Effects of foliar application of chemical agents and shading levels on growth and physiological aspects of "malagueta" pepper plants

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Abstract

Foliar application of chemical agents and cultivation in different levels of shading can modify pepper plants. The objective of this study was to evaluate the effect of the application of chemical agents in the growth, partition of photoassimilates and photosynthetic pigments of malagueta pepper in different growing environments. The treatments were arranged in a randomized design with a 2x5 factorial scheme under two cultivation environments [eucalyptus structures measuring 5.0 x 5.0 x 2.5 m (length x width x height), coated with type coverage screens (Sombrite®) with 18% and 35% shading], and five treatments with chemical agents [1 - C - without application; 2 - PBZ (50 mg L⁻¹) + Nut. (1%); 3 - Gib. (50 mg L⁻¹) + Nut. (1%); 4 - Amin. (50 mg L⁻¹) + Ant. (50 mg L⁻¹) and 5 - Gib. (50 mg L⁻¹) + A (50 mg L⁻¹) + Amin. (50 mg L⁻¹) + Ant. (50 mg L⁻¹)] in four replications. We evaluated the stem diameter, the plant height, number of leaves, leaf dimensions, leaf area, accumulation and dry matter partition and chlorophyll contents. The cultivation of pepper plants in environment with 18% of shading provides plants with a higher number of leaves and dry matter, as environment protects plants against climatic agents by controlling temperature, humidity and solar radiation. The application of the Paclobutrazol + Nitrogen promotes shoot growth and increases the green pigmentation in pepper plants.

Keywords: Capsicum frutescens L., light incidence, partition of photoassimilates, photosynthetic pigments, protected environments.

Abbreviations: A_ Auxin; Amin._ Amino acid; Ant._ Antioxidant; C._ Control; DAT._ Days after transplanting; ELL_ Expanded leaves length; ELW_ Expanded leaves width; FA_ Foliar area; GA_ Gibberellic acid; Gib._ Gibberellin; IBA_ Indole-butyric acid; N._ Nitrogen; NL_ Number of leaves; N.S._ Not significant; Nut._ Nutrient; P_ Phenylalanine; PBZ_ Paclobutrazol; PH_ Plant height; R._ Riboflavin; RAGR_. Ratio of above-ground dry phytomass and dry phytomass of the root system; RHD_. Ratio of the plant height and stem diameter; RHM_. Ratio of the plant height and dry phytomass; SD_ Stem diameter; SGI_ Speed of germination index.

Introduction

Malagueta pepper (Capsicum frutescens L.) is a species from the Capsicum gender widely cultivated and used by human as a condiment or in the form of processed sauces, canned or dehydrated chips (Costa and Henz, 2007). The foliar application of chemical agents based on plant growth regulators, nutrients, amino acids and antioxidants, and the use of cultivation in protected environments with different levels of shading are alternatives to improve the productive system of the malagueta pepper.

According to Castro (2006), biostimulants are mixtures of plant growth regulators or mixture of plant growth regulators with other compounds with different chemical nature, such as nutrients, amino acids and vitamins. Plant growth regulators are organic compounds other than nutrients, applied to the plant to promote, inhibit or modify morphologic and physiologic processes in the plant, for instance auxins, gibberellins, cytokinins, retarders, and ethylene inhibitors. Biostimulants are able to improve efficiency in the use of nutrients and increase plant tolerance to biotic and abiotic stresses (Bulgari et al., 2015).

Proper supply of nutrients is an essential requirement for crops to express their productive potential. Nutrient deficiencies can cause growth limitations and development of hot malagueta pepper crop (Flores et al., 2012). Amino acids can be used on protein synthesis and as intermediate compounds of endogenous hormones. They are also capable of increasing plant resistance to drought stress and high temperature. Likewise, they can be used as plant bio activators and as completing substances to promote more efficient or narrower absorption of ions or molecules through foliar application (Castro and Carvalho, 2014). Antioxidants are a heterogeneous group of substances formed by minerals, vitamins, natural pigments and other plant compounds and enzymes that hinder the harmful effects of free radicals, and are used to slow oxidative changes that cause deterioration (Food Ingredients Brazil, 2009). Cultivation in protected environments can be an alternative to maximize the productivity of malagueta pepper crop. According to Purquerio and Trivelli (2014), protected cultivation is characterized by building a
framework that protects plants against weather agents, allowing passage of the light essential to perform photosynthesis. In a specialized agricultural production system, we are able to control temperature, humidity, and solar radiation to provide optimal condition for development of crops during periods that would not be suitable for the field production. The improvements gained from using foliar application of chemical agents and protected cultivation can be quantified by the growth of malagueta pepper plants. According to Benincasa (2003), the plant growth analysis is based on the fact that 90% of the accumulated drying biomass by plants throughout their development is the result of photosynthetic activity. This allows the evaluation of the final plant growth and the participation of the several bodies in the whole development process. This study aimed to evaluate the effect of foliar application of chemical agents and the cultivation at different levels of shading on growth and partitioning of photoassimilates and the concentration of photosynthetic pigments of malagueta pepper plants (*Capsicum frutescens* L.).

**Results and discussion**

**Number of leaves, length and width of expanded leaves and leaf area**

Regarding crop environmental factors and foliar application with chemical agents, no interactions concerning the number of leaves, length and width of expanded leaves and leaf area were verified. In this case, they have been presented alone (Table 1).

A decreased number of leaves in environment with lower incidence of light was observed. However, the shading level had no influence on the length and width of the leaves and leaf area (Table 1).

Foliar application with Gib. + A + P + R stimulated the pepper plant to produce leaves with greater length and width in relation to the evidence and chemical agents without gibberellin. The results show that increments in relation to the length and width of pepper leaves may be due to the action of gibberellin to promote stretching and cell expansion for exercising effects on the cell wall, while gibberellins are promoters of cell elongation preferably in the young ones. The results for foliar dimensions resemble Lucchesi et al. (1982) work that evaluated the influence of gibberellic acid (GA₃) in the development, production and fruit quality in bell pepper culture (*Capsicum annuum* L.). They found that bell pepper plants had higher vegetative growth in treatments, in which high concentrations of GA₃ were applied and the plant leaves in these treatments were larger due to the increase of cell expansion promoted by this phytoregulator. Less leaf area was observed with the use of chemicals PBZ+N e P+R compared to Gib.+A+P+R.

**Dry phytomass of the root system, stalk, leaves and total mass**

Interactions were not observed between environmental factors of growing and foliar application of chemical agents for dry phytomass of the root system, stalk and leaves. They were presented separately (Table 2). Malagueta pepper plants grown in an environment with 18% of shading produced greater dry phytomass of the root system, stalk and leaves and total mass (Table 2) than plants grown in an environment with 35% of shading. The plants use sunlight to perform photosynthesis and produce organic solutes needed as an energy source for their metabolism. The use of shade screens in protected environments reduces photosynthetic radiation incident to the cultivated plants, and the decrease in light intensity that directly affects plant’s photosynthesis process impacting on growth and development. Malagueta pepper plants grown in an environment with 18% of shading showed less sunlight restriction that plants grown in an environment with 35% of shading, making up for a greater photosynthetic activity, forming a higher number of leaves and producing higher levels of dry matter. Similar results were obtained by Díaz-Pérez (2013), where the author found that in bell pepper plants (*Capsicum annuum* L.) the number of leaves per plant was increased with the decrease of the shading level where the plants were being grown.

The application of PBZ + N provided plants with lower dry phytomass of stem compared to the use of chemical agents with gibberellin. It is due to the fact that paclobutrazol is a growth regulator that interferes directly with the biosynthesis of gibberellin in plants, and this plant hormone exerts influence on stem growth and consequently its dry phytomass.

**Stem diameter, plant height and relations RHM, RHD and RAGR**

Using growing environmental factors and foliar application with chemical agents, there was no interaction for stem diameter, plant height, ratio of the plant height and above-ground dry phytomass (RHM), ratio of the plant height and stem diameter (RHD) and ratio of above-ground dry phytomass and dry phytomass of the root system (RAGR), all acted separately (Table 3).

The shading levels tested did not affect the stem diameter, plant height, RHD and RAGR. However larger shading favored plants with higher RHM, verifying that the decline in photosynthesis as a result of lack of light may have caused higher loss on above-ground dry phytomass production than the plant height composition, in addition to morphogenesis (structural control of plant development) which is controlled by light.

Foliar application of PBZ + N provided more "compressed" malagueta pepper plants, with shorter leaves (Table 1), producing lower dry phytomass of the stem in relation to the evidence and the application of gibberellic acid (Table 2), lower plant height (Table 3), lower RHD for the application of chemical agents. These results are justified since the growth retardant PBZ, substance of the group of triazoles, reduces the gibberellin’s biosynthesis by inhibiting the action of kaurene oxidase, involved in the formation of kaurenic acid, a gibberellic acid precursor. The growth retarding chemicals are substances that have inhibitory effects on the growth and development of the plant and being applied under suitable conditions affecting the elongation of the branches, and plant height.
| Treatments | NL | ELL | ELW | FA |
|----------|----|-----|-----|----|
| **Environment (levels of shading)** | | | | |
| 18% | M103.70 a | 14.70 a | 6.54 a | 563.95 a |
| 35% | 63.25 b | 14.60 a | 6.75 a | 487.25 a |
| **Chemical Agents (foliar)** | | | | |
| Evidence | 93.25 a | 14.31 bc | 6.29 b | 563.95 a |
| PBZ+N | 104.25 a | 11.75 d | 5.41 b | 398.75 b |
| Gib.+N | 84.38 a | 16.10 ab | 7.38 a | 550.88 ab |
| P+R | 72.25 a | 14.15 c | 6.23 b | 401.00 b |
| Gib.+A+P+R | 63.25 a | 16.91 a | 7.93 a | 667.38 a |

**Table 2.** Dry phytomass of root system, stalk and leaves, and total, of *Capsicum frutescens* depending on the level of shading and foliar application of chemical agents. UEMS, Cassilândia (MS), 2015

| Treatments | Dry Phytomass (7dias) | | |
|-----------|-----------------------|---|---|---|
| **Environment (level of shading)** | Root | Stalk | Leaf | Total |
| 18% | M3.74 a | 3.15 a | 3.13 a | 10.02 a |
| 35% | 2.73 b | 2.10 b | 2.46 b | 7.29 b |
| **Chemical Agents (foliar)** | | | | |
| Evidence | 3.64 a | 2.81 a | 2.87 a | 9.32 a |
| PBZ+N | 2.41 a | 1.51 b | 2.48 a | 6.40 a |
| Gib.+N | 3.41 a | 3.20 a | 2.90 a | 9.51 a |
| P+R | 3.02 a | 2.60 ab | 2.43 a | 8.05 a |
| Gib.+A+P+R | 3.68 a | 3.03 a | 2.30 a | 10.00 a |

Means followed by lowercase letters in columns within the environment and chemical agents factor, statistically differ among each other by F and Turkey tests at 5% of probability, respectively; ** significant at 1% probability; * significant at 5% probability; N.S. Not significant; PBZ - Paclobutrazol; N - Nitrogen; Gib. - Gibberellin; P - Phenylalanine; R - Riboflavin; A - Auxin.
Table 3. Stem diameter (SD), plant height (PH), ratio of the plant height and above-ground dry phytomass (RHM), a relation of plant height and stem diameter (RHD), and ratio of above-ground dry phytomass and dry phytomass of the root system (RAGR) of Capsicum frutescens, depending on the level of shading and foliar application of chemical agents. UEMS, Cassilândia (MS), 2015

| Treatments | SD     | PH     | RHM    | RHD    | RAGR   |
|------------|--------|--------|--------|--------|--------|
| Environment (level of shading) |        |        |        |        |        |
| 18%        | 7.00 a | 22.03 a| 3.62 b | 3.16 a | 1.86 a |
| 35%        | 6.55 a | 19.54 a| 4.60 a | 3.00 a | 1.75 a |
| Chemical Agents (foliar) |        |        |        |        |        |
| Evidence   | 7.13 ab| 21.46 a| 3.98 a | 3.02 ab| 1.75 a |
| PBZ+N      | 6.00 b | 14.21 b| 3.61 a | 2.37 b | 1.92 a |
| Gib.+N     | 7.13 ab| 21.58 a| 3.92 a | 3.18 a | 1.91 a |
| P+R        | 6.13 b | 20.48 a| 4.37 a | 3.28 a | 1.65 a |
| Gib.+A+P+R | 7.50 a | 26.19 a| 4.66 a | 3.57 a | 1.81 a |

Table 4. Chlorophyll b in Capsicum frutescens, depending on the level of shading and foliar application of chemical agents. UEMS, Cassilândia (MS), 2015

| Treatment | Chemical Agents (foliar) |        |        |        |        |
|-----------|--------------------------|--------|--------|--------|--------|
| Evidence  | PBZ+N                    | 842.00 a| 645.75 c| 759.62 b| 826.00 a|
|           | Gib.+N                   |        |        |        |        |
|           | P+R                      |        |        |        |        |
|           | Gib.+A+P+R               |        |        |        |        |
| 18%       | 784.25 b                 |        |        |        |        |
| 35%       |                          |        |        |        |        |
| DMS       | Environment              |        |        |        |        |
|           | Chemical                 | 26.95  |        |        |        |
| C.V. (%)  | 2.41                     |        |        |        |        |

Table 5. Unfolding of the interaction regarding the chlorophyll a, depending on foliar application with different chemical agents and levels of shading, in Capsicum frutescens. UEMS, Cassilândia (MS), 2015

| Chemical Agents (foliar) | Environment (level of shading) |
|--------------------------|---------------------------------|
| Testemunha               |                                  |
| PBZ+N                    | 1142.50 aA                      |
| Gib.+N                   | 1078.00 bA                      |
| P+R                      | 1086.75 bA                      |
| Gib.+A+P+R               | 1074.50 bB                      |
| DMS                      | Chemical Agents                 |
| C.V. (%)                 | 1.11                            |

The results are in agreement with those obtained by Mutlu and Agan (2015), in peppers of the Capsicum gender, where the PBZ promoted reduction in plant height and Benett et al. (2014), on tomato seedlings (Solanum lycopersicum L.), where the use of PBZ inhibited the above-ground dry phytomass of the seedlings.
Photosynthetic pigments

Pepper grown in an environment with 18% of shading showed higher concentration of chlorophyll b than the plants grown in an environment with 35% of shading (Table 4). Foliar application of PBZ + N and Gib. + A + P + R provided plants with higher content of chlorophyll b in the chili leaves. Foliar application of Gib. + N in pepper plants provided higher leaf chlorophyll b. Berova and Slatev (2000), in tomato culture (Lycopersicon esculentum Mill.) found improvement in photosynthetic activity of plants with foliar application of PBZ. Monge et al. (1994) analyzed the effect of the application of GA³ in photosynthetic pigments in adult peach trees (Prunus persica L. Batsch), verifying that the use of GA³ significantly reduced the concentration of chlorophyll b. Growing environmental factors and foliar application with chemical interactions were not observed for chlorophyll b, where they acted individually (Table 4).

Malagueta pepper plants grown in an environment with 18% of shading showed higher concentration of chlorophyll a, when they were subjected to foliar application with the different treatments studied in relation to the evidence, especially the use of PBZ + N which promoted the highest concentration of chlorophyll a compared to other treatments analyzed (Table 5). Results are in agreement with those obtained by Melo et al. (2014), which reviewed the use of the growth retardant PBZ in the film-coating of tomato seeds (Solanum lycopersicum L), verifying that independent from the concentration adopted, the use of PBZ increases the concentration of chlorophyll. Pepper grown in an environment with 35% of shading showed higher concentration of chlorophyll a compared to control, when they were subjected to foliar application of Gib. + A + P + R, and the use of P + R and Gib. + N inhibited the concentration of chlorophyll a compared to control (Table 5). Ouzounidou et al. (2010) found that the concentration of chlorophyll a of green pepper leaves was inhibited by using GA³ in Capsicum annuum L. Significant interactions between growing environmental factors and foliar application with chemical agents were found in chlorophyll a (Table 5).

In control and application of Gib. + A + P + R the environment with larger shading led to plants with increase of chlorophyll a content in the leaves. However, other chemical agents under 18% of shading, plants showed a higher content of chlorophyll a. Souza et al. (2011) evaluated the effect of shading on chlorophyll levels in young guaco plants (Mikania laeavigata Sch. Bip. ex Baker) and verified that the shaded treatments showed higher chlorophyll a concentrations compared to plants exposed direct to the sun.

Materials and methods

Conduction of experiment

The experiment was carried out in the experimental area of the State University of Mato Grosso do Sul (UEMS), in the University Unit of Cassilândia (UUC), located in Cassilândia county - MS (19º07’21” S; 51º43’15” W; 516 m asl) within the period from January to July 2015.

Plant material

The chilli pepper (Capsicum frutescens L.) is the species tested in the experiment. The "malagueta" pepper belongs to the family Solanaceae and is characterized by having a pivoting radicular system with a high number of lateral branches, reaching a depth of 70-120 cm. The leaves are green, the flowers are hermaphrodite, usually composed of 5 petals and 5 sepalas, with a greenish-white corolla and blue, purple or violet anthers, the seeds are of straw color and the fruits are of the berry type (Costa and Henz, 2007).

Seed testing

Capsicum frutescens L. seeds were used for analysis of the physiological quality before setting up the experiment. The seeds had 12% of moisture content, 41% of normal seedlings in the 1st germination count and 86% of final germination and 6.2 speed of germination index (SGI).

Treatments and experimental design

The experimental design was completely randomized with foliar application of five treatments with chemical agents [1 - witness - without application; 2 - PBZ (50 mg L⁻¹) + Nut. (1%); 3 - Gib. (50 mg L⁻¹) + Nut. (1%); 4 - Amin. (50 mg L⁻¹) + Ant. (50 mg L⁻¹)] and 5 - Gib. (50 mg L⁻¹) + A (50 mg L⁻¹) + Amin. (50 mg L⁻¹) + Ant. (50 mg L⁻¹)] executed in four repetitions. As there were no repetitions of the growing environments, each one was considered an experiment. For each growing environment (shading of 18 and 35%) the experimental design was adopted completely randomized to evaluate leaf chemical agents. The environments were evaluated by the analysis of groups of experiments (Banzatto and Kronka, 2013).

Commercial products used

The products used to prepare the chemical agents were: paclobutrazol - as Cultar 250 SC; nutrient - nitrogen in the form of urea (45% N); gibberellin – in the form of gibberellic acid (GA³); aminoacid - in the form of phenylalanine; antioxidant – in the form of vitamin B2 (riboflavin) and auxin - in the form of indole-butyric acid (IBA).

Seedling production

For the seedling production, we used 12 trays of 72 expanded polystyrene cells, each tray presented length of 68 cm, width 34.5 cm and high 11.5 cm and each cell of the trays was filled with Bioplant® commercial substrate. Three pepper seeds were sown per cell, pointing up that the thinning was done after seedling emergence, remaining only one seedling per cell. The trays were watered daily with the use of a manual water can. Nutrient solution and foliar nitrogen fertilization were applied over the seedlings during the production. Modified nutrient solution applications were prepared (Hoagland and Arnon, 1950). The nutrient solution was composed by calcium nitrate (5 ml L⁻¹), potassium nitrate (5 ml L⁻¹), potassium dihydrogen phosphate (1 ml L⁻¹), magnesium sulphate (2 ml L⁻¹) and a micronutrient’s solution (1 ml L⁻¹). The micronutrient’s solution was composed of boric acid (1
ml L⁻¹), zinc sulfate (1 ml L⁻¹), copper sulfate (1 ml L⁻¹) and ammonium molybdate (1 ml L⁻¹). We applied 250 ml of solution to each vessel in the morning at 47, 32 and 05 days before transplanting the seedlings. Foliar applications were made with N solution (1%), the source used was urea nitrogen with 125 ml of solution for each vessel, applied in the afternoon at 47, 32 and 15 days before transplanting the seedlings.

Transplanting the seedlings

Transplanting the seedlings to the 15 L-capacity plastic vessels was applied at day 77 after seeding, being two seedlings conditioned per vessel. The vessels contained substrate consisting of 7.5 L of fine vermiculite + 7.5 L of composted manure. The manure was acquired from the by-product from a slaughterhouse in the region. It is composted in a process of revolving and hydration in two-day intervals, for a period of 30 days. After this process, the manure was sifted and bagged. The composted manure analysis showed the following characteristics: 1.2% of N; 0.6% of P₂O₅; 0.3% of K₂O; 0.6% of Ca; 0.1% of Mg; 0.2% of S; 30% humidity at 65°C; 25% of MO; 14% of C; 966 mg kg⁻¹ of Na; 8 mg kg⁻¹ of Cu; 2765 mg kg⁻¹ of Fe; 113 mg kg⁻¹ of Mn; 90 mg kg⁻¹ of Zn, C/N relation of 12/1 and pH of 5.1.

Growing environments

The vessel with seedlings were conditioned in two growing environments that were characterized as screened environments which were covered and shading screens (Sombrite®). The difference between the two environments is that the A1 set maintained, with an eucalyptus structure measuring 5.0 x 2.5 m (length x width) and the A2 set 5.0 x 2.5 m (length x width x height) with cover and shading screens (Sombrite®). The average relative humidity was 61.7% in the environment 1 and 64.4% in the environment 2, and the higher temperature was 24.2 °C (35%) was 25.1 °C and the A2 (35%) was 24.2 °C. The average relative humidity was 61.7% in the environment 1 and 64.4% in the environment 2, and the higher average of photosynthetic active radiation (measured just above the sheet used as a reference for each portion), the lower average of photosynthetic active radiation (measured just below the sheet used as a reference for each portion) and direct in the A1 setting were 564; 82; 1012 μmol m²s⁻¹ respectively and in A2 setting were respectively 405; 68; 995 μmol m²s⁻¹.

Foliar applications in plants

The chemical agents were applied via foliar at 13 days after transplanting (DAT). The application was made with a manual sprayer and the volume of chemical agents applied 50 ml per vessel (25 ml per plant). The application was done in the late afternoon. At 25 DAT the thinning was performed, leaving only one plant per vessel and staking up some plants. At 54 DAT the adapted nutrient solution (Hoagland and Arnon, 1950) was applied and at 55 DAT, the N solution (1%).

Non-destructive, destructive analysis and relations

A non-destructive analysis was performed at 59 DAT and the destructive analysis was performed at 66 DAT. For repetitions were performed for each analysis, with each repetition consisting of a plastic vessel containing a single pepper plant.

Non-destructive analysis

Stem diameter - To obtain the stem diameter, we used a digital caliper. Results were expressed in mm. Plant height - to measure the plant height, we used a ruler graduated in millimeters, along the stem and considering the measure between the substrate and the nearest branch of the plant apex. The results were expressed in cm. Number of Leaves - To determine the number of leaves per plant, we counted all the leaves of the plants. Leaf Dimensions - To measure the length and width of the leaves, two expanded leaves per plant were analyzed for each evaluation period. To perform the analysis, we used a ruler graduated in mm. The results were expressed in cm.

Destructive analysis

Partitioning of photoassimilates - Plants were collected to determine the dry phytomass of the roots, stem, leaves and the total (sum) that was determined after subjecting the samples to drying in a forced-air circulation oven at 65 °C until it reached constant weight. Chlorophyll content - a and b chlorophyll contents were determined according to the method of Arnon (1949). A 0.5g sample of leaf tissue was macerated in 10ml of acetone. Subsequently, the extract was centrifuged at 1500 rpm for 10 minutes and held by spectrophotometer readings at wavelengths at 645 and 663 nm. These readings were used in the equations for the quantification of: chlorophyll a (mg L⁻¹)= 12.7 x DO663 – 2.69 x DO645 and chlorophyll b (mg L⁻¹)= 22.9 x DO645 – 4.69 x DO663. Foliar area - To obtain the foliar area, all plant leaves were removed and scanned, whose images have been stored and processed in a specialized software. The results were expressed in cm² per plant.

Relations

RHM - ratio of the plant height and dry phytomass (stems and leaves)
RHD - ratio of the plant height and stem diameter.
RAGR - ratio of above-ground dry phytomass and dry phytomass of the root system.

Statistical analysis

Data were submitted to analysis of variance (F test) and the averages compared by Tukey test at 5% probability factor to chemical agents and even by the F test for the growing environments. The SANEST software (Zonta and Machado, 1986) was used.

Conclusion

The cultivation of malagueta pepper plants in a protected environment with 18% of shading provides plants with a higher number of leaves and dry phytomass of root system, stalk, leaves and total mass and recommended for the vegetative growth. Application of PBZ + N modifies the The
partition of photo-assimilates, occurring a reduction in dry phytomass accumulation in the stalk, not affecting other parts of the plant. The use of PBZ + N in malagueta pepper plants for ornamental purposes is an alternative to provide the a decreased growth of the above-ground part, with shorter leaves, and enhancing the green pigment because of the higher chlorophyll b content.

Acknowledgements
We thank CAPES for the financial support necessary to carry out the research and the "field team" of UEMS of Cassilândia - MS for the valuable contribution during the development of the project.

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