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**PHYSIOLOGICAL PARAMETERS OF PADDY RICE UNDER INFESTATIONS OF *TIBRACA LIMBATIVENTRIS* (HEMIPTERA: PENTATOMIDAE) ADULTS****PARÂMETROS FISIOLÓGICOS DE ARROZ IRRIGADO SOB INFESTAÇÕES DE ADULTOS DE *TIBRACA LIMBATIVENTRIS* (HEMIPTERA: PENTATOMIDAE)**

**Resumo:** A alimentação por percevejos podem modificar a atividade de trocas-gasosas de plantas hospedeiras. Assim, o objetivo desse estudo foi avaliar os efeitos da alimentação por adultos de *Tibraca limbativentris* sobre parâmetros fisiológicos de arroz irrigado. O experimento foi realizado em condições de casa telada. As plantas de arroz foram infestadas por dois e quatro insetos adultos. Parcelas controle foram mantidas sem infestação. A taxa fotossintética, condutância estomática, transpiração e CO<sub>2</sub> intercelular foram medidas em diferentes tempos. As taxas de fotossíntese e condutância estomática foram reduzidas com a maior densidade de *T. limbativentris* somente 72 h após a infestação. CO<sub>2</sub> não foi afetado pela alimentação dos percevejos; no entanto, a inibição da fotossíntese provavelmente foi devida a uma redução da taxa de transpiração. Estes resultados indicam que é possível um impacto substancial na fisiologia de arroz irrigado por adultos de *T. limbativentris*.

**Palavras-chave:** : trocas-gasosas, injúria, *Oryza sativa* L., Pentatomidae, percevejo-do-colmo-do-arroz.

**Abstract:** Feeding by stink bugs may change gas-exchange activity of host plants. Thus, the aim of this study was to evaluate the effects of feed by *Tibraca limbativentris* adults on the physiological parameters of paddy rice. The experiment was performed in screenhouse conditions. Rice plants were infested for two and four insects adult. Control plots were maintained with no insect infestation. Photosynthetic rate, stomatal conductance, transpiration rate and intercellular CO<sub>2</sub> were measured at different times. The photosynthesis and stomatal conductance rates were reduced with the highest density of *T. limbativentris* only at 72 h after infestation. Intercellular CO<sub>2</sub> was not affected by stink bugs feeding; however, inhibition of photosynthesis likely was due to a reduction of transpiration rate. These results indicate that substantial physiological impact on paddy rice by *T. limbativentris* adults is possible.

**Keywords:** gas-exchange, injury, *Oryza sativa* L., Pentatomidae, rice stalk stink bug.

**Introduction**

Rice (*Oryza sativa* L.) is one of the most important agricultural crops worldwide (Cassman, 1999). However, yield losses could occur due to attack of insect pests. The rice stalk stink bug *Tibraca limbativentris* Stal, 1860 (Hemiptera: Pentatomidae) is the most important pest in paddy rice in South America (Alves et al., 2016; Ferreira et al., 1997; Krinski & Foerster, 2017; Panizzi et al., 2000; Pantoja et al., 2007; Pasini et al., 2018). *T. limbativentris* is an invasive pest in many countries, including the

Argentina, Bolivia, Brazil, Colombia, Costa Rica, Dominican Republic, Ecuador and Uruguay (Fernandes & Grazia, 1998; Panizzi et al., 2000; Pantoja et al., 1995; Trujillo, 1970). Total rice yield loss by *T. limbativentris* feeding can occur (Ferreira et al., 1997; Pantoja et al., 2007).

Feeding by 12 to 24 h of *T. limbativentris* can result in death of the stalk (Ferreira et al., 1997). The literature reports contradictory findings regarding *T. limbativentris* injury and damage. Besides, the quantification of rice stalk stink bug injuries on rice is difficult because *T. limbativentris* feeding may occur over an extended period of time and the initial injury (such as chlorosis) to the plant is inconspicuous. An alternative method to quantify plant injury is through physiological measures such as gas-exchange (Hoback et al., 2015). Feed by insect on specific tissues, such as phloem and xylem, can cause physiological responses in plants (Nabity et al., 2009). For example, photosynthetic rates of soybean (*Glycine max* L.) were significantly affected by *Aphis glycines* (Macedo et al., 2003).

Alterations in gas exchange of plants in response to insect attack are crucial determinants of plants growth, development and fitness (Peterson, 2000). However, previous research has not addressed the contribution of photosynthesis to studies of response to infestations of *T. limbativentris* on paddy rice. Therefore, this study aimed to examine whether the injury caused by different densities of *T. limbativentris* adults affects the gas-exchange parameters of paddy rice.

## Material and Methods

The experiment was carried out under screen-house conditions at the Embrapa Rice and Beans, located in Santo Antônio de Goiás, Goiás, Brazil (16°40'43"S; 49°15'14"W). Experimental pots were exposed to natural light and photoperiod. The temperature ranged from 19.70 to 44.10 °C (mean 26.90 °C), and relative humidity from 46.20 to 79.10% (mean 63.20%).

The rice cultivar 'BRS Pampeira' was planted at 0.5 cm depth in trays of 200 cells containing substrate. After 10 days, ten seedlings were transplanted to plastic pots of 5 L (30 cm diameter, 25 cm height, without holes in the bottom), previously filled with a Oxisol soil. The cultural treatments were carried out according to recommendations, except for the application of insecticides. Plants were irrigated daily, maintaining the soil flooded to simulate paddy rice fields. Flooded irrigation was established 20 days after emergence of rice and all plots remained flooded permanently.

The experimental design was a randomized

complete block with six replications. Each pot containing ten plants was considered an experimental unit, which was covered with a net supported by a metal frame. To establish the initial populations, plants inside cages were infested with two and four *T. limbativentris* adults with 10 days age. Control plots were maintained with no insect infestation. The plants were infested at 30 days after emergence.

The insects used in the experiment were obtained from a colony of *T. limbativentris* established in a different greenhouse. The colony was initiated with field-collected adults found on sentinel plants set in the borders of areas previously cultivated with rice at the Embrapa Experimental Station near Goianira, in the state of Goiás, Brazil (16° 25' S; 49° 24' W). *T. limbativentris* were reared on rice plants (*Oryza sativa* L., cv. BR-IRGA-409) grown in 5 L pots covered with a nylon screen. Insect density in each caged pot consisted of 100 adults (sex ratio 1 male: 1 female) with the same age. Colonies were free of any chemical treatment.

Photosynthetic rate, stomatal conductance, intercellular CO<sub>2</sub> concentration and transpiration rate were measured using a portable gas exchange instrument (LCpro-SD, ADC BioScientific, UK) at 8:00-11:00 hours in sunny days. The following settings were used: blue light source at 1 200 μmol m<sup>-2</sup> s<sup>-1</sup> photosynthetic photon flux density (PPFD), 500 μmol of CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>. All gas exchange readings were taken on the top of leaf of the last fully expanded leaf; two plants in each pot were measured. The assessments were performed at 0, 1, 12, 24, 48, 72 and 144 hours after first infestation. Stink bugs were allowed to feed and develop normally during all the evaluations. After the infestation period the numbers of dead-hearts were recorded per plot.

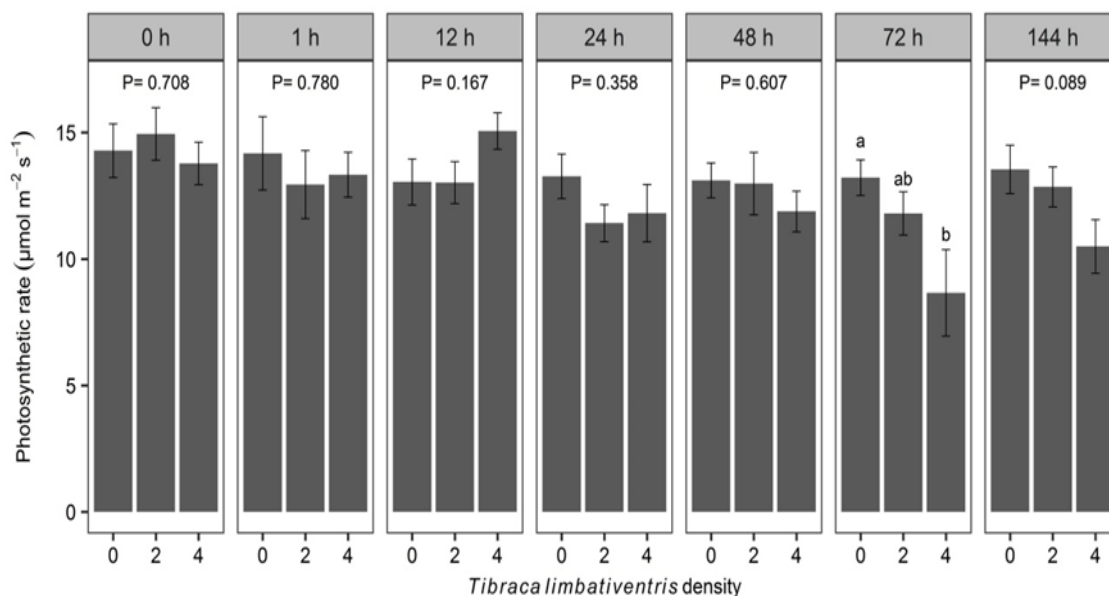
Data of photosynthetic rate, stomatal conductance, intercellular CO<sub>2</sub> concentration, transpiration rate and dead-heart were subjected to studentized residual analysis to confirm the assumption of normality with the Shapiro-Wilk test. The data were then subjected to one-way analysis of variance (ANOVA) followed by the LSD test ( $\alpha < 0.05$ ) using the ExpDes package (Ferreira et al., 2013). All analyses were created in R version 3.4.0 (R Development Core Team, 2017).

## Results and Discussion

At 72 h after infestation, the lowest photosynthetic rate was observed in the plants with four stink bugs per plot ( $F = 3.92$ ,  $df = 2,15$ ,  $P = 0.043$ ; Fig.1). However, no significant differences were observed in photosynthetic rate at 0, 1, 12, 24, 48 and 144 hours after infestation with different densities of *T. limbativentris*

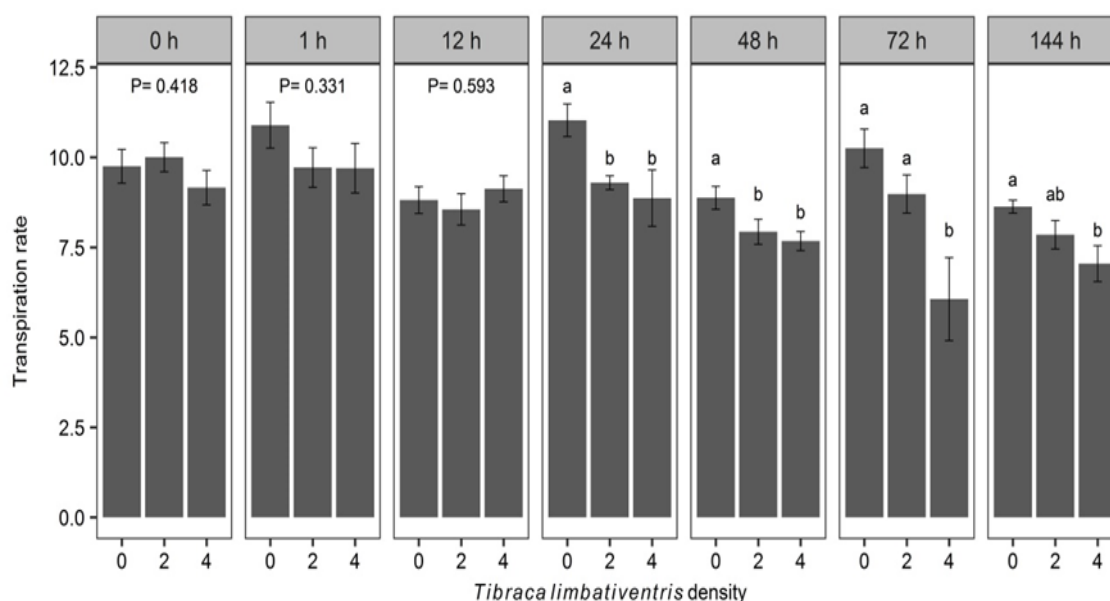
adults (0 h:  $F = 0.35$ ,  $df = 2,15$ ,  $P = 0.708$ ; 1 h:  $F = 0.25$ ,  $df = 2,15$ ,  $P = 0.780$ ; 12 h:  $F = 2.02$ ,  $df = 2,15$ ,  $P = 0.167$ ; 24 h:  $F = 1.10$ ,  $df = 2,15$ ,  $P = 0.358$ ; 48 h:  $F = 0.52$ ,  $df = 2,15$ ,  $P = 0.607$ ; 144 h:  $F = 2.86$ ,  $df = 2,15$ ,  $P = 0.089$ ; Fig.1).

**Figure 1.** Photosynthetic rate of paddy rice in different time after infestation with *Tibraca limbativentris* (Hemiptera: Pentatomidae) adults.



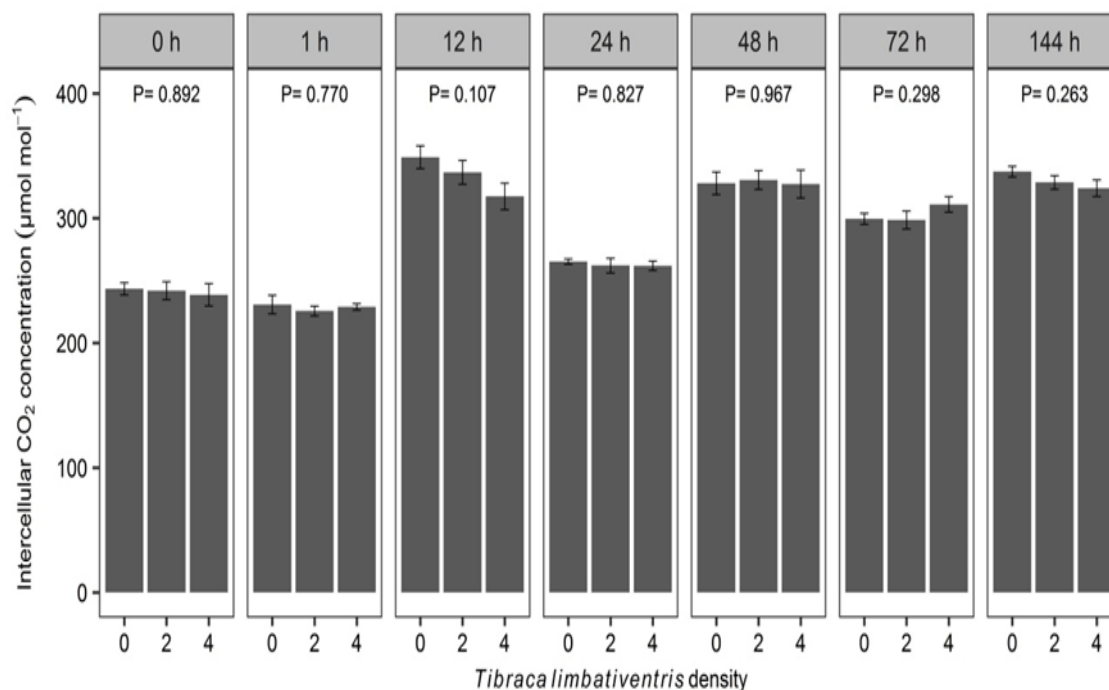
The transpiration rate was affected after 24 h of infestation (24 h:  $F = 4.59$ ,  $df = 2,15$ ,  $P = 0.028$ ; 48 h:  $F = 4.09$ ,  $df = 2,15$ ,  $P = 0.038$ ; 72 h:  $F = 7.29$ ,  $df = 2,15$ ,  $P = 0.006$ ; 144 h:  $F = 4.29$ ,  $df = 2,15$ ,  $P = 0.034$ ; Fig.3). Two and four stink bugs promoted the lowest transpiration rate at 24 and 48 h, and four stink bugs at 72 and 144 h. However, at 0, 1 and 12 h after infestation there was not effect of *T. limbativentris* adults on transpiration rate (0 h:  $F = 2.06$ ,  $df = 2,15$ ,  $P = 0.161$ ; 1 h:  $F = 0.45$ ,  $df = 2,15$ ,  $P = 0.647$ ; 12 h:  $F = 1.66$ ,  $df = 2,15$ ,  $P = 0.223$ ; Fig.3).

**Figure 3.** Transpiration rate of paddy rice in different time after infestation with *Tibraca limbativentris* (Hemiptera: Pentatomidae) adults.



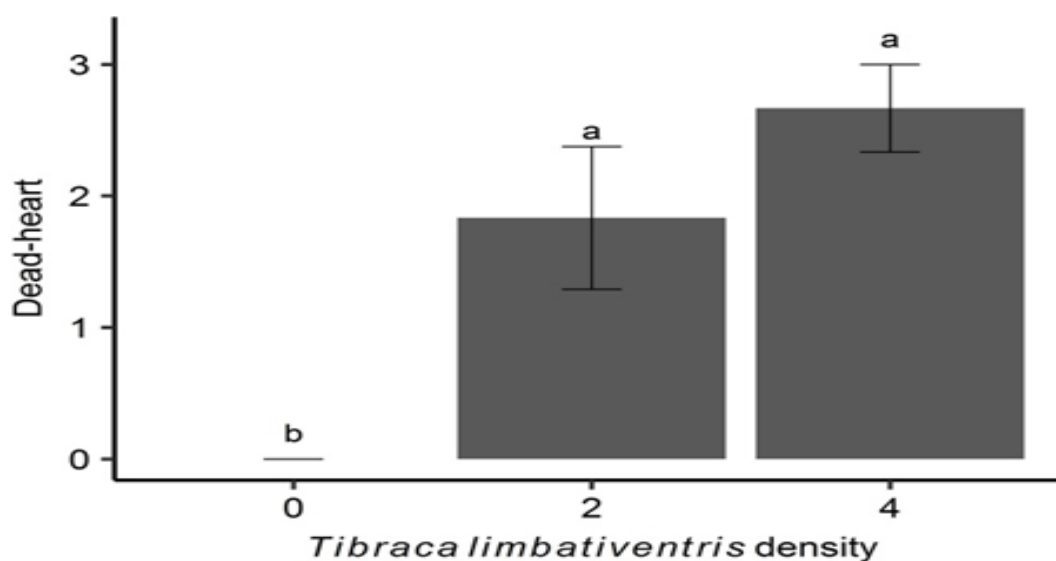
Intercellular CO<sub>2</sub> was not altered with different densities of *T. limbativentris* adults in any time (0 h:  $F=0.11$ ,  $df=2,15$ ,  $P=0.892$ ; 1 h:  $F=0.27$ ,  $df=2,15$ ,  $P=0.770$ ; 12 h:  $F=2.61$ ,  $df=2,15$ ,  $P=0.107$ ; 24 h:  $F=20.68$ ,  $df=2,15$ ,  $P=0.827$ ; 48 h:  $F=0.03$ ,  $df=2,15$ ,  $P=0.967$ ; 72 h:  $F=1.31$ ,  $df=2,15$ ,  $P=0.298$ ; 144 h:  $F=1.46$ ,  $df=2,15$ ,  $P=0.263$ ; Fig.4).

**Figure 4.** Intercellular CO<sub>2</sub> concentration of paddy rice in different time after infestation with *Tibraca limbativentris* (Hemiptera: Pentatomidae) adults.



Infestations per two and four stink bugs of *T. limbativentris* adults promoted higher number of dead-hearts than the treatment control ( $F=13.77$ ,  $df=2,15$ ,  $P<0.001$ ; Fig.5).

**Figure 5.** Dead-heart after infestation with *Tibraca limbativentris* (Hemiptera: Pentatomidae) adults on paddy rice.



Piercing-sucking herbivores may feed on sap of xylem, phloem, or other plant cells (Lucini & Panizzi, 2018; Walling, 2000). Their feeding site and the amount of tissue damage may vary considerably (Lucini & Panizzi, 2018; Walling, 2000). The mechanical action of stylets and the injection of digestive enzymes within plant tissue result in different degrees of damage in vegetative and reproductive tissues; the resulting damage is strongly correlated to the strategies and tactics of feeding used by stink bug (Lucini & Panizzi, 2018). In general, the initial symptoms are bleaching and local lesions, with later development of secondary symptoms and physiological disarray of the plant from stylet insertion and saliva injection (Lucini & Panizzi, 2018).

In the current study, gas-exchange revealed substantial injury to the photosynthetic apparatus of paddy rice caused by *T. limbativentris*. Photosynthesis and stomatal conductance decreased with the highest density of *T. limbativentris* only at 72 h after infestation. Intercellular CO<sub>2</sub> did not decrease proportionally with stink bugs increase; then, inhibition of photosynthesis likely can be explained due the reduction of transpiration rate. Velikova et al. (2010) found similar results with a negative impact of pentatomidae feeding damage on leaves photosynthesis of *B. oleracea* and *P. vulgaris*. However, an increase of transpiration was observed in sugar maple (*Acer Saccharum*) seedlings attacked by thrips *Taeniothrips inconsequens* (Kolb et al., 1991). This increase was attributed to the larger conductance of gases both at stomatal and cuticular level caused by the insect punctures, and explained an unexpected increase of photosynthesis in damaged leaves (Kolb et al., 1991).

Plant stress often is absent when feeding is limited to a few hours. For example, photosynthesis injuries do not develop when cotton leaves are attacked by aphids for few hours (Gomez et al., 2006), but occur after herbivores attack a plant for several days (Reddall et al., 2004). Furthermore, photosynthesis decreased rapidly and substantially in *Brassica oleracea* and *Phaseolus vulgaris* infested by stink bugs (Velikova et al., 2010). The damage caused on *B. oleracea* leaves by *Murgantia histrionica* (Heteroptera: Pentatomidae) adults is visible immediately, whereas that provoked by *Nezara viridula* (Heteroptera: Pentatomidae) is not (Velikova et al., 2010). In this study was observed that the reduction of transpiration rate by two insect adults occurred faster than the reduction of photosynthetic rate and stomatal conductance.

The gas-exchange data offers important insights into the effect of *T. limbativentris* on rice physiology. Results reported in this work indicate that injury

by *T. limbativentris* adults can affect paddy rice physiology, even at low densities. Once the recommended economic thresholds for *T. limbativentris* in upland rice was between 2 and 4 stink bugs per 15 stalks sampled in the vegetative stage, and between 1 and 2 stink bugs per 15 stalks sampled at the beginning of reproductive stage (R3/R4) (Krinski & Foerster, 2017). Yet, our results indicate that the photosynthetic rate of paddy rice might be not immediately impacted by *T. limbativentris* injury. Instead, transpiration rate may be more immediately impaired by *T. limbativentris* feeding. This finding is valuable in focusing additional research efforts and may provide a physiological target for developing stink bugs-resistant rice (Macedo et al., 2003). Additional research on mechanisms underlying photosynthetic responses of rice to *T. limbativentris* injury is needed, as well as the time of infestation (Santana et al., 2018). Beyond further work regarding the role of *T. limbativentris* injury on photosynthetic responses, studies detailing rice responses across phenological stages and at different stink bugs intensities are needed.

## Conclusion

The results of present study indicate that substantial physiological impact on paddy rice by *T. limbativentris* adults is possible. Effect in the photosynthetic rate is observed only after 72 h of infestation with four *T. limbativentris* adults. However, there is no effect in the photosynthetic rate and stomatal conductance of paddy rice with infestations of up to two insect adults per ten stalks. Transpiration rate is affected 24 h after infestation with both two and four *T. limbativentris* adults per ten stalks. These findings indicate that infestation time and population level of *T. limbativentris* adult during early stages of paddy rice should be considered in management of this pest.

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