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OCCURRENCE OF DIAPAUSE AND THE ROLE OF *ANDROPOGON BICORNIS* (POACEAE) TUSsockS ON THE SEASONAL ABUNDANCE AND MORTALITY OF *TIBRACA LIMBATIVENTRIS* (HEMIPTERA: PENTATOMIDAE)

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ABSTRACT

The rice stalk stink bug, *Tibraca limbativentris* Stål (Hemiptera: Pentatomidae), is a major pest of rice crops throughout Latin America. We investigated the occurrence of diapause in *T. limbativentris*, as well as the role of West Indian foxtail, *Andropogon bicornis* L. (Poaceae), in its seasonal abundance and mortality. This plant grows spontaneously in grasslands from Mexico to Argentina, including at the edges of rice fields in southern Brazil. Tussocks of *A. bicornis* were collected in Eldorado do Sul, State of Rio Grande do Sul, Brazil throughout one yr and examined to sample rice stalk stink bugs. We collected in 2,355 *T. limbativentris* adults from 208 tussocks, totalling 2,205 live and 150 dead individuals. Live insects were dissected to determine the reproductive stage and to assess the presence of the fat body. We describe the occurrence of imaginal diapause and the use of *A. bicornis* as a shelter for this rice pest. Overwintering lasted 7 mo; arrival at the refuge occurred in early autumn (late Mar); the permanence period began in Jun, and extended to the end of Sep. From Oct the population decreased gradually until total departure from the tussocks in Jan. Thus, here we highlight the role of *A. bicornis* as an hibernation site for *T. limbativentris* in southern Brazil.

Key Words: Diapause, rice stalk stink bug, subtropics, tussocks

RESUMO

O percevejo-do-colmo do arroz, *Tibraca limbativentris* Stål (Hemiptera: Pentatomidae), é considerada uma das principais pragas do arroz irrigado na América Latina. A ocorrência de diapausa em *T. limbativentris* foi investigada, bem como o papel do capim-rabo-de-burro, *Andropogon bicornis* L. (Poaceae), na abundância sazonal e mortalidade. Esta planta cresce espontaneamente em pastagens do México a Argentina, incluindo o entorno do cultivo de arroz no sul do Brasil. Touceiras de *A. bicornis* foram coletadas em Eldorado do Sul, Estado do Rio Grande do Sul, Brasil, durante um ano e examinadas para a amostragem os percevejos. Foram coletados 2.355 adultos de *T. limbativentris* provenientes de 208 touceiras, totalizando 2.205 indivíduos vivos e 150 mortos. Insetos vivos foram dissecados afim de determinar o estágio de desenvolvimento dos órgãos reprodutivos e avaliar a presença de corpos gordurosos. A ocorrência de diapausa imaginal e a utilização de *A. bicornis* como abrigo foram descritas. A hibernação durou 7 meses: a entrada no refúgio ocorreu no início do outono; o período de permanência iniciou em Junho, e estendeu-se até o final de Setembro. A partir de Outubro, a população decresceu gradualmente até o abandono total das touceiras em Janeiro. Desta forma, destacamos o papel de *A. bicornis* como um sítio de hibernação para *T. limbativentris*, no sul do Brasil.

Palavras-Chave: Diapausa, percevejo-do-grão-do-arroz, subtropical, touceiras

The role of grass tussocks as shelter for invertebrates has long been documented (Pearce 1948; Luff 1966). Vegetation structure of areas surrounding agricultural fields may be a factor essential to the survival of certain insects during winter (Dennis et al. 1994), by offering favorable microclimatic conditions and sites for hibernation (Geiger et al. 2009; Helden et al. 2010).

The life history of pentatomids living on soybean crops is well documented, including information on wild host plants, overwintering strategies and diapause characterization (Panizzi & Vivan 1997). On the other hand, there is little knowledge about diapause and overwintering sites of heteropterans that feed on rice (*Oryza sativa* L.) (Poaceae) in Brazil. *Oebalus poecilus* (Dallas)

(Hemiptera: Pentatomidae) is the only species that has been studied in detail in this regard (Albuquerque 1993; Santos et al. 2003, 2006).

The rice stalk stink bug, *Tibraca limbativentris* Stål (Hemiptera: Pentatomidae), is distributed throughout Latin America, including Argentina, Brazil, Colombia, Peru, Ecuador, the Dominican Republic, and Venezuela (Pantoja et al. 2007). This insect is a major pest of irrigated rice crops in southern Brazil (Botton et al. 1996) where it can be found on rice stems during the entire cycle of cultivation. The most significant losses due to this insect occur from emergence to panicle formation (Pantoja et al. 1995, 2007). Because the feeding habits of *T. limbativentris* differ from those of other rice stink bugs, we hypothesized that its life strategy throughout the year is distinct also.

In spite of the economic importance of *T. limbativentris*, there are no studies on its phenology on wild plants, especially when rice is not available in the field. *Tibraca limbativentris* was recorded supposedly hibernating on spontaneous vegetation around rice crops, especially grasses, rice stubble and tussocks (Link et al. 1996).

The West Indian foxtail, *Andropogon bicornis* L. (Poaceae), occurs in grasslands and rocky outcrops from Mexico to Argentina (Zanin & Longhi-Wagner 2006). It is a caespitose, perennial grass, with erect culms, 150-250 cm long. *Andropogon* species are frequently found in disturbed areas at early successional stages (Zanin & Longhi-Wagner 2006). In southern Brazil, tussocks of this plant dominate the landscape, growing along field perimeters and roadsides throughout the year. Thus, we investigated the occurrence of diapause and the role of *A. bicornis* tussocks on the seasonal abundance and mortality of *T. limbativentris*.

MATERIALS AND METHODS

Specimen Collection

The study was carried out in Eldorado do Sul (S 30° 02' W 51° 23'), southern Brazil. This region has extensive lowlands (10 m asl) and a subtropical climate. Irrigated rice is the main crop cultured during spring to early autumn, alternating with pastures in the winter.

The study area is composed of rice fields from Oct to Mar. *Andropogon bicornis* is the dominant weed along the boundaries of these rice fields. This species is the only weed in the area forming tussocks dense enough to host stink bugs.

Sampling was performed fortnightly on *A. bicornis* tussocks from Apr 2010 to Mar 2011 on 4 transects, each 30-50 m long and 4-20m wide. The sites were located on the boundaries of rice fields adjacent to roadsides. For each transect and each sampling date, we removed 2 tussocks from op-

posite ends, at least 4 m apart and up to 30 m from the field.

Laboratory Analysis

In the laboratory, adults of *T. limbativentris* were visually inspected, counted and sexed. The insects were kept at 4 °C for dissection for up to 4 d after being collected. We dissected up to 32 specimens on each occasion, taking care to dissect similar numbers of insects from different tussocks, as well as even numbers of males and females.

The urosternites were removed for examination of reproductive organs and fat body quantification. We quantified the amount of fat body according to the proportion occupying the abdominal cavity: 1 = one third; 2 = two thirds and 3 = completely filled. Diapause was defined by a high level (completely filled) of fat body and immature reproductive organs, as described by Mielitz et al. (1996).

Vouchers were deposited in the collection of Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre, Brazil (MCNZ).

Statistical analysis

Data were evaluated for normality using the Shapiro-Wilk and D'Agostino-Pearson tests. The arrival, permanence and departure from refuge were determined by comparison of mean abundance among grouped sampling occasions by using the Kruskal-Wallis test. Differences between seasons were tested using the Mann-Whitney U-test. The correlation between the mean number of individuals and crop distance or tussock diam was analyzed using Spearman's rank correlation coefficient. Sex-ratio was compared by the chi-square test of adherence. In all analyses, we used a 0.05 significance level; all analyses were performed using Bioestat 5.0 (Ayres et al. 2007) or Past 2.02 (Hammer et al. 2001) software.

RESULTS

We collected 2,355 adults of *T. limbativentris* from 208 tussocks, totalling 2,205 live and 150 dead individuals. Distribution of the sexes was similar across tussocks; the sex-ratio was 0.5043 (1,112 females and 1,093 males), $P = 0.6858$.

Live individuals were most abundant in the tussocks during the winter ($n = 1,454$ individuals), followed by the spring ($n = 455$), autumn ($n = 292$) and summer ($n = 4$). The number of live individuals did not differ significantly between spring and autumn ($P = 0.3785$; $U = 12.5$).

Tussocks were 11-47 cm wide. A weak correlation was found between tussock diam and mean

abundance ($r_s = 0.273$; $P = 0.0006$). Distance from the crop varied from 1 to 30 m. There was no association of this variable with the mean number of individuals found on the tussocks ($r_s = 0.095$; $P = 0.2419$).

The hibernation period lasted 7 mo. Individuals arrived at the refuge around the end of Mar. The permanence period began at the end of Jun, extending through to the end of Sep. From mid-Oct the population decreased gradually. Total departure from the tussocks was observed in Jan 2011 with no new individuals being captured (Fig. 1).

We dissected 466 adults of *T. limbiventris*, corresponding to 21.1% of the total number collected. The abdominal cavity of both males and females was more than two-thirds filled with fat body at the beginning of the refuge arrival period (Apr) (Fig. 2). Aside from the large amount of fat body, females invading the hibernation refuge exhibited immature reproductive organs, with co-alescent ovarioles. We found 7 post-reproductive females bearing wrinkled (corrugate) ovarioles, suggesting that their oocytes had discharged. In contrast, hibernating males did not show such evident variation in testes development.

An increasing proportion of dead individuals was detected from Aug until Jan, when the highest mortality was reported (Fig. 1). Most of the dead individuals (86%) presented mycelial growth on the external body surface, 5.3% were parasitized and 8.7% showed no alterations. The fungus was identified as *Beauveria bassiana* (Vuill.).

DISCUSSION

In this study, we investigated the life strategies of *T. limbiventris*, and assessed the role that the West Indian foxtail, *A. bicornis*, plays in its life history. Analysis of the fat body and reproductive stage, in the context of its population dynamics, provided evidence for the first time that *T. limbiventris* undergoes an imaginal diapause in *A. bicornis* tussocks. Both males and females arrived sexually immature at the refuge, with a large amount of fat body.

In southern Brazil, diapause has been recorded for some insects such as *O. poecilus*, the grain rice stink bug associated with rice crops (Albuquerque 1993). This pentatomid begins to arrive at refuge in mid-Mar and concludes the departure in Oct (Albuquerque 1993). At our latitude, studies have shown that the hibernation of *O. poecilus* lasts 7 to 8 mo (Santos et al. 2006).

Oebalus poecilus leaves its hibernation sites during the panicle formation (Jan) and grain filling (Feb) stages (Santos et al. 2006). In contrast, the rice stalk stink bug initiates departure in mid-Sep, thus colonizing the rice crop before *O. poecilus*. Damage caused by *T. limbiventris* is recorded in plants older than 20 d (Panizzi et al. 2000) and the insect stays on the crop during its entire cycle. Another insect that is frequently seen in rice crops, *Oryzophagus oryzae* (Costa Lima) (Coleoptera: Curculionidae), also shows diapause in the studied region. The arrival of this rice water weevil occurs from Feb to Mar and its

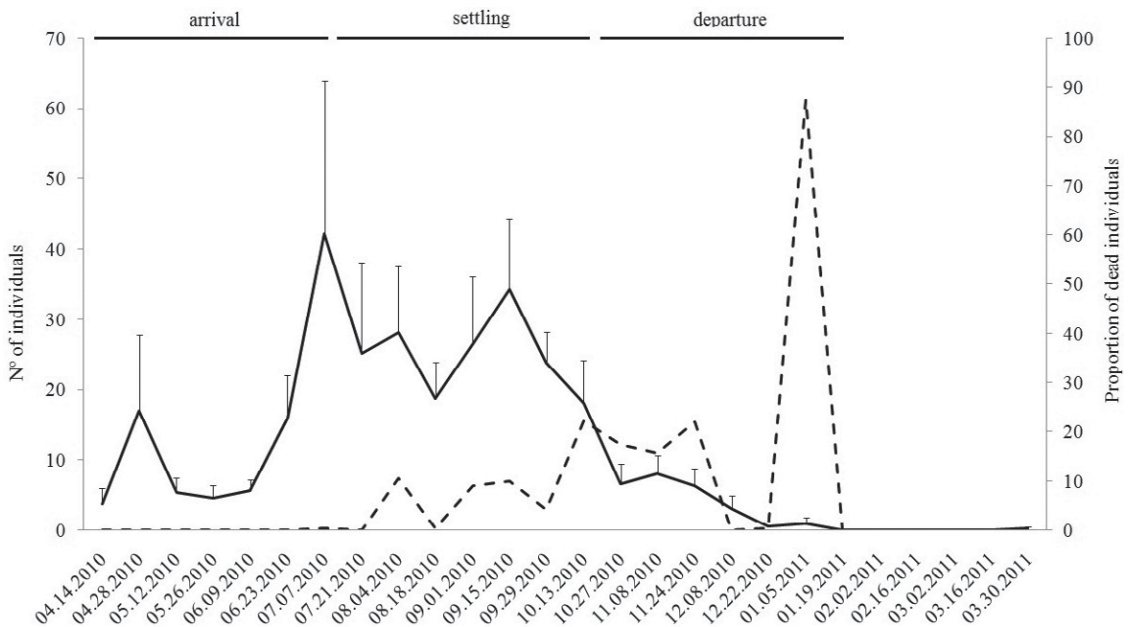


FIG. 1. Temporal variation in the mean number of adults (—) and the proportion of dead individuals (----) of *Tibraca limbiventris* sampled in *Andropogon bicornis* tussocks per sampling occasion (Eldorado do Sul, RS, Brazil: 2010-2011).

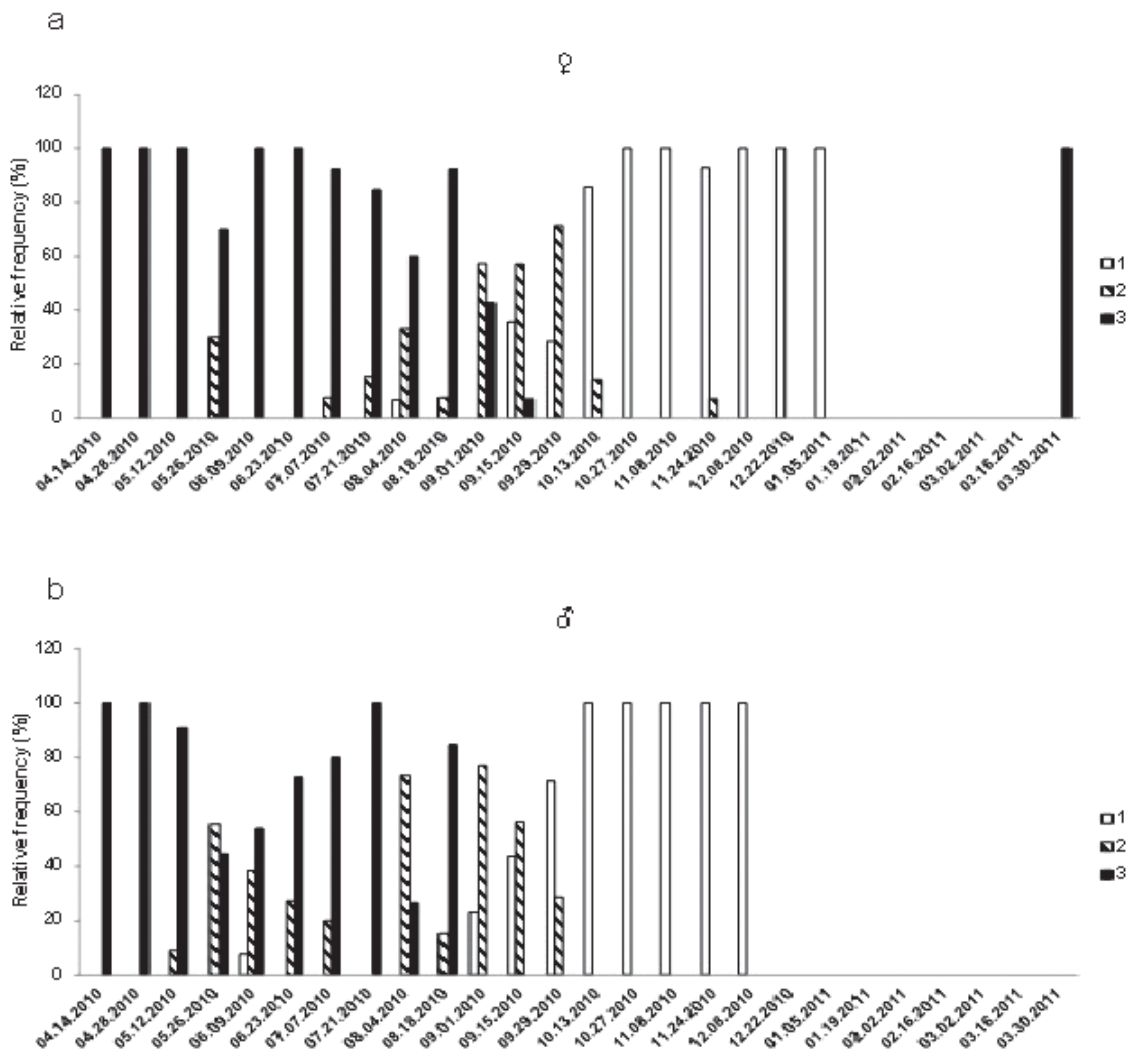


FIG. 2. Percentages of *Tibraca limbativentris* individuals with fat body levels 1, 2 and/or 3 from Apr 2010 to Mar 2011, where 1, 2 and 3, respectively, indicate 1/3 rd, 2/3 rds and 3/3 rds of the abdominal cavity filled with the fat body; a. females and b. males.

departure begins in mid-Aug (Mielitz et al. 1996). The seasonal synchrony among these species may be related to their food specificity, all of them having rice as their main host.

Insects in imaginal diapause may show physiological changes, such as fat body storage, reduced oxygen consumption, and cessation of development and cessation of gametogenesis (Denlinger 2002; Tauber et al. 1986). These traits have been used to describe diapause in other stink bugs, e.g. *Oebalus pugnax* (Fabricius) (Nilakhe 1976), *Euschistus heros* (Fabricius) (Mourão & Panizzi 2000) and *O. poecilus* (Santos et al. 2003).

The accumulation of reserve substances before entering into the hibernation site was pointed out by Tauber et al. (1986) and Santos et al. (2003).

The predominance of individuals with only one-third of their abdomens filled with body fat from mid-Sep indicates that they had consumed most of the reserves during overwintering. Redaelli et al. (1995) found a reduction in the amount of fat body near the time of total departure from the refuge by the rice water weevil. A similar observation was made by Santos et al. (2003) for *O. poecilus*.

The female reproductive organs remained immature during the entire hibernation period. The presence of post-reproductive females suggests copulation had occurred before hibernation. Insects with imaginal diapause usually copulate after this period (Tauber et al. 1986). A similar pattern was observed in hibernating *O. poecilus*

in which females also became mature after leaving hibernation sites (Santos et al. 2003). Copulation before winter was associated with enhanced female survival during overwintering in *Orius* species (Hemiptera, Anthocoridae). In these bugs, only females hibernate. Their success may be related to nutrient transfer from the male during copulation (Kobayashi & Osakabe 2009).

We did not observe seasonal dimorphism between hibernating and non-hibernating in *T. limbiventris*, as reported for *E. heros* (Mourão & Panizzi 2000) and *O. poecilus* (Albuquerque 1993). In both species, active individuals are darker and have more prominent humeri.

Increased mortality during diapause followed the pattern described for *O. poecilus* (Santos et al. 2006) and *O. oryzae* (Mielitz et al. 1996). These authors pointed out that natural mortality increases because of the lack of sufficient reserves to survive through this period. Nevertheless, in our study, the low proportion of dead individuals found compared to the total number of individuals sampled, indicates that a high number of individuals survive during the winter and are able to colonize the next rice crop.

The greater proportion of dead bugs infected by *B. bassianais* probably related to moisture in the tussocks, which favours the establishment and dissemination of pathogens. The same fungus was isolated from hibernating rice water weevils by Mielitz et al. (1996) and from *O. poecilus* by Santos et al. (2006). However, we do not know if this fungus is actually entomopathogenic or whether it simply uses the dead insect as substrate for its mycelial development. This fungus species has been studied for biological control in rice production areas (Martins et al. 1997). The role of natural enemies in regulation of hibernating *T. limbiventris* populations is poorly understood. Management procedures that increase mortality during hibernation, such as a biological control could minimize the current use of chemicals on rice.

Andropogon bicornis is a permanent and widespread plant in southern Brazil. It is a permanent shelter resource for various arthropod species. This grass is a more stable environment for *T. limbiventris* than rice stubble, which is removed as an agricultural practice. West Indian foxtail, a perennial weed, serves as a refuge for *T. limbiventris* when rice is not available in the field.

Considering the importance of the rice stalk stink bug in other latitudes of Latin America, further studies are necessary to investigate its life strategies under different climatic conditions and rice crop management practices.

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