Effect of light stress on *Crotalaria spectabilis* (Fabaceae) and on its herbivore insect, the moth *Utetheisa ornatrix* (Erebidae: Arctiinae)

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**ABSTRACT.** The Plant Stress Hypothesis predicts that stressed plants are more attacked by herbivorous insects. In this work, we investigated the influence of light stress on *Crotalaria spectabilis* Roth (Fabaceae) and on its main herbivore, the moth *Utetheisa ornatrix* (L., 1758) (Erebidae: Arctiinae). Specifically, we verified whether plants stressed by shading differ from non-stressed plants in terms of productivity, morphological characteristics and water percentage. We also evaluated the performance of moths in stressed and non-stressed plants. Seeds were sown in pots. When the plants reached 50 cm in height, they were randomly divided into two groups: stressed plants (treatment group) and non-stressed plants (control group). The stressed plants were covered by a black mesh, providing 50% of shading. Eight characteristics of stressed and non-stressed *C. spectabilis* plants were evaluated: height, fresh and dry aerial biomass, number of pods and seeds, leaf hardness, number of trichomes, leaf area, specific leaf mass and percentage of leaf water. Moths were raised individually on leaves of stressed and non-stressed plants and we obtained the larval survival, larval development time, pupal weight and female fecundity. The non-stressed plants had significantly higher percentage of water in the leaves, greater fresh aerial biomass and a higher number of trichomes than the stressed plants. The survival rate was 98% for larvae raised on leaves from stressed plants and 92% on leaves from non-stressed plants. The larval developmental time was significantly shorter and the weight of female pupae significantly higher in non-stressed plants than in stressed plants. Thus, the Plant Stress Hypothesis was only corroborated by the treated variables: number of trichomes (lower in stressed plants) and larval survival (higher in stressed plants). Trichomes are among the main types of plant defenses against herbivory and reducing their number on leaves would make stressed plants more susceptible to attack by moth larvae, a fact corroborated by a greater larval survival. One of the possible explanations for the lack of corroborating the Plant Stress Hypothesis for most of the variables tested is that other characteristics can be changed under stress conditions, such as the concentration of secondary compounds.

**KEYWORDS.** Lepidoptera, performance, Plant Stress Hypothesis, trichomes.

**RESUMO.** Efeito do estresse luminoso sobre *Crotalaria spectabilis* (Fabaceae) e seu inseto herbívoro, a mariposa *Utetheisa ornatrix* (Erebidae: Arctiinae). A hipótese do estresse da planta prevê que as plantas estressadas são mais atacadas por insetos herbívoros. Neste trabalho, investigamos a influência do estresse luminoso em *Crotalaria spectabilis* Roth (Fabaceae) e em seu principal herbívoro, a mariposa *Utetheisa ornatrix* (L., 1758) (Erebidae: Arctiinae). Especificamente, verificamos se as plantas estressadas por sombreamento diferem das plantas não estressadas em termos de produtividade, características morfológicas e porcentagem de água. Também avaliamos o desempenho das mariposas em plantas estressadas e não estressadas. As sementes foram semeadas em vasos. Quando as plantas atingiram 50 cm de altura, foram divididas aleatoriamente em dois grupos: plantas estressadas (grupo de tratamento) e plantas não estressadas (grupo controle). As plantas estressadas foram cobertas por uma malha preta, proporcionando 50% de sombreamento. Foram avaliadas oito características de plantas estressadas e não estressadas de *C. spectabilis*: altura, biomassa aérea fresca e seca, número de vagens e sementes, dureza foliar, número de tricomas, área foliar, massa foliar específica e porcentagem de água foliar. As mariposas foram criadas individualmente em folhas de plantas estressadas e não estressadas. Obtivemos a sobrevivência larval, o tempo de desenvolvimento larval, o peso pupal e a fecundidade das fêmeas. As plantas não estressadas apresentaram porcentagem significativamente maior de água nas folhas, maior biomassa aérea fresca e um maior número de tricomas do que as plantas estressadas. A taxa de sobrevivência foi de 98% para larvas criadas em folhas de plantas sem estresse e 92% em folhas de plantas sem estresse. O tempo de desenvolvimento larval foi significativamente menor e o peso das pupas femininas significativamente maior em plantas sem estresse do que em plantas estressadas. Assim, a hipótese de estresse em plantas foi corroborada apenas por duas variáveis testadas: número de tricomas (menos nas plantas estressadas) e sobrevivência larval (maior nas plantas estressadas). Os tricomas estão entre os principais tipos de defesa das plantas contra a herbivoria e a redução de seu número nas folhas tornaria as plantas estressadas mais suscetíveis ao ataque de larvas de mariposas, fato corroborado por uma maior sobrevivência larval. Uma das possíveis explicações para a falta de corroboração da hipótese de estresse de planta para a maioria das variáveis testadas é que outras características podem ser alteradas sob condições de estresse, como a concentração de compostos secundários.

**PALAVRAS-CHAVE.** Desempenho, Lepidoptera, Teoria do Estresse da Planta, tricomas.
Many hypotheses have been proposed to explain the patterns of interaction among insects and plants. One of these is the Plant Stress Hypothesis (PSH) (White, 1969), which predicts that stressed plants are more attacked by herbivorous insects. White (1969) formulated this hypothesis based on observations of population outbreaks of mealbugs in eucalyptus trees subjected to water stress. For the author, the population outbreaks of these insects were due to changes in the physiology of stressed plants, which increased the availability of nitrogen in their tissues during the prolonged hydric deficit. Since nitrogen is one of the most important nutrients for insects (Mattson, 1980), White (1969, 1984) suggested that an increase in nitrogen concentration during periods of water stress would result in increased growth and reproduction of herbivorous insects, generating population outbreaks. A basic premise of PSH is that different types of stress induce similar responses in plants (i.e., increased concentration of soluble nitrogen), and therefore different types of stress affect the development of insects in the same way.

Light is the primary source of energy used in photosynthesis, which is the main factor that influences the growth of plants (Campos & Uchida, 2002). Light is also important in the morphogenetic processes of the life cycle of plants, such as seed germination, seedling development, flower formation and seed production (Taiz et al., 2017). In this way, luminosity is considered one of the limiting factors for the development, growth and adaptive characteristics of plants (Gazolla-Neto et al., 2013). Despite this, few studies that address the relationship between plant stress and the performance of its herbivorous insect have tested the light stress, and most of these (76%) used tree species as a model (Koricheva et al., 1998). In plant species adapted to the sun, shading usually makes them more susceptible to herbivory because they reduce structural and chemical resistance characteristics (Agrawal et al., 2012).

Although many studies have observed higher density, greater herbivory, greater preference and better performance of herbivorous insects in stressed plants than in non-stressed plants, many other studies have found results contrary to PSH (e.g. Mopper & Whitham, 1992; Koricheva et al., 1998; Huberty & Denno, 2004). One of the possible explanations for the lack of corroboration of PSH is that, in addition to nitrogen concentration, other variables are changed under stress conditions. For example, despite the increase in nitrogen in stressed plants, sap pressure and water content generally decrease and the concentration of secondary compounds can increase under stress conditions (Hsiao, 1973; Inbar et al., 2001; Zobayed et al., 2007).

In addition, plants under stress may have leaf size (Stone & Bacon, 1994), leaf hardness (Foggo et al., 1994) and architecture (Waring & Price, 1990) altered. All these characteristics influence the selection of plants by herbivorous insects and their performance (Schoonhoven et al., 2005) and, consequently, the relationship between plant stress and the herbivore’s fitness.

In this study, the influence of light stress on Crotalaria spectabilis Roth (Fabaceae) and on its main herbivore, the moth Utetheisa ornatrix (L., 1758) (Erebidae: Arctiinae), was investigated. Specifically, (1) we verified whether plants stressed by shading differ from plants not stressed in terms of productivity (biomass and total number of seeds), morphological characteristics (height, hardness, leaf area, specific leaf mass and number of trichomes) and percentage of water and (2) we evaluated the performance (survival, larval development time, pupal weight and female fecundity) of moths in stressed and non-stressed plants.

**MATERIAL AND METHODS**

**Studied species.** Crotalaria species are perennial or annual, shrub, sub-shrub or herbaceous plants, ranging from 3 cm to 3 m in height (A. S. Flores, unpubl. data). They can be erect or branched and have simple or typetrigoliolate leaves (A. S. Flores, unpubl. data). The genus has about 600 species in the tropics and subtropics of the world (Pollhill, 1982). In Brazil, 31 native and 11 exotic species were recorded. The Cerrado biome has the largest number of native species (A. S. Flores, unpubl. data). These plants reproduce by seeds, and they are commonly found in disturbed environments such as roadsides, abandoned land and pastures (Lorenzi, 1982).

Crotalaria spectabilis is an Asian species used in green fertilization (A. S. Flores, unpubl. data). This species has also been used to control nematodes of agricultural importance (Silveira & Rava, 2004). It is considered the most toxic species of the genus (Lorenzi, 1982). This species ranges from 1 to 1.5 m high, its leaves are simple and with trichomes on the dorsal surface. Plants of this species produce 15 to 31 flowers in each terminal raceme (A. S. Flores, unpubl. data). Crotalaria spectabilis prefers fertile and deep soils. This species has a high tolerance to drought and has a vegetative cycle of 120 to 150 days (A. S. Flores, unpubl. data).

Utetheisa ornatrix (Erebidae: Arctiinae) is found mainly in areas with some degree of anthropization, occurring from North America to Chile and Argentina (Pease, 1968). The larva of this species consumes mainly plants of the genus Crotalaria, which are extremely rich in pyrrolizidine alkaloids, which are found with higher concentrations in green seeds (Johnson et al., 1985). These alkaloids are extremely important in protecting this moth against its predators and are retained throughout the individual’s development (Bogner & Eisner, 1991).

**Plant cultivation.** The study was conducted in a greenhouse at the Samambaia Campus of the Federal University of Goiás, located in the city of Goiânia, state of Goiás, Brazil. Seeds from the same population of C. spectabilis were sown individually in pots with vegetable garden soil and vermiculite (1:1). After reaching 50 cm in height, these plants were randomly divided into two groups: stressed plants (treatment group) and non-stressed plants (control group). The stressed plants were covered by a 170 cm high, 70 cm wide and 70 cm deep PVC structure covered by a black mesh, providing 50% shading. The stressed plants...
remained shaded until the end of the experiment. The non-stressed plants did not receive the coverage described above. All plants were watered four times a day for 5 min, twice in the morning (6 a.m. and 8 a.m.) and twice at night (8 p.m. and 10 p.m.), for their complete vegetative cycle.

The position of each plant in the greenhouse (Fig. 1) was defined by drawing lots. To prevent the group of non-stressed plants from being shaded by the coverings of the treatment group, a distance of 1 m was established between the plants on the same bench and from one bench to another.

The plants did not suffer any type of damage until the pods fully matured, neither natural (consumption by herbivorous insect or presence of pathogen) nor artificial (for example, removal of leaves to create larvae), since many works (e.g. Marquis, 1984) prove that herbivory (natural or artificial) alters the fitness of plants.

**Plant characteristics.** Eight characteristics of stressed and non-stressed *C. spectabilis* plants were evaluated: height, fresh and dry aerial biomass, number of pods and seeds, leaf hardness, number of trichomes, leaf area, specific leaf mass and percentage of leaf water. We measured these characteristics on 15 plants of both, the treatment group and the control group.

When the pods were ripe, the height (shortest distance between the upper limit of the photosynthetic tissues and the soil) of each plant was measured with a telescopic ruler. Then, the plants were cut close to the soil. All aerial plant material was considered for accounting for fresh aerial biomass. To calculate the dry aerial biomass, the fresh aerial part of the plants was dried at 60°C for 24 h and then they were weighed. The total number of pods and seeds for each plant were also counted.

Leaf hardness was measured using a penetrometer. A new fully expanded leaf was removed from each plant. Each leaf was perforated in the central region with a penetrometer on the right side and on the left side of the central rib and, afterwards, the average was calculated. For the quantification of trichomes, a 28 mm² disc was demarcated at the distal end of a new fully expanded leaf from each plant. The number of trichomes on the dorsal face (the ventral is glabrous) was measured using a microscope.

To calculate the leaf area, a fully expanded leaf was selected from each plant. These leaves were scanned, and the leaf area was measured using the Image J® software (Rasband, 2006). The specific leaf mass (SLM) was measured using the same leaves that we used to calculate the leaf area. After being scanned, the leaves were dried to obtain dry biomass. The SLM value was obtained by dividing the dry mass (g) and the leaf area (cm²) (Cornelissen et al., 2003). For the water percentage, a new fully expanded leaf from each plant was weighed, and then placed in a drying oven for 24 h at 60°C. The dry material was weighed again to obtain the percentage of water in the leaves.

The influence of light stress on each variable related to the plant (height, fresh and dry aerial biomass, number of pods and seeds, leaf hardness, number of trichomes, leaf area, specific leaf mass and percentage of leaf water) was analyzed through t tests in the R software.

**Moth performance.** To obtain the moths, 100 green pods of *Crotalaria spectabilis* with herbivory marks were collected in the Leolídio di Ramos Caiado Municipal Park, in the northern region of Goiânia, GO, Brazil. The collected pods were taken to the Insect Ecology Laboratory of the Universidade Federal de Goiás, where the moths were raised on *C. spectabilis* leaves. The eggs obtained from this breeding were placed in Petri dishes until they hatched.

The newly hatched larvae were randomly placed individually in glass vials containing stressed or non-stressed *C. spectabilis* leaves from plants grown in the greenhouse. The vials were cleaned and the leaves were changed daily. The vials were kept under controlled conditions of temperature and photoperiod (28°C and 12 h, respectively). The dates of the larva’s death or its moult to the pupal stage were recorded. Thus, the percentage of larval survival and developmental time was obtained. The pupae were weighed on a precision balance.

![Fig. 1. Distribution of stressed plants (with mesh cover) and non-stressed plants of Crotalaria spectabilis Roth in the greenhouse.](image-url)
After the hatching of the adults, couples belonging to the same experimental group (that is, one male and one female raised on stressed leaves or one male and one female raised on unstressed leaves) were transferred to cylinders made of cardboard (10 cm in diameter and 20 cm in height). There were 15 couples from non-stressed leaf creations and 18 couples from stressed leaves. The adults were fed with a 1:4 honey and water solution. The eggs deposited by the females were counted daily in order to measure their fertility. The influence of light stress on each variable related to the performance of the moth (time of larval development, pupal weight, female fecundity) was analyzed using t tests in the R software.

RESULTS

The non-stressed plants showed significantly higher percentage of water in the leaves, higher fresh aerial biomass and a higher number of trichomes than the stressed plants (Tab. I). There was no difference between the types of plants for the other variables tested (Tab. I).

Tab. I. Mean values of plant characteristics in non-stressed and stressed plants of Crotalaria spectabilis Roth. N = 15 for both types of plants for all variables. Numbers in parentheses represent the range. The asterisks mean the significant p-values.

<table>
<thead>
<tr>
<th>Variables</th>
<th>t values</th>
<th>P values</th>
<th>Non-stressed plant</th>
<th>Stressed plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (m)</td>
<td>0.19</td>
<td>0.84</td>
<td>1.36 (1.02-1.68)</td>
<td>1.35 (1.21-1.69)</td>
</tr>
<tr>
<td>Total number of pods</td>
<td>0.68</td>
<td>0.49</td>
<td>25 (13-63)</td>
<td>24 (7-58)</td>
</tr>
<tr>
<td>Total number of seeds</td>
<td>0.39</td>
<td>0.69</td>
<td>527 (401-1221)</td>
<td>519 (351-1319)</td>
</tr>
<tr>
<td>Leaf water (%)</td>
<td>2.21</td>
<td>0.03*</td>
<td>27.1 (18.3-34.9)</td>
<td>22 (10.1-37.5)</td>
</tr>
<tr>
<td>Fresh aerial biomass (g)</td>
<td>2.47</td>
<td>0.01*</td>
<td>328.7 (118.7-615.9)</td>
<td>229.1 (7.9-391.4)</td>
</tr>
<tr>
<td>Dry aerial biomass (g)</td>
<td>1.50</td>
<td>0.14</td>
<td>116 (67-189)</td>
<td>97 (62-179)</td>
</tr>
<tr>
<td>Leaf hardness (N)</td>
<td>0.63</td>
<td>0.52</td>
<td>17.5 (14-18.5)</td>
<td>17.5 (14-22)</td>
</tr>
<tr>
<td>Leaf area (cm²)</td>
<td>0.71</td>
<td>0.48</td>
<td>14.1 (11.4-18.3)</td>
<td>14.3 (7.3-17.9)</td>
</tr>
<tr>
<td>Specific leaf mass (g/cm²)</td>
<td>1.87</td>
<td>0.07</td>
<td>0.003 (0.001-0.007)</td>
<td>0.003 (0.002-0.004)</td>
</tr>
<tr>
<td>Number of trichomes (28 mm²)</td>
<td>3.02</td>
<td>0.005*</td>
<td>287 (229-392)</td>
<td>252 (198-315)</td>
</tr>
</tbody>
</table>

Fig. 2. Survival of Utetheisa ornatrix (L., 1758) larvae raised in the leaves of Crotalaria spectabilis Roth from light stressed plants (black circle) and non-stressed plants (white circle). N = 50 larvae for both types of plants.

Fig. 3. Development time of the larvae of Utetheisa ornatrix (L., 1758) reared with leaves of Crotalaria spectabilis Roth from light stressed plants and non-stressed plants. N = 49 for stressed plants and N = 46 for non-stressed plants. Different letters indicate statistical difference (t = 2.27; p = 0.02).
Females’ fertility did not differ between the two types of diet (stressed and non-stressed leaves) \((t = 0.07; p = 0.94)\) (Fig. 5). The fertility of females from non-stressed plant leaves was 288.72 eggs on average. Females fed with stressed plant leaves laid an average of 286.67 eggs.

**DISCUSSION**

Trichomes are among the main types of plant defenses against herbivory (Gilbert, 1971; Levin, 1973; Smith et al., 1975; Ramalho et al., 1984; Woodman & Fernandes, 1991; Agrawal & Fishbein, 2006). Morphologically, trichomes exhibit a wide range of variation, from flattened plaques to elongated hairs; some are unicellular and others multicellular; some are glandular and others are non-glandular and some develop thick secondary walls, sometimes impregnated with silica and calcium carbonate, in order to form strong hooks (Taiz et al., 2017). Glandular trichomes affect herbivores through chemical (toxic substances) and physical (adhesive substances) properties. Non-glandular structures act to prevent fixation, blocking access to the leaf surface, serving as a mechanical barrier against herbivores (Taiz et al., 2017). Non-glandular trichomes, which are present in *Crotalaria spectabilis*, are also related to the reduction of water loss through transpiration. Therefore, the lower amount of trichomes in the group of stressed plants can be explained by the lower rate of light. In this way, stressed plants would be more susceptible to attack by herbivorous insects. Other studies have also observed a reduction in structural resistance levels in stressed plants. Agrawal et al. (2012), for example, found that shaded plants produced leaves with less trichomes than plants in full sun. A similar result was observed by Pérez-Estrada et al. (1998), Fini et al. (2014) and Dardengo et al. (2017). Although *U. ornatrix* larvae are mostly found in seeds, leaf characteristics, such as the amount of trichomes, can be important for first and second instar larvae and also in the selection for oviposition sites (females’ oviposit in the leaves).

The only variable of the insect’s performance that corroborated the Plant Stress Hypothesis was larval survival, since larvae had a little higher survival in stressed plants. The Plant Stress Hypothesis predicts that stressed plants are more attacked by herbivorous insects, as there is an increase in nitrogen in their tissues. Since nitrogen is one of the most important nutrients for insects (Matson, 1980), White (1969) suggested that an increase in nitrogen concentration during periods of stress would result in an increase in the performance of herbivorous insects.

One of the variables that measure the fitness of an insect is the time of larval development. Larvae that take a longer time to develop have a greater chance of being attacked by predators, parasitoids and pathogens (Schoonhoven et al., 2005). So, the leaves of stressed plants, as they provide a significantly slower development than the non-stressed plants, allow the larvae more time exposed to natural enemies and other sources of mortality, contrary to the Plant Stress Hypothesis.

The weight of pupae is also a factor that can contribute to the reproductive success of adults. Several studies have found a strong positive correlation between the weight of pupae and the fertility of Lepidoptera females (e.g. Spurgeon et al., 1995; Tammaru et al., 1996). Female pupae from stressed plants weighed significantly less than those from non-stressed plants. Therefore, it is disadvantageous to feed on stressed plant leaves, as lighter pupae can originate less fertile females, not corroborating the Plant Stress Hypothesis.

The females of *Utetheisa ornatrix* showed high individual variability in the total number of eggs deposited, regardless of the larval food. Thus, the fertility of the females does not seem to be affected by the larval diet. Lamunyon (1997) found that the fertility of *Utetheisa ornatrix* females increased after the third mating. The variation in the number of matings may have contributed to the high variability in
the number of eggs deposited in the present study, since the couples remained together until they died and we did not observe the number of matings.

One of the possible explanations for the lack of corroboration of the Plant Stress Hypothesis for most of the variables tested in our study is that other characteristics can be changed under stress conditions, such as the concentration of sap and the concentration of secondary compounds. These characteristics also influence the selection of plants by herbivorous insects and their performance (SCHOONHOVEN et al., 2005) and, consequently, the relationship between plant stress and the herbivore’s fitness. In the future, we intend to analyze chemically the leaves, as well as the female’s preference in relation to the oviposition site and herbivory levels in stressed and non-stressed plants of C. spectabilis.

Acknowledgements. To FAPEG (Fundaçao de Amparo à Pesquisa do Estado de São Paulo) for funding this project (PUBLIC CALL No. 05/12 - FAPEG/UNIVERSAL) and for the Scientific Initiation scholarships granted to Leticia Barbosa and Lorena Lima. To Dr. Leandro Maracahipes for his assistance in calculating the leaf area, Pedro Batista for helping in the greenhouse and Dr. Marcus Cianciaruso for equipment lending.

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