First report of *Monalonion bondari* (Hemiptera: Miridae) as the causal agent of lateral shoot dieback in *Eucalyptus* spp. in Brazil and its control strategies

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Abstract

Lateral shoot dieback has emerged as a significant disorder affecting *Eucalyptus* spp. plantations in Brazil, with increasing incidence and unclear etiology. During field observations, plant bug species (Miridae) were consistently found in symptomatic areas, raising the hypothesis of their involvement in the onset of the disease. This study aimed to identify the insect species associated with dieback symptoms, evaluate their ability to induce damage under controlled conditions, and test the efficacy of insecticides for population control. Morphological and molecular analyses confirmed the presence of *Monalonion bondari*, a mirid species previously reported as a pest in cocoa (*Theobroma cacao*), now observed infesting young Eucalyptus trees. Experimental infestations with *M. bondari* led to the development of characteristic shoot dieback symptoms, confirming its role as a causal agent. Furthermore, field trials demonstrated that applications of thiamethoxam and bifenthrin significantly reduced the insect population, with protective effects for up to 15 days post-application. These findings represent the first report of *M. bondari* damaging eucalypt in Brazil and provide evidence-based strategies for its chemical control. Implementing targeted monitoring and pest management can mitigate damage and enhance plantation sustainability

Keywords: Eucalypt disease, sucking pest, integrated pest management.

1 INTRODUCTION

Eucalypt is a tree of significant economic value in various regions worldwide, including Brazil. However, the emergence of pests and diseases has been impacting commercial plantations, leading to considerable crop losses (Wingfield et al. 2008). A recently identified and severe disease, initially attributed to *Pseudoplagiostoma eucalypti* (Fungi: Ascomycota: Pseudoplagiostomataceae), has been affecting plantations across several Brazilian states, including Bahia, Espírito Santo, and Minas Gerais (Zauza et al. 2023). Typical symptoms of the disease include leaf spots, branch cankers, shoot blight, defoliation, and dieback (Zauza et al. 2023). These symptoms have been observed in nearly all hybrid clones of *Eucalyptus* spp.

Although Zauza et al. (2023) demonstrated that *P. eucalypti* can cause lateral shoot die-back under high humidity conditions (relative humidity above 90%), this fungus, as an endophyte often co-occurring with other pathogens, typically plays a secondary role in disease development (Crous et al. 2019). Therefore, it is unlikely to cause significant damage in a short time frame. In February 2023, in a field inspection of highly infested eucalypt trees in the South of Bahia, we observed near the lesions insect eggs and alive insects (Figure 1), belonging to the Miridae family (plant-bugs). This observation has led to the question whether these insects could be associated with or if potentially could cause the disease. Disease is defined here as "any abnormal condition of a plant that interferes with its normal functioning, growth, or reproduction"(Agrios 2009). Indeed, plant-bugs are insects that cause diseases in several crops (Muhamad and Way 1995; Montilla Pérez et al. 2014; Roy et al. 2015; Kodakkadan et al. 2020). In Indonesia, for example, the species *Helopeltis theivora* (Heteroptera: Miridae) have been reported causing lateral shoot dieback, curling, deformation, and drying usually, irrespective of the clones (Kodakkadan et al. 2020). In Brazil, plant-bugs have not yet been reported as causing diseases in eucalypt trees. However, the state of Bahia, a key region for cocoa production (*Theobroma cacao* L.: Malvaceae), faces significant damages by *Monalonion bondari* Costa Lima 1938, a Miridae species. This insect is one of the main pests affecting cocoa plantations, where it is responsible for both lateral shoot dieback of the trees and damage to the fruits. The damage caused by this pest in cocoa production emphasizes its potential risk to other crops, including eucalypt, particularly due to its feeding behavior, which can lead to significant physiological stress in plants.

Chemical control has been the predominant method for managing insects in the Miridae family, particularly for species that cause significant damage to crops (Rahib Chowdhury et al. 2013; Zhang et al. 2015; Dumont and Provost 2019). Among the most effective insecticides are molecules such as thiamethoxam and bifenthrin, which have shown high efficiency in controlling *Helopeltis theivora*, a major pest in tea crops (Rahib Chowdhury et al. 2013; Zhang et al. 2015). Their success in tea plantations highlights their potential application for managing similar pests in other crops, including plant-bugs, such as *Monalonion bondari*. These insecticides are expected to be effective to reduce significantly the insect population in eucalypt plantations. The objectives of this study were to identify the insect species collected in *Eucalyptus* spp. in southern Bahia, assess the potential impact of *M. bondari* feeding on the health of the plants inducing the shoot dieback symptons, and evaluate the effectiveness of insecticides for its control.

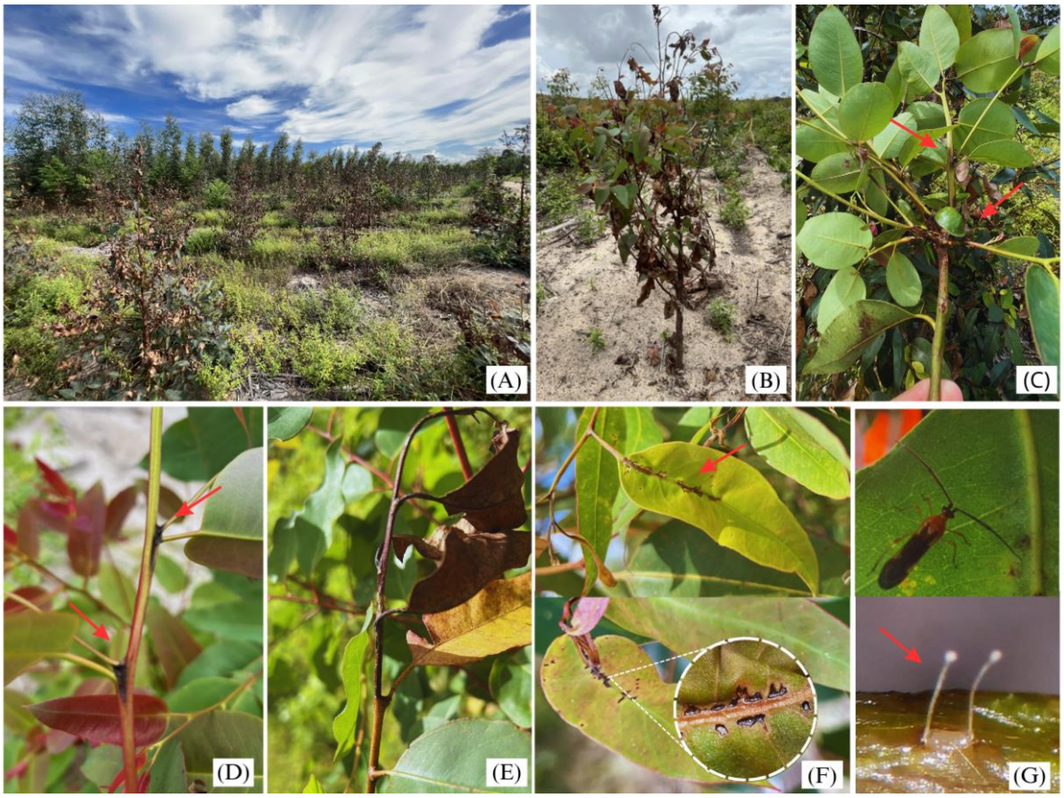

Figure 1 – Symptoms of lateral shoot dieback in *Eucalyptus* spp in the field, caused by *Monalonion bondari*: A- Affected trees; B- Detail of lateral shoot dieback of a single tree; C – Minicankers and secondary shoot emergence (arrow) at the insect feeding site; D- Dark lesions at the point of insect attack; E – Generalized dieback in a severely affected tree. F- Lesions on the main leaf vein; G – Detail of the adult insect and eggs (arrow) on the underside of the leaf.

2 MATERIAL AND METHODS

Morphological characteristics

A total of thirty insect specimens were collected from eucalypt plantations exhibiting severe disease symptoms (Figure 2). These specimens were carefully preserved in 90% ethanol to maintain their integrity for further analyses. They were then sent to the Department of Animal Biology at the Universidade Federal de Viçosa, where they underwent a detailed morphological examination and identification. This step was crucial to determine the species involved and assess their potential role in the disease progress. The symptoms of lateral shoot dieback include necrotic lesions and mini cankers at branch insertions, petiole necrosis, excessive sprouting, and lesions along the veins (Zauza et al. 2023). These symptoms are most commonly observed in plants between 2 to 6 months after planting, age that we collected the insects in the field.

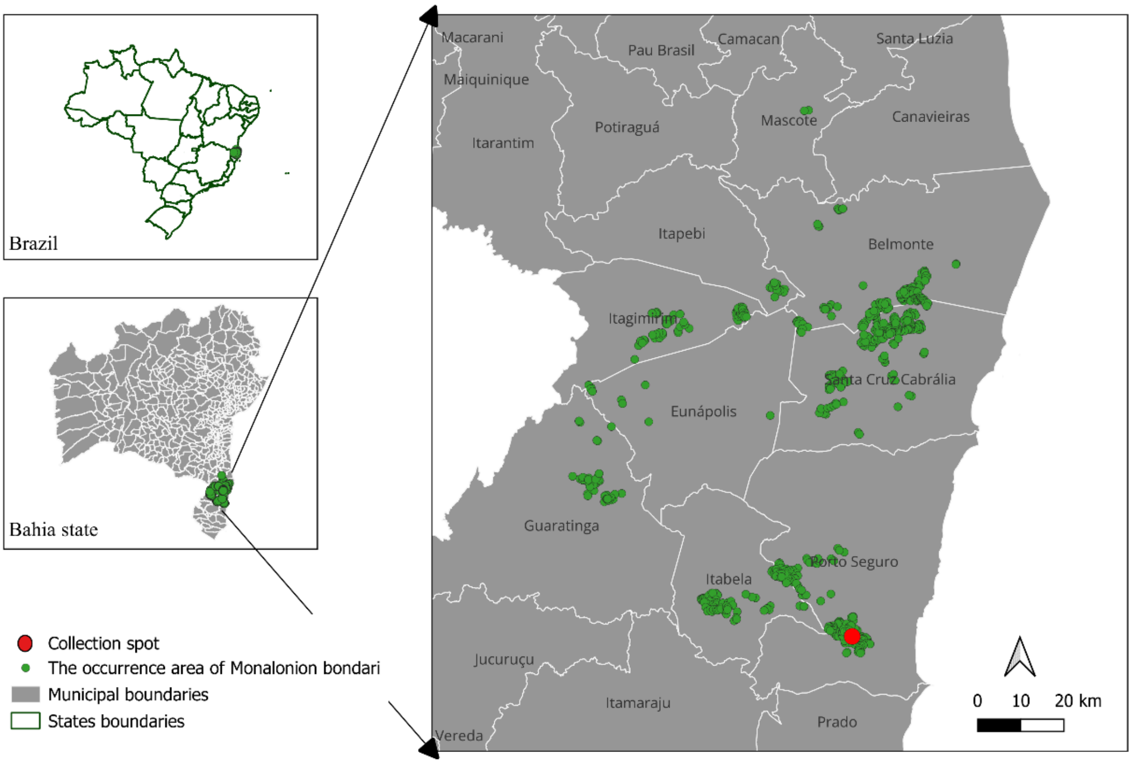

Figure 2. Distribution of *Monalonion bondari* infesting eucalypt plantations, along with the insect collection areas located in regions displaying lateral shoot dieback. The map highlights the specific zones where the insect was observed and collected, coinciding with the presence of dieback symptoms in the affected plantations.

Molecular analysis

Total genomic DNA was extracted from five *M. bondari* previously conserved in ethanol 99.5%. The extraction followed the recommendation from the Wizard® Genomic DNA Purification Kit from Promega.

The cytochrome c oxidase subunit I (COI) gene fragment was amplified by polymerase chain reaction (PCR) using the universal primers LCO1490 (5′ GGTCAACAAATCATAAAGATATTGG 3′) and HCO2198 (5′ TAAACTTCAGGGTGACCAAAAAATCA 3′) (Folmer et al., 1994). The PCR was performed in a total of 25 μL containing 3 μL genomic DNA (gDNA), 10.7 μL MilliQ-H_2O, 2.5 μL 10X PCR Buffer Mg^{2+} free (Sinapse Inc) 2.0 μL $MgCl_2$ (25 mM) (Sinapse Inc), 2.0 μL dNTP (2.5 mM) (Sinapse Inc), 2 μL of each primer (5 μM), and 0.3 μL Taq DNA Polymerase (5 U μL^{-1}) (Sinapse Inc). The PCR amplification conditions used were 94 °C for 3 min for primary denaturation, then 35 cycles at 94 °C for 30 s, 53 °C for 45 s, 72 °C for 2 min, with a final extension at 72 °C for 10 min. PCR amplicons were visualized under ultraviolet light after electrophoresis using 5 μL amplicon in 1.5% (w v^{-1}) agarose gel stained with SYBR Safe (Life Technologies). The subsequent purification process was performed using a mix of Exo-Sap (Exonuclease/Alkaline Phosphatase) (Cellco Biotech). The bidirectional sequencing was performed by the Sanger method using the same PCR primers at the Molecular Biology Laboratory CEBTEC at ESALQ, University of São Paulo.

Induction of disease symptom

Three *M. bondari* insects at different developmental stages (1 adult and 2 nymphs), collected from the field, were taken to the Forest Health laboratory at Veracel and Pulp Company and Suzano (Eunápolis, Bahia) for further analysis. The insects were then introduced into an entomological cage containing five healthy, 3-month-old rooted cuttings of *Eucalyptus urophylla* x *Eucalyptus grandis* (clone 2123). These saplings were chosen to represent young, healthy plants for monitoring potential damage. To ensure accurate results, another set of five samplings from the same clone was placed outside the cage, completely separated from the insects to prevent any contact. After 24 h, the plants inside the cage were inspected for the development of dieback symptoms, including the presence of necrotic lesions, cankers, or other symptoms of injury. This setup allowed for the assessment of the potential impact of *M. bondari* on the health of the plants under controlled conditions.

Effect of insecticides for disease control

The plot of 4-month-old of *E. urophylla* x *E. grandis* (clone 2123) with severe dieback symptoms was selected. The plot was divided into three blocks containing 10 planting rows. In each block, 20 plants were randomly selected to be sampled in order to evaluate the initial insect infestation.

Before insecticide spray, the insects present in the plants were counted (Census) and the treatments (Table 1) were subsequently applied according to the manufacturer's recommendations.

Table 1. Insecticides tested against *Monalonion bondari*

Treatment	Dose	Volume of spray solution
Thiamethoxam	200 mL/100 L	200L/ha
Bifenthrin	300g/ 100 L	200L/ha
Control*	-	-

*There was no spraying of insecticides.

After 24 h, the insects were counted again to ascertain the effectiveness of the treatment. In addition, a control group of insects was maintained without the application of the treatment, to ensure that the reduction of the insect population did not occur naturally. To evaluate the symptoms after the application of the treatment, counts of the insects present in the plants were performed at 1, 7 and 15 days after application.

A generalized linear model of quasipoisson (GLM) was used to investigate the effect of treatments and time (days) after spraying on the reduction of the insect number in each block. The selection of the best model was based on the lowest AIC value among the models. The quality of the GLMs was evaluated by checking the normality of the model's residual distribution through a Q-Q plot and plotting the model's residuals against the fitted values to verify their homoscedasticity. All analyses were performed, and graphics generated with R 3.4.1 (R Core Team 2023). The package multcomp (Hothorn et al. 2008) for the multiple comparison analysis, and the package MuMIn (Barton 2018) were used to select a model through AIC.

3 RESULTS

Morphological characteristics

Monalonion differs from the other genera of the Monaloniini tribe by the following characters (Carvalho 1972; Gamboa et al. 2020): large size (more than 8 mm); elongated and shiny body with a short, broad head. Eyes occupying the anterior region of the head with posterior region tapering towards the thorax, making a short neck. Labium not reaching the middle coxa. Antennal segment I pear-shape, and shorter than the length of the head; antennal segments II–IV filiforme. Hemelytron fully covering the abdomen, membrane with a single very elongate cell; tarsi with large, fleshy, divergent pseudopulvilli weakly dilated or thickened; antenna as long or longer than body to apex of membrane.

Monalonion bondari Costa Lima, 1938 is characterized and distinguished from other species of the genus by the following characteristics (Carvalho 1972): general dark brown color with reddish abdomen; black pronotum with three light bands, generally reddish brown; scutellum black with a pale longitudinal stripe; dark hemelytra with pale spots at the base of the clavus and embolium; cuneus and membrane veins dark brown to reddish; darkened membrane. Antennal segment I globose with a lighter color in the basal half (male); antennal segment III cylindrical, the same thickness or thinner than segment II. Legs with posterior femurs without a white ring or stripe, more tapered in the median portion and with a uniform light color (sometimes darker at the apex or base), anterior and medial tibiae predominantly pale, and posterior tibiae dark. Male genitalia with left paramere enlarged in the median region and sharply tapering towards the apical region, ending in a lobe with a sharp apex; right paramere narrow with median sinuosity and blunt apex.

Molecular characterization

Forward and reverse sequences were analyzed together, but as they generated different sizes, a consensus sequence was made, according to the usual procedure for generate the barcode COI sequences. The sequences were edited in a consensus size of 685 pb. Evidence of NumtS presence was not found in the sequences. The five sequences of *M. bondari* resulted in four haplotypes (NCBI accession numbers H1: OR961527; H2: OR961528; H3: OR961529; H4: OR961530; H4: OR961531).

Induction of disease symptom

At 24 h of the introduction of the insects in the cage (Figure 3 A), all plants expressed dieback symptoms (Figure 3 B and C) as observed in the field and described by Zauza et al. (2023). Differently, plants outside the cage did not show any symptoms and remained healthy. After five days of incubation, all plants showed dryness in all apical young shoots

(Figure 3C). That insects introduced into the cage successfully inserted their stylets into the plants, suggesting potential feeding activity. Moreover, female insects were observed depositing eggs within the plant tissues (Figure 3B).

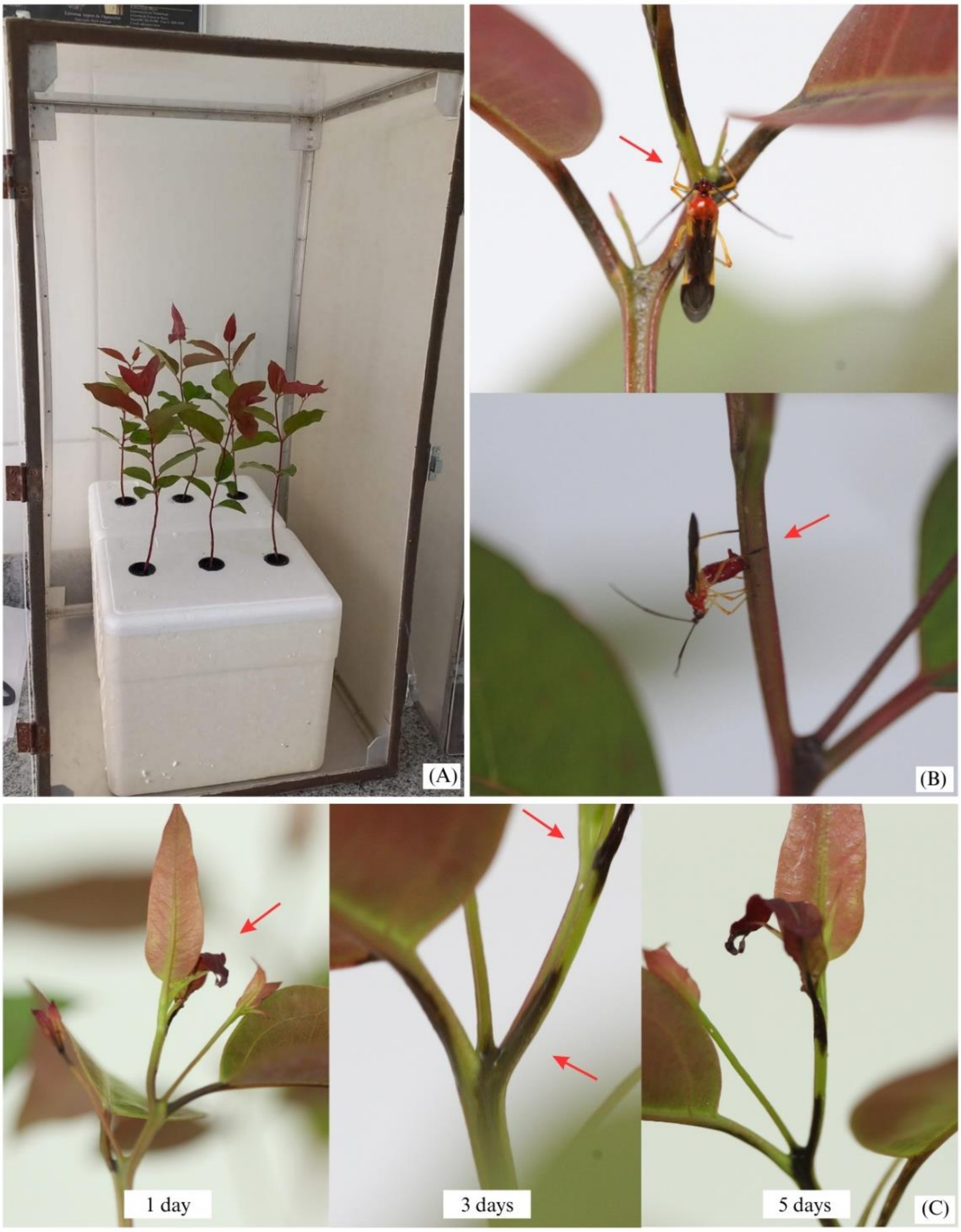

Figure 3. Reproduction of disease symptoms caused by *Monalonion bondari* on a hybrid clone of *Eucalyptus grandis* × *E. urophylla* under controlled conditions. A – Cage containing healthy plants exposed to insect attack. B – Insect feeding on a rooted eucalypt cutting by inserting its stylets. C – Evolution of disease symptoms at 1, 3, and 5 days, respectively, after the introduction of the insects into the cage.

Effect of insecticides for disease control

The number of insects after spraying the insecticides was significantly lower than that from the census (insects counted before spraying) and the control (area without spraying insecticides) ($p<0.001$), regardless of the time after application (Figure 4). The average number of insects found in the insecticide treatments did not differ from each other, Thiamethoxam 0.22 ± 0,076 (mean ± Stantard error), Bifentrin 0,0 ± 0.017, Censo 2.85 ± 0.418; Control 3.5 ± 0.367.

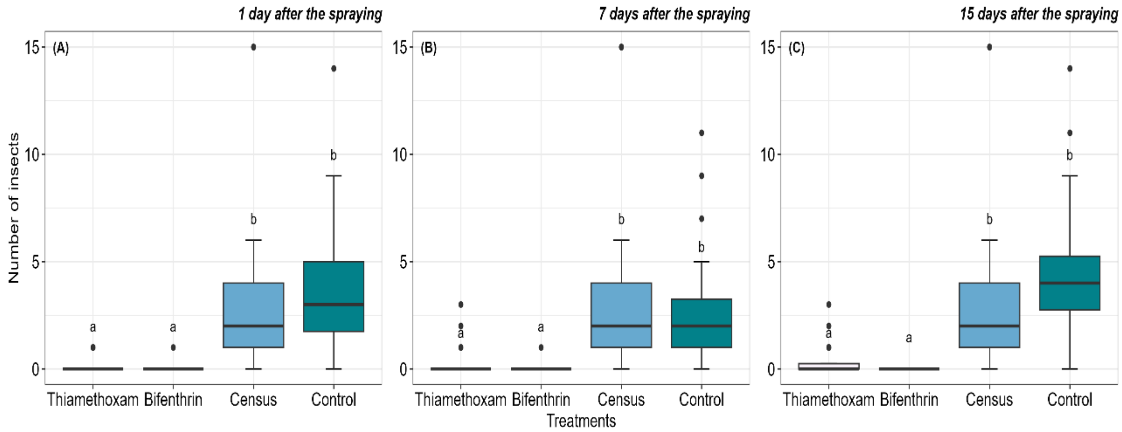

Figure 4. Number of *Monalonion bondari* insects found in eucalypt plants in each treatment over 1 (A), 7 (B) and 15 (C) evaluation after spraying the insecticides. In (A), (B) and (C) the bars bearing the same letters indicate similar values at the 5% significance level (Tukey post-hoc test). The lines within the boxplots represent the median, the lower and upper boundaries of the boxes represent, respectively, the 25th and 75th percentiles, while the whiskers extend to the smallest and largest values within 1.5 box lengths. The dots represent the outliers.

4 DISCUSSION

Lateral shoot dieback is a disease that has gained increasing importance in recent years due to its significant potential for causing damage and reducing productivity in *Eucalyptus* spp. plantations. In this study, we have identified the disease as being caused by *M. bondari*, an endemic mirid species from the Bahia region of Brazil, which has previously been documented as a pest in cocoa plantations. Our results demonstrate that *M. bondari* is not only a threat to cocoa crops but is also capable of infesting *Eucalyptus* spp., feeding on its tissues and inducing the development of lateral shoot dieback symptoms. This is consistent with findings from Indonesia, where related mirid species have been reported to have a broad host range, affecting various crops, including *Eucalyptus* spp. (Muhamad and Way 1995; Roy et al. 2015). This highlights the potential for *M. bondari* to become a more widespread pest in eucalypt plantations, with significant implications for crop management and disease control. This discovery enhances our understanding on the biology and ecology of *M. bondari* and shows its potential host range plasticity. This suggests that, like other mirid species, *M. bondari* may adapt to different host plants, potentially influencing its populations and the economic impacts in several agricultural crops.

Based on distinct morphological features highlighted in previous taxonomic studies (Carvalho 1972; Gamboa et al. 2020) and molecular identification, it has been established that the insects collected in this work belong to the tribe Monaloniini, genus *Monalonion*, that is known for its endemic distribution in the Neotropics (Cassis and Schuh 2012; Gamboa et al. 2020). The species *M. bondari* has been described and reported inly in Brazil, in the state of Bahia (Gamboa et al. 2020). This insect species is recognized for its specific host association, with cocoa (*T. cacao*) as its primary host plant (Cassis and Schuh 2012; Gamboa et al. 2020). This mirid often feeds on the vegetative tissues of cocoa leaves and shoots, causing significant damage to plantations (Muhamad and Way 1995; Moubarak et al. 2022). The presence of *M. bondari* in the cages with *Eucalyptus*, along with the evidence of feeding, allows us to conclude that this insect can cause dieback symptoms. Indeed, plant-bugs are known to cause diseases in various crops (Carvalho 1972; Kodakkadan et al. 2020; Muhamad and Way, 1995; Roy et al. 2015). In our case, Cocoa plantations are situated near the areas where *M. bondari* was collected in *Eucalyptus* spp. plantations, with symptoms of the disease becoming more prominent in *Eucalyptus* trees_after periods of heavy rainfall, which is when the symptoms typically emerge (Wu et al. 2002; Demirel 2022;). Increased rainfall tends to boost the population of *Monalonion* in plantations, and it is possible that competition within the cocoa plantations prompted the pest to migrate and adapt to the conditions of eucalypts. This suggests that an insect highly specialized for cocoa may also exploit other plant species under specific conditions, potentially influencing its ecology and interactions within agricultural ecosystems.

The relationship between the insect and the fungus *Pseudoplagiostoma eucalypti*, which has been previously identified as a causal agent of dieback in *Eucalyptus* spp. (Zauza et al. 2023), remains insufficiently known and requires further investigation. While it is established that the insect injects a toxin that leads to the death of leaf tissue (Sarker and Mukhopadhyay 2006; Kodakkadan et al. 2020), it is still unclear whether the interaction between the insect and the fungus exacerbates the symptoms of the disease, or if the fungus enters the infection process at a later stage, acting as an opportunistic pathogen. Additionally, it is important to explore whether the presence of the fungus amplifies the effects of the insect's feeding damage. To fully comprehend the role of each organism in the onset and progression of dieback, comprehensive studies are essential to elucidate their interactions and determine the extent of their combined impact on Eucalyptus plantations.

The molecules bifenthrin and thiamethoxam proved to be effective in controlling *M. bondari*, as anticipated. This efficacy aligns with previous observations where these insecticides have demonstrated successful control of other insects within the same genus (Zhang et al. 2015; Dumont and Provost 2019; Amarasekare and Shearer 2020). Previous studies have reported an efficiency of 86% to 90% in controlling *Monalonion velezangeli* and *Helopeltis theivora* using the same insecticides, bifenthrin and thiamethoxam. These results demonstrate the effectiveness of these chemical treatments in managing these mirid pests, as observed by Montilla Pérez et al. (2014) and Rahib Chowdhury et al. (2013). Both studies highlighted the ability of these insecticides to significantly reduce pest populations, providing valuable insights for the control of similar pests, such as *M. bondari*, in different agricultural crops. Broad-spectrum insecticides reduce pest populations and prevent reinfestation for up to 15 days after application. As previously reported for other species of plant-bugs, chemical insecticides lose efficacy over time, with their effectiveness halving 17 days after application (Amarasekare and Shearer 2020). In the present study, however, we did not observe any reinfestation within the 15-day period following insecticide spraying. This is advantageous because the eggs of *M. bondari* are laid inside the branches of eucalypt trees, which prevents direct contact with the insecticide. However, even after the eggs hatch, the insect can still be affected due to the broad-spectrum activity of the insecticides, which target various stages of insect development. This makes them suitable for managing *M. bondari* infestations in eucalypt plantations.

Although this study presents promising results for identifying the causal agent of lateral shoot dieback disease and controlling *M. bondari* in eucalypt plantations, it remains essential to define an economic injury level (EIL), develop effective monitoring methods, and explore alternative pest control strategies. Establishing an EIL will help determine the threshold at which pest populations cause significant economic damage, thereby informing management decisions. Furthermore, investigating alternative control methods that are less harmful to natural enemies in the ecosystem is crucial. For example, integrating biological control agents, such as microbiological or macrobiological organisms, could offer a sustainable and effective approach to managing.

AUTHOR CONTRIBUTIONS

Rafael Ferreira Alfenas: Conceptualization, investigation, methodology, data curation, visualization, writing – original draft, review, and editing. **Jessica Josefa Sanches:** Investigation, methodology, processing datasets, data curation, visualization, writing – original draft, review, and editing. **Acelino Couto Alfenas:** Investigation, supervision, writing – original draft, review, and editing. **Nilmara Pereira Caires:** Methodology, visualization of data, writing – review and editing. **Frederico Nanini:** Methodology, writing - original draft, review, and editing. **Cristiano Feldens Schwertner:** Methodology, writing - original draft, review, and editing. **Maurício Magalhães Domingues:** Methodology, visualization of data, writing – review and editing. **Taís Aparecida Machado dos Santos:** Preparation and editing of figures, visual representation of data, writing – review and editing.

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DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon request and can also be accessed via the Zenodo public repository at the following link https://doi.org/10.5281/zenodo.16884343

CONFLICT OF INTEREST STATEMENT

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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