

# Use of Leaves Treated with Hydrogen Peroxide in Case-Building by *Phylloicus* spp. (Trichoptera: Calamoceratidae)\*

Thyago Augusto da Silva Vidovix<sup>✉</sup>, Renato Tavares Martins, Cleber Duarte & Ana Maria Oliveira Pes

Instituto Nacional de Pesquisas da Amazônia, e-mail: [thyagovidovix@gmail.com](mailto:thyagovidovix@gmail.com) (Autor para correspondência<sup>✉</sup>), [martinsrt@gmail.com](mailto:martinsrt@gmail.com), [cleberduarte08@gmail.com](mailto:cleberduarte08@gmail.com), [anampes@gmail.com](mailto:anampes@gmail.com).

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**Abstract.** The objective of this study was to assess if larvae of *Phylloicus* spp. show any preference for leaves with or without microorganisms when building cases. Two experiments were realized at municipality of Presidente Figueiredo (Amazonas – Brazil), containing leaves treated with Hydrogen peroxide ( $H_2O_2$ ), a compost that has an antimicrobial action, and another one with untreated leaves. In the first were observed the case-building by larvae of *Phylloicus* spp. and in the second was tested the preference by treated or untreated leaves. The larvae were monitored during 24 hours and to statistical analysis was used the t-test. Were not observed significant differences in the number of discs used by the larvae in the first experiment ( $p=0.24$ ;  $t=-0.73$ ) and no preference by type of leaf in the second experiment ( $p=0.41$ ;  $t=0.23$ ). Therefore, we do not recommend the use of hydrogen peroxide in studies that aim to eliminate microorganisms from decomposing leaves.

**Keywords:** Amazon; aquatic insects; larvae; leaf litter; microorganisms.

## Uso de Folhas Tratadas com Peróxido de Hidrogênio na Construção de Abrigos por *Phylloicus* spp. (Trichoptera: Calamoceratidae)

**Resumo.** O objetivo deste estudo foi avaliar se larvas de *Phylloicus* spp. possuem preferência por folhas com ou sem micro-organismos para a construção de abrigos. Dois experimentos foram realizados no município de Presidente Figueiredo (Amazonas – Brasil), contendo folhas tratadas com peróxido de hidrogênio ( $H_2O_2$ ), composto que possui ação antimicrobiana, e folhas não tratadas. No primeiro observaram-se a construção de abrigos pelas larvas de *Phylloicus* spp. e no segundo testou-se a preferência por folhas tratadas ou não tratadas. As larvas foram monitoradas durante 24 horas e para a análise estatística foi utilizado o teste-t. Não foram observadas diferenças significativas no número de discos usados pelas larvas no primeiro experimento ( $p=0,24$ ;  $t=-0,73$ ) e nem na preferência pelo tipo de folha no segundo experimento ( $p=0,41$ ;  $t=0,23$ ). Portanto, não recomendamos o uso do peróxido de hidrogênio em estudos realizados em laboratório que visem à eliminação de micro-organismos de folhas em decomposição.

**Palavras-chaves:** Amazônia; folhicho; insetos aquáticos; larvas; micro-organismos.

The larvae of *Phylloicus* spp. (Calamoceratidae) are usually found in bodies of water of low flow and with submerged leaves (WANTZEN & WAGNER 2006). The leaves are used by these organisms as food or for case-building (RINCÓN *et al.* 2005). Although energetically demanding, case-building provides protection against predators (BOYERO *et al.* 2006). Since *Phylloicus* cases are relatively large, the larvae usually build cases with less palatable leaves and with less microbial presence to minimize the influence of leaf shredding invertebrates (GRAÇA 2001).

Leaf palatability is increased by fungal and bacterial activity on this material (GRAÇA & CRESSA 2010). The crustacean *Gammarus minus* Say 1818 (Amphipoda) showed preference for leaves with fungi rather than leaves without fungi (KOSTALOS & SEYMOUR 1976). JABIOL & CHAUVET (2012) verified that the consumption rate of *Gammarus fossarum* Koch was higher in leaves that had associated fungi, and also showed preference for certain types of fungi. Larvae of *Hesperophylax* sp. and *Psychoglypha* sp. (Trichoptera: Limnephilidae) have the ability to locate fungi on leaves, and the degree of success in locating them varied according to the level of fungal colonization (ARSUFFI & SUBERKROPP 1985).

Hydrogen peroxide ( $H_2O_2$ ) is used to eliminate microorganisms from the leaf surface, and thus make the leaves less palatable to shredding invertebrates. Due to the formation of free hydroxyl radicals,  $H_2O_2$  can be detrimental to the development of these

microorganisms since it disrupts the cytoplasmic membrane, DNA and other essential cellular structures (REES & ORTH 1986). Since the objective of this study was to test if *Phylloicus* spp. larvae show preference for leaves with or without microorganisms for case-building, hydrogen peroxide was used to eliminate microorganisms from the leaves to test this preference. Two questions were posed: (1) Do larvae of *Phylloicus* spp. use leaves with and without microorganisms for case-building? (2) Do larvae of *Phylloicus* spp. prefer leaves without microorganisms for case-building?

## MATERIAL AND METHODS

The experiments took place in October/2011 in one of the tributaries of the Igarapé da Onça (2°02'S, 59°50'W), Presidente Figueiredo, Amazonas, Brazil. In this study 40 larvae were collected with D-nets, removed from their cases and placed in plastic containers covered with a 1 mm mesh screen. The containers were then placed in a low flow area of the stream. Submerged leaves conditioned by microorganisms were obtained, from the same area as the larvae, and were fragmented into 60 mm diameter discs.

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Two treatments were used, on the first experiment to observe the construction of cases by *Phylloicus* spp.: leaves treated in H<sub>2</sub>O<sub>2</sub> for an hour and untreated leaves. There were 10 replications for each treatment, with eight leaf discs in each container. The difference between the numbers of discs used by the larvae in both treatments was verified by the t-test through the statistical analysis software R (R DEVELOPMENT CORE TEAM 2012).

The preference for H<sub>2</sub>O<sub>2</sub> treated or untreated leaf was tested on the second experiment. There were 20 replications with 16 discs (eight treated and eight untreated) in each container. The t-test was also used to test their preference for one of the treatments. In both experiments the larvae were monitored for 24 hours.

## RESULTS AND DISCUSSION

Most of the *Phylloicus* spp. larvae (77.5%) built their cases 3 hours after they started to manipulate the leaves. The behavior while constructing their cases was the same as observed by NORWOOD & STEWART (2002) for *Phylloicus ornatus* Banks, and for *Phylloicus* sp. (MORETTI et al. 2009). The cases made during the current experiments had size and shape similar to the original cases, an observation also made by NORWOOD & STEWART (2002). But, in the current study, one of the larvae used 11 discs, much more than the original size of its case, which can make this specimen more susceptible to the action of leaf-shredding organisms and predators (GRAÇA & CRESSA 2010).

In the first experiment, *Phylloicus* spp. larvae constructed cases with H<sub>2</sub>O<sub>2</sub> treated and untreated leaves. The larvae used a mean of 2.7 untreated and 3.2 H<sub>2</sub>O<sub>2</sub> treated leaves, with no significant difference observed for the number of discs used (p=0.24; t=-0.73). H<sub>2</sub>O<sub>2</sub> did not inhibit the use of the discs for the construction of cases by the larvae.

In the second experiment a mean of 3.81 discs were used by the larvae. No significant difference was observed in the preference for either type of leaf (p=0.41; t=0.23), being 48.7% untreated and 51.3% H<sub>2</sub>O<sub>2</sub> treated leaves. Therefore, we assume that the disc choice was not affected by the use of hydrogen peroxide.

The reduced preference was expected due to the antibacterial action of H<sub>2</sub>O<sub>2</sub> MATTOS et al. (2003). However, THÉRAUD et al. (2004) did not observe the efficiency of H<sub>2</sub>O<sub>2</sub> against the fungus *Candida albicans* (Robin) Berkhout 1923. Taking into account that fungi are a major part of the decomposers biomass BALDY et al. (1995), H<sub>2</sub>O<sub>2</sub> might not have been efficient in their elimination, which would explain the lack of significant difference between the two treatments. Another hypothesis is that the hydrogen peroxide was not efficient in eliminate other microorganisms from the leaves surface, which did not alter their palatability. Furthermore, disc choice might have been influenced by the use of leaves from different plant species and in different stages of decomposition. According to RINCÓN et al. (2005) and BASTIAN et al. (2007), *Phylloicus* sp. larvae and *Anisoxentropus* sp. (Calamoceratidae) prefer tougher leaves, and with higher quantity of polyphenols, for case construction.

Our results indicate that the H<sub>2</sub>O<sub>2</sub> treatment did not influence in the leaf disc choice of *Phylloicus* spp. larvae. Therefore, we do not recommend the use of H<sub>2</sub>O<sub>2</sub> for studies in laboratories that aim at eliminating microorganisms from decomposing leaves.

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