, since *gs* regulates the gas flow in the stomata, and the reduction of the stomatal opening causes a higher concentration of carbon in the intercellular spaces, (ZOBIOLE et al., 2010). In the present study, it was

observed that the efficiency of the Calvin cycle (BERTOLLI; SOUZA; SOUZA, 2015) and, consequently, *A* resulted in reduced carboxyl efficiency. Gulzar and Siddiqui (2015) studied the allelopathic effects of *Eclipta alba* on plants of *Arachis hypogaea* and *Vigna radiata*, and verified significant reductions in physiological parameters such as chlorophyll content, *A*, intercellular concentration of CO<sub>2</sub> (*Ci*), *gs* and *E*.

At 50 DAT, there was no interference of the allelopathic species in the Fv/Fm, ΔF/Fm', ETR and NPQ of the pequi plants grown with residues incorporated in the soil compared to the control, but at 100 DAT, the Fv/Fm and ETR were affected. In this evaluation, the plants cultivated in the presence of residues of *M. minutiflora* had an increase in NPQ values compared to the control.

In some cases, the ETR curve may be correlated to the crude photosynthetic rate (GA), presenting the same pattern of the CO<sub>2</sub> assimilation curve. Considering the absolute values, it can be seen that at 50 DAT, both *A* and ETR have values below the control, and at 100 DAT, this difference increases, resulting in statistical difference.

It can be seen that at 50 DAT, the *gs* in pequi plants was affected by residues of *U. decumbens* and *M. minutiflora*, but this did not occur at 100 DAT. This is possibly because plants at more advanced stages can better regulate gas flow in the stomata, reducing the deleterious effects of allelopathy for this variable.

Low values of Fv/Fm (0.51, 0.68 and 0.59 in plants grown on *U. decumbens*, *M. minutiflora* and *P. notatum*, respectively) were observed at 100 DAT. The optimal Fv/Fm values were between 0.75 and 0.85 (MAXWELL; JOHNSON, 2000). This is why the light absorbed in FSII is used to reduce plastoquinone A (KRAUSE; WEIS, 1991). Values below this range are indicative of photoinhibition or photooxidation, causing damage to the photosynthetic apparatus. These results indicate that while these values of Fv/Fm are normal at 50 DAT, over time there was damage to FSII caused by allelopathic substances.

The use of energy by pequi plants to perform photochemical processes (CO<sub>2</sub> fixation and reduction of NADPH) is directly related to the ETR, and the lower values observed for this variable in relation to the control at 100 DAT indicates a lower efficiency in the use of energy from light. Inefficient use of light energy can lead to the formation of reactive oxygen species, such as singlet oxygen, hydroxyl radical, superoxide and hydrogen peroxide. These substances damage all types of cellular structures, also causing loss of FSII activity and the degradation of protein D1 (AHSAN et al., 2008; TAKAHASHI; MURATA, 2008; QIAN et al., 2009; POSPÍŠIL; PRASAD, 2014).

**Table 2.** Photosynthetic rate ( $A$ ), stomatal conductance ( $g_s$ ), transpiratory rate ( $E$ ), relationship between internal and external  $\text{CO}_2$  concentration ( $C_i/C_a$ ), maximum quantum yield ( $F_v/F_m$ ), effective quantum yield of FSII ( $\Delta F/F_m'$ ), electron transport rate (ETR), non-photochemical quenching (NPQ) and chlorophyll content index of chlorophyll  $a$ ,  $b$  and total in pequi plants evaluated at 50 and 100 days after transplanting (DAT) and the relative production of the dry matter (RPDM) at 100 DAT as a function of the establishment on residues of *Urochloa decumbens* (URODE), *Melinis minutiflora* (MELMI) and *Paspalum notatum* (PASNO).

Variables	URODE	MELMI	PASNO	TEST	CV%	F <sub>A</sub>	F <sub>B</sub>	F <sub>AxB</sub>	F <sub>fatxadditional</sub>
	50 DAT								
$A$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	4.32 b (-)	5.25 b (-)	8.58 a	9.21	36.45	10.82*	3.68*	1.36 <sup>ns</sup>	14.24*
$g_s$ ( $\text{mol m}^{-2} \text{s}^{-1}$ )	0.14 a (-)	0.15 a (-)	0.17 a	0.25	44.31	0.45 <sup>ns</sup>	0.85 <sup>ns</sup>	0.63 <sup>ns</sup>	12.71*
$E$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	2.01 a	1.98 a	2.12 a	2.85	39.14	0.09 <sup>ns</sup>	1.14 <sup>ns</sup>	0.43 <sup>ns</sup>	8.79*
$C_i/C_a$	0.85 a	0.83 a	0.74 b	0.80	9.43	7.94*	1.83 <sup>ns</sup>	0.54 <sup>ns</sup>	0.89 <sup>ns</sup>
$F_v/F_m$	0.74 a	0.76 a	0.76 a	0.79	5.98	0.60 <sup>ns</sup>	1.76 <sup>ns</sup>	2.04 <sup>ns</sup>	3.68 <sup>ns</sup>
$\Delta F/F_m'$	0.23 b	0.24 b	0.30 a	0.35	18.95	10.52*	2.69 <sup>ns</sup>	2.07 <sup>ns</sup>	26.59*
ETR	89.38 b	101.79 ab	119.15 a	141.36	19.97	7.81*	2.84 <sup>ns</sup>	2.02 <sup>ns</sup>	21.83*
NPQ	1.62 a	1.42 b	1.41 b	1.88	32.70	4.91*	0.62 <sup>ns</sup>	1.21 <sup>ns</sup>	0.36 <sup>ns</sup>
Chlorophyll $a$	241.50 b (-)	260.19 ab (-)	287.56 a	317.37	12.30	7.84*	6.52*	0.83 <sup>ns</sup>	19.83*
Chlorophyll $b$	62.06 b (-)	64.56 b (-)	89.94 a (-)	121.50	29.35	7.65*	7.05*	2.49 <sup>ns</sup>	19.05*
Chlorophyll total	303.56 b (-)	324.75 b (-)	377.50 b (-)	438.87	15.03	8.69*	7.70*	0.75 <sup>ns</sup>	17.79*
Variables	100 DAT								
$A$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	6.10 a (-)	4.72 a (-)	5.42 a (-)	8.56	53.42	1.58 <sup>ns</sup>	34.91*	2.39 <sup>ns</sup>	8.94*
$g_s$ ( $\text{mol m}^{-2} \text{s}^{-1}$ )	0.10 a	0.11 a	0.08 a	0.12	50.63	1.15 <sup>ns</sup>	8.93*	1.49 <sup>ns</sup>	2.37 <sup>ns</sup>
$E$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	1.20 a	1.47 a	1.00 a	1.58	49.08	1.71 <sup>ns</sup>	8.41*	1.33 <sup>ns</sup>	2.38 <sup>ns</sup>
$C_i/C_a$	0.47 b	0.77 a (+)	0.43 b	0.56	29.26	14.51*	11.12*	1.91 <sup>ns</sup>	1.22 <sup>ns</sup>
$F_v/F_m$	0.51 b (-)	0.68 a (-)	0.59 b (-)	0.78	28.25	3.70*	14.60*	2.13 <sup>ns</sup>	3.60 <sup>ns</sup>
$\Delta F/F_m'$	0.18 b	0.17 b	0.23 a	0.33	29.04	3.72*	27.77*	2.17 <sup>ns</sup>	20.02*
ETR	72.07 a (-)	65.98 a (-)	89.96 a (-)	130.05	29.30	2.29 <sup>ns</sup>	29.34*	1.68 <sup>ns</sup>	22.73*
NPQ	0.93 ab	1.49 a (+)	0.65 b (-)	0.96	51.86	7.69*	3.50*	2.84 <sup>ns</sup>	0.17 <sup>ns</sup>
Chlorophyll $a$	179.00 a (-)	249.58 a (-)	216.67 a (-)	318.33	33.31	2.66 <sup>ns</sup>	10.42*	2.14 <sup>ns</sup>	7.91*
Chlorophyll $b$	55.58 a (-)	65.33 a (-)	76.33 a (-)	141.50	44.65	1.15 <sup>ns</sup>	9.39*	1.52 <sup>ns</sup>	25.53*
Chlorophyll total	234.58 a (-)	314.92 a (-)	293.00 a (-)	459.83	34.67	1.91 <sup>ns</sup>	10.69*	2.04 <sup>ns</sup>	15.53*
RPDM (%)	77.83 b (-)	84.03 ab	90.37 a	100.00	12.13	5.87*	17.27*	2.02 <sup>ns</sup>	9.23*

Averages followed by the same letter in a row are not statistically different by the Tukey test ( $p \leq 0.05$ ) of probability. Means followed by (-) or (+) are lower or higher, respectively, than the control by the Dunnett test ( $p \leq 0.05$ ). FA = factor A (species), FB – factor B (concentration), FAxB – factorial interaction, Ffatxradicial – analysis considering additional treatment. \*Significant by F test ( $p \leq 0.05$ ), ns – not significant.

A decrease in photosynthetic efficiency is often accompanied by an increase in NPQ (MAXWELL; JOHNSON, 2000), but in the present study, a higher value was observed for this variable only at 100 DAT in pequi plants grown under *M. minutiflora* residues. Possibly in this treatment, the

response of pequi plants to the high voltage of light in the reaction centres was provided by non-photochemical dissipation as a way to reduce damage to these centres. Part of the absorbed energy may have been dissipated by non-photochemical and photochemical processes, among which is thermal

dissipation, measured by NPQ. This fact indicates a loss in the use of excitation energy for photochemical quenching.

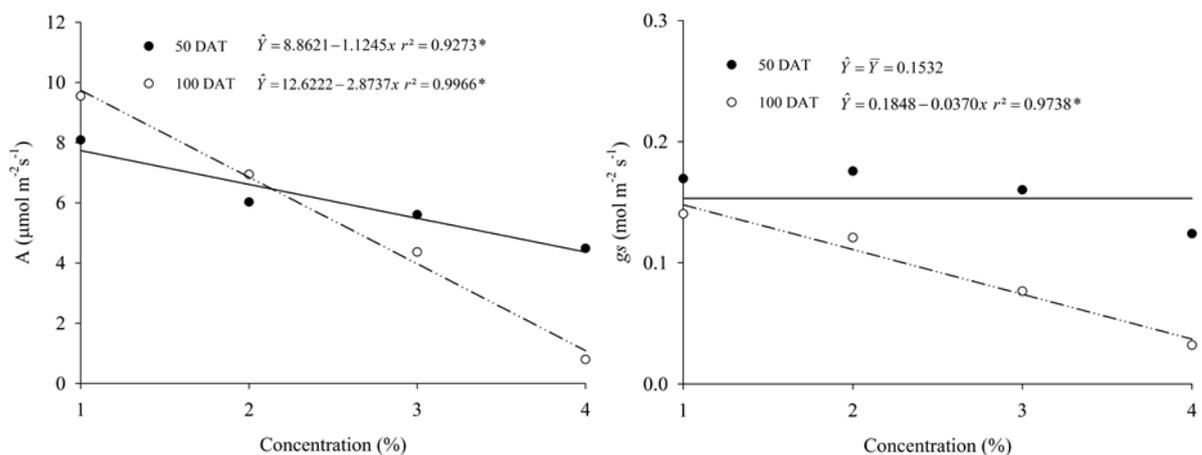
Except for pequi plants cultivated in soils with *P. notatum* plant extracts for chlorophyll content at 50 DAT, in both evaluations, pequi plants cultivated on substrates with residues of the species *U. decumbens*, *M. minutiflora* and *P. notatum* were affected in terms of chlorophyll *a*, *b* and total contents. Chlorophyll is one of the most important components of plants, present in all plant tissues, and is directly linked to photosynthetic efficiency and, consequently, to the growth and adaptation of plants to the most diverse environments (ENGEL; POGGIANI, 1991). It is located in chloroplasts and its function is to channel the energy of sunlight to be converted into chemical energy (TAIZ; ZEIGER, 2013). As a consequence, the reduction of these pigments causes a reduction in photosynthesis, as observed in pequi plants grown in the presence of the forage residues. Zhang et al. (2016) studied the allelopathic effect of *Bidens pilosa* root exudates in *Pteris multifida*, and found inhibitory effects on photosystems I and II, reducing chlorophyll *a*, *b* and total.

A reduction in relative dry matter was observed at 100 DAT compared to control pequi plants grown on *U. decumbens* residues, indicating that, in addition to affecting plant physiology, this allelopathic species also affected dry matter gain (Table 2). This species has already been reported to have allelopathic effects in several works (SOUZA et al., 2006; RODRIGUES et al., 2012). The main effect caused by *U. decumbens* is the reduction of nitrogen in the soil solution and consequently in the plant (SOUZA et al., 2006). On the other hand, the phytotoxic effect caused by this species may be due to (6R, 9S)-3-oxo- $\alpha$ -ionol (KOBAYASHI; KATO-NOGUCHI, 2015). The species *M. minutiflora* and *P. notatum*, while causing damage to

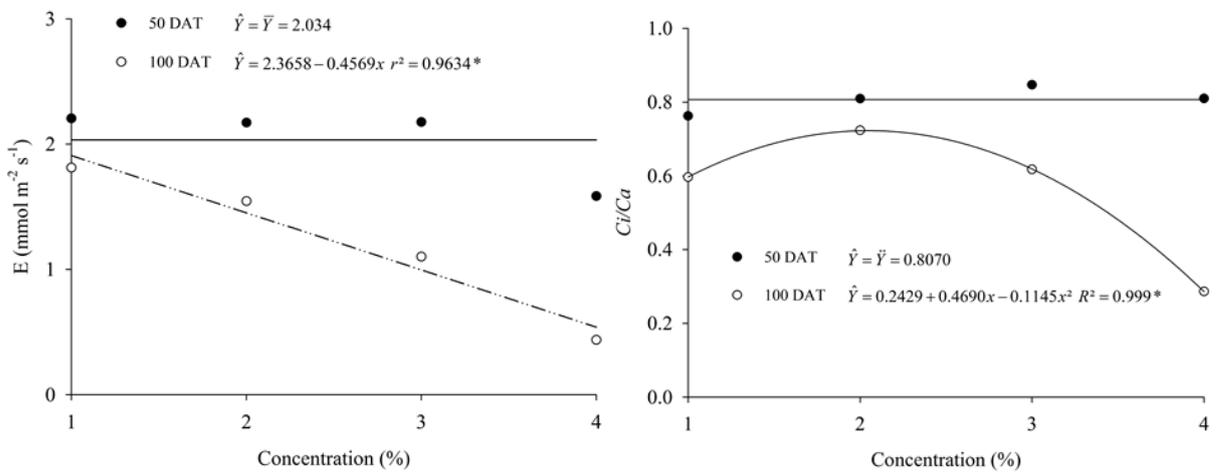
the photosynthetic variables of the pequi plants, did not affect the dry matter gain of this species.

The comparison between pequi plants cultivated in the presence of *U. decumbens*, *M. minutiflora* and *P. notatum* strata showed that pequi cultivated in the presence of the latter was less impaired than the plants cultivated in the presence of the others. In this evaluation, the ETR was also higher in the plants cultivated in the presence of *P. notatum* residues; however, it did not statistically differ from the cultivated pequi plants in the presence of residues of *M. minutiflora*. At 100 DAT, higher relative dry matter production was observed in pequi plants grown in the presence of *P. notatum* residues, but this was not statistically different from *M. minutiflora*.

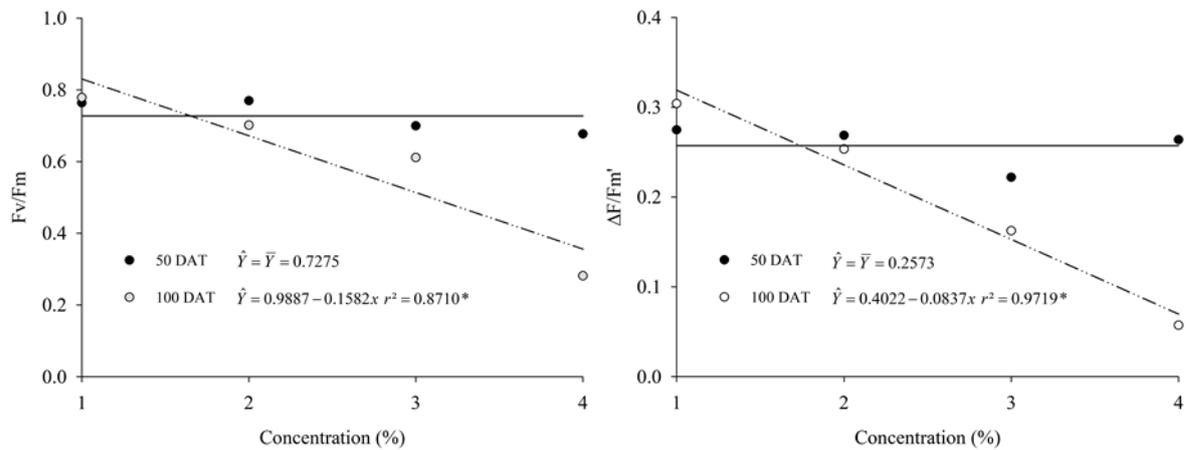
In the evaluations made at 50 and 100 DAT, there was a linear reduction of *A*, ETR and chlorophyll indexes for the total chlorophyll and *a* and *b* in pequi plants grown on soils with grass residues, as the concentrations of these residues increased (Figures 1, 4 and 5). However, for *Ci/Ca*, NPQ, Fv/Fm and  $\Delta F/Fm'$ , there was also a decrease in the density of the straw, but only at 100 DAT (Figures 1, 2, 3 and 4). As observed in Table 2, changes in pequi plants in the presence of grass residues caused changes in stomatal development. However, these changes did not affect the variables mentioned at 50 DAT with increasing concentrations of residues. However, with the development of the plants, together with their photosynthetic system, at 100 DAT, these variables were affected, which may imply losses in the development of these plants in the long term, due to being more susceptible to competition with other plants. In addition, at 100 DAT, there was a longer contact time of the roots of the small plant with the residues, which may have favoured the absorption of compounds released during the decomposition of the residues.



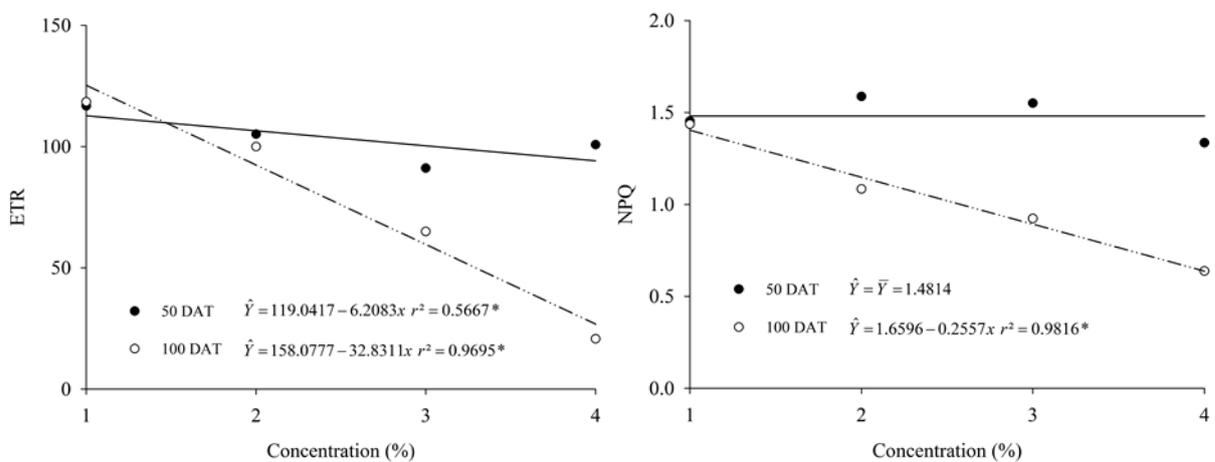
**Figure 1.** Photosynthetic rate (*A* in  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and stomatal conductance (*gs* in  $\text{mol m}^{-2} \text{s}^{-1}$ ) in pequi plants evaluated at 50 and 100 days after transplanting (DAT) as a function of the establishment on residues of *U. decumbens*, *M. minutiflora* and *P. notatum*.



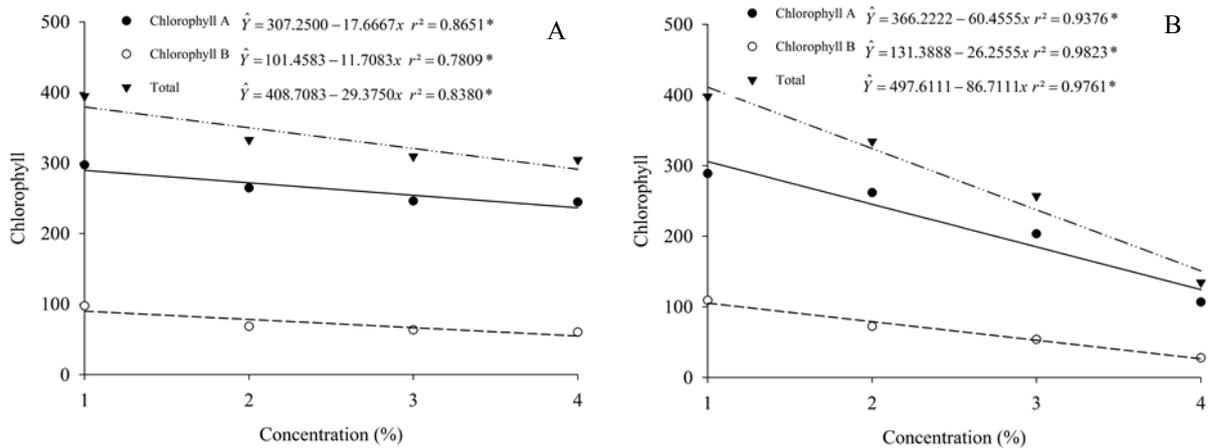
**Figure 2.** Transpiration rate ( $E$  in mmol m<sup>-2</sup> s<sup>-1</sup>) and relationship between internal and external CO<sub>2</sub> concentration ( $C_i/C_a$ ) in pequi plants evaluated at 50 and 100 days after transplanting (DAT) as a function of establishment on residues of *U. decumbens*, *M. minutiflora* and *P. notatum*.



**Figure 3.** Maximum quantum yield ( $F_v/F_m$ ) and effective quantum yield of FSII ( $\Delta F/F_m'$ ) in pequi plants evaluated at 50 and 100 days after transplanting (DAT) as a function of their establishment on residues of *U. decumbens*, *M. minutiflora* and *P. notatum*.



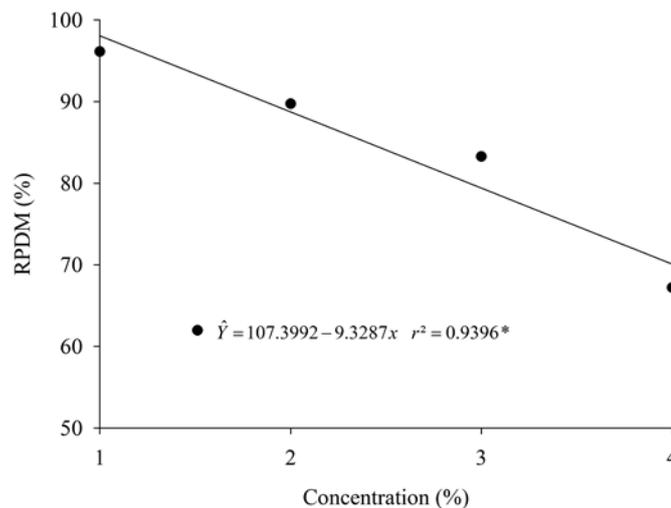
**Figure 4.** Electron transport rate (ETR) and non-photochemical quenching (NPQ) in pequi plants evaluated at 50 and 100 days after transplanting (DAT) as a function of their establishment on *U. decumbens*, *M. minutiflora* and *P. notatum* residues.



**Figure 5.** Chlorophyll index for chlorophyll *a*, *b* and total in pequi plants evaluated at 50 (A) and 100 (B) days after transplanting according to their establishment on residues of *U. decumbens*, *M. minutiflora* and *P. notatum*.

Regarding the production of relative dry matter, there was also a linear reduction, indicating that for this variable the concentration of residues of the tested species affected pequi plants (Figure 6).

These results indicate that, in general, pequi plants were affected by both the allelopathic species and the concentration of residues of these species in the soil.



**Figure 6.** Relative yield for the total dry mass of the weevil (RPMS) evaluated at 100 days after transplanting according to its establishment on *U. decumbens* (URODE), *M. minutiflora* (MELMI) and *P. notatum* (PASNO) residues.

## CONCLUSION

The physiological characteristics of pequi plants, such as photosynthetic rate, maximum quantum yield, electron transport rate and chlorophyll *a*, *b* and total, were negatively altered by the presence of residues of the species *Melinis minutiflora*, *Paspalum notatum* and *Urochloa decumbens*. These also affected dry matter production.

The increase in the density of the residues of *M. minutiflora*, *P. notatum* and *U. decumbens* in the soil negatively affected the physiological characteristics of pequi plants, such as stomatal conductance, transpiratory rate, relationship between internal and external  $\text{CO}_2$  concentration,

non-quenching photochemical, maximum quantum yield, effective quantum yield of FSII and dry matter production.

In general, pequi plants were less affected by allelopathic substances when cultivated in the presence of *P. notatum* residues.

## ACKNOWLEDGMENTS

We thank the Foundation for Support to Research and Innovation of Espírito Santo (FAPES) for financial support.

## REFERÊNCIAS

- AHSAN, N. et al. Glyphosate-induced oxidative stress in rice leaves revealed by proteomic approach. **Plant Physiology and Biochemistry**, Paris, v. 46, n. 12, p. 1062-1070, 2008.
- ALVES JR., J. et al. Respostas do pequizeiro à irrigação e adubação orgânica. **Global Science Technology**, Rio Verde, v. 8, n. 1, p. 47-60, 2015.
- BARBOSA, E. G.; PIVELLO, V. R.; MEIRELLES, S. T. Allelopathic Evidence in *Brachiaria decumbens* and its Potential to Invade the Brazilian Cerrados. **Brazilian Archives of Biology and Technology**, Curitiba, v. 51, n. 4, p. 825-831, 2008.
- BERTOLLI, S. C.; SOUZA, J.; SOUZA, G. M. Caracterização fotossintética da espécie isohídrica pata-de-elefante em condições de deficiência hídrica. **Revista Caatinga**, Mossoró, v. 28, n. 3, p. 196-205, 2015.
- BILGER, W.; BJÖRKMAN, O. Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbency changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. **Photosynthesis Research**, The Hague, v. 25, n. 3, p. 173-185, 1990.
- ENGEL, V. L.; POGGIANI, F. Estudo da concentração de clorofila nas folhas e seu espectro de absorção de luz em função do sombreamento em mudas de quatro espécies florestais nativas. **Revista Brasileira de Fisiologia Vegetal**, Londrina, v. 3, n. 1, p. 39-45, 1991.
- GENTY, B.; BRIANTAIS, J. M.; BAKER, N. R. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. **Biochimica et Biophysica Acta**, Amsterdam, v. 990, n. 1, p. 87-92, 1989.
- GOMES, B. R. et al. The effects of dopamine on antioxidant enzymes activities and reactive oxygen species levels in soybean roots. **Plant Signaling & Behavior**, Austin, v. 9, n. 12, p. 1-6, 2014.
- GULZAR, A.; SIDDIQUI, M. B. Root-mediated allelopathic interference of bhringraj (*Eclipta alba* L.) Hassk. on peanut (*Arachis hypogaea*) and mung bean (*Vigna radiata*). **Applied Soil Ecology**, Amsterdam, v. 87, n. 3, p. 72-80, 2015.
- KOBAYASHI, A.; KATO-NOGUCHI, H. Phytotoxic Substance with Allelopathic Activity in *Brachiaria decumbens*. **Natural Product Communications**, Sendai, v. 10, n. 5, p. 761-763, 2015.
- KRAUSE, G. H.; WEIS, E. Chlorophyll fluorescence and photosynthesis: the basics. **Annual Review Plant Physiology. Plant Molecular Biology**, New York, v. 42, s/n., p. 313-349, 1991.
- MAXWELL, K.; JOHNSON, G. N. Chlorophyll fluorescence – a practical guide. **Journal Experimental Botany**, Oxford, v. 51, n. 345, p. 659-668, 2000.
- POSPÍŠIL, P.; PRASAD, A. Formation of singlet oxygen and protection against its oxidative damage in Photosystem II under abiotic stress. **Journal of Photochemistry and Photobiology B: Biology**, Lausanne, v. 137, n. 8, p. 39-48, 2014.
- QIAN, H. et al. Allelochemical stress causes oxidative damage and inhibition of photosynthesis in *Chlorella vulgaris*. **Chemosphere**, Oxford, v. 75, n. 3, p. 368-375, 2009.
- RASCHER, U.; LIEBIG, M.; LÜTTGE, U. Evaluation of instant lightresponse curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field. **Plant Cell Environ**, Oxford, v. 23, n. 12, p. 1397-1405, 2000.
- RICE, E. L. Allelopathy: an update. **The Botanical Review**, New York, v. 45, n. 1, p. 15-109, 1979.
- RICE, E. L. **Allelopathy**. 2. ed. Orlando, Florida: Academic Press, 1984. 422 p.
- RODRIGUES, A. P. D. C. et al. Alelopatia de duas espécies de braquiária em sementes de três espécies de estilosantes. **Ciência Rural**, Santa Maria, v. 42, n. 10, p. 1758-1763, 2012.
- SANTOS, P. et al. Avaliação físico-química e sensorial do Pequi (*Caryocar brasiliensis* camb.) submetido à desidratação. **Revista Brasileira de Produtos Agroindustriais**, Campina Grande, v. 12, n. 2, p. 115-123, 2010.
- SILVA NETO, S. P.; COSTA, C. J. **Importância econômica, social e ambiental do pequizeiro**. Planaltina, DF: Embrapa Cerrados, 2010. Disponível em: <<http://www.cpac.embrapa.br/noticias/artigosmidia/publicados/279>>. Acesso em: 03 mai. 2016.
- SINGH, H. P. et al. Herbicidal activity of volatile oils from *Eucalyptus citriodora* against *Parthenium hysterophorus*. **Annals of Applied Biology**, London, v. 146, n. 1, p. 89-94, 2005.
- SOUZA, C. L. M. et al. Efeito inibidor dos extratos hidroalcoólicos de coberturas mortas sobre a germinação de sementes de cenoura e alface. **Planta Daninha**, Viçosa, v. 17, n. 2, p. 263-272, 1999.

SOUZA, L. S. et al. Efeito alelopático de capim-braquiária (*Brachiaria decumbens*) sobre o crescimento inicial de sete espécies de plantas cultivadas. **Planta Daninha**, Viçosa, v. 24, n. 4, p. 657-668, 2006.

TAIZ, L.; ZEIGER, E. **Fisiologia Vegetal**. 5. ed. Porto Alegre, RS: Artmed, 2013. 954 p.

TAKAHASHI, S.; MURATA, N. How do environmental stresses accelerate photoinhibition?. **Trends Plant Science**, Oxford, v. 13, n. 4, p. 178-182, 2008.

VALLE, C. B.; JANK, L.; RESENDE, R. M. S. O melhoramento de forrageiras tropicais no Brasil. **Revista Ceres**, Viçosa, v. 56, n. 4, p. 460-472, 2009.

ZHANG, K. M. et al. Photosynthetic electron-transfer reactions in the gametophyte of *Pteris multifida* reveal the presence of allelopathic interference from the invasive plant species *Bidens pilosa*. **Journal of Photochemistry and Photobiology B: Biology**, Lausanne, v. 158, n. 5, p. 81–88, 2016.

ZOBIOLE, L. H. S. et al. Glyphosate reduces shoot concentration of mineral nutrients in glyphosate-resistant soybeans. **Plant and Soil**, Dordrecht, v. 328, n. 1, p. 57–69, 2010.