The Psyllids *Triozoida Limbata* Supplant the Defenses of *Psidium Guajava* Plants to Guarantee Feeding and Protection

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Abstract

**Background:** Herbivores cause biochemical, physiological, and morphological changes on plants. The ultimate effects of the herbivore activities are damages on the plant's body that can lead from local tissues death to the plant death. The intensity of herbivore attack and the plant's capability to defend itself determine the outcomes of herbivory. When the plant under attack has economic importance, such damages can diminish the productivity of the plantation and compromise the economy of families and the local communities. Although the adverse effects of herbivory on plants and economy are entirely known, some biological mechanisms underlying these damages remain unclear, especially at the anatomical and hormonal levels. The psyllid *Triozoida limbata* (Hemiptera: Triozidae) is a critical guava (*Psidium guajava*) pest in the Neotropical region, causing productivity plantation losses up to 55%. Psyllids cause visible morphological changes on guava leaves; however, there is a lack of detailed studied of the cellular, anatomical, and hormonal aspects of the subject. Thus, this work aimed to determine the alterations caused in guava leaves by *T. limbata* through anatomical and hormonal analyzes.

**Results:** We found that the psyllid's nymphs feeding causes cells hypertrophy and hyperplasia determining the formation of the leaf-roll galls. Although there was no anatomical differentiation of the mesophilic tissues, the cells accumulate starch. Along with the anatomical changes, we observed increasing in the jasmonic acid and reduction of zeatin concentrations in the leaves.

**Conclusions:** The alteration of phytohormone contents and the anatomical changes indicates that the feeding of *T. limbata* activates plant defenses, but it is not active toward the insects off. The insect nymphs live inside the leaf-roll galls until adults' phase, feeding on the guava leaves xylem and phloem. In commercial guava orchard, the control of *T. limbata* nymphs have been difficult. Thus, the understanding of the composition of this protective layer can help to find the most effective and efficient control solution for this pest.

1. **Background**

"Why is the world green?" is a classical question in plant ecology not yet fully responded by the scientists [1, 2]. The core of this puzzle question relies on the fact that although many herbivores, mainly insects, can reduce the plant growth and reproduction and increase their mortality, in its majority plants cover the terrestrial world. One partial response for this question is that most herbivores species cannot obtain the quantity and or quality of food resources required to grow, survive, or reproduce at high rates [2, 3] because plants synthesize secondary metabolites and proteins that are toxic, repellent and or that inhibit insect herbivores digestion. These chemical defenses have been evolving throughout the arms race between plants and insects and gave the plants the ability to recognize signals from damaged cells and activate their immune response against the attackers [4].
In turn, plant resistance imposes selection pressures on herbivores. Many herbivores evolved adaptations to overcome the adverse effects of plant defense metabolites by adopting specific behaviours of foraging and choice the feeding local. Moreover, after that, to deal with the harmful chemical compounds by excretion, detoxification through chemical conjugation and breakdown [5, 6]. As a result of the ongoing arms race, many herbivores can feed on defended plants and even maintain its healthy development without obvious negative fitness consequences [6], making the world less green. It seems the case of the herbivore *Triozoida limbata*.

The psyllid *Triozoida limbata* Enderlein 1918 (Hemiptera: Psylloidea: Triozidae) is critical guava (*Psidium guajava* L.) pest in the neotropical region, occurring in Argentina, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, El Salvador, French Guiana, Mexico, Panama, Peru, and Trinidad and Tobago [7, 8]. These tiny insects, popularly known as jumping plant-louse, are monophagous on guava and were of minor economic importance until becoming a key pest in commercial plantations [9-11].

Due to the delicious guava taste and high nutritional content, like sugars, vitamins A, C and B, phosphorus, potassium, iron, and calcium, fibre, and antioxidants, recently there is an increase of the fruit consumption worldwide [12]. In consequence, its economic importance was drive up along with the enlargement of its cultivated area [13-16]. Guava orchards generate net income of US$ 6000 per hectare, six direct jobs, and three indirect jobs per hectare, and is cultivated mainly by small farmers, which have a tremendously positive social impact, especially for developing countries [17]. Also, the guava leaves and fruits are used for a variety of therapeutic purposes in alternative medicine [18, 19], having high cultural value for many communities in different countries.

The guava plants attacked by *T. limbata* lose productivity up to 55% due to deformations in leaves and losses of leaf photosynthetic area [20]. To control the damages in the leaves and decrease the economic losses the guava producers apply pesticides each 15 to 20 days [21] with up to 600L/ha of pesticide, mainly imidacloprid, a neonicotinoid [22, 23] which is not ecologically desirable for the health of the plantation workers nor the health environment.

Despite the economic and social importance of guava plantation for the developing countries in neotropical and tropical zones, surprisingly little is known about their interaction. There is a lack of scientific information concerning the biochemical, physiological, and morphological changes that guava plants face when under psyllids attack. Thus, it is necessary to understand the guava-psyllid interaction more deeply to find effective ways to control the insect herbivory on the plants.

The understanding of the relationship between pest insects and their host plants makes it possible to determine critical points in the bioecology of these herbivores. The knowledge of these critical points enables the planning of efficient and environmentally sustainable control strategies to be included in integrated pest management programs [24]. Therefore, this work aimed to describe the morphological, anatomical, and biochemical changes caused by psyllids in guava leaves, as well as describe the feeding behaviour of the herbivore insects.
2. Methods

2.1. Experimental conditions

This work was carried out at the Universidade Federal de Viçosa (UFV), in Viçosa municipality (20º48'45" S, 42º56'15" W), Minas Gerais State, Brazil.

We bought guava plants var Paluma with six months old from the local commerce and transferred them to polyethene pots with eight litres capacity filled with a mixture of latosol and Tropstrato® (1:1) and kept the plants inside a greenhouse, cultured according to recommended practices (Gonzaga Neto & Soares, 1995). The average temperature was 27ºC, and the average relative humidity of air was 70% inside the greenhouse.

Inside another greenhouse, we kept 60 guava plants to rear the psyllids.

2.2. Psyllid rearing

We collected leaves containing psyllid nymphs from the guava trees cultivated in the UFV orchard and placed them on the leaves of guava plants cultivated inside the greenhouse. Along with the psyllid nymphs, we also collected specimens of psyllid adults and stored it in vial glasses (10 mL) containing 92% ethanol-water solution. These vials were sent to Dr Daniel Burckhardt of the Department of Biosciences of the Natural History Museum in Basel, Switzerland to confirm the insects' identification. Details of guava plants growing in pots, roll galls and T. limbata are in Figure 1.

2.3. Morpho-anatomical investigations on leaves attacked by the psyllids

We choose 30 plants infested with psyllid, each one containing at least five leaves with freshly laid eggs and another five plants from the group not exposed to the psyllids attack and transferred all of them to another greenhouse. From this day on, we photographed one leaf from the guava plant infested with psyllid and one leaf of the guava plant not infested to register and evidence the morphological changes.
caused by the herbivory. Also daily we collected leaves from the attacked plants that were cut parallel to the margin in the attacked region and then transferred individually to FAA 50 (formaldehyde: acetic acid: ethyl alcohol 50%, 2: 1: 18 v / v) solutions for 48 hours and after that stored in 70% ethanol [25, 26].

We repeated these procedures for 24 days, and at the end of this experimental time, we classified the damages in the leaves in 3 sequential stages. Then, from the material previously stored in 70% ethanol and adequately labelled by day, we choose ten leaves from each damage stage to proceed with the anatomical investigation.

To do so, we hand-cut the fixed leaves in cross-sections using a razor blade and double-stained it with safranin/Astra blue to evidence the development of the cell wall using the contrast with safranin that will stain lignin regardless of whether cellulose is present whereas Astra blue will stain cellulose only in the absence of lignin [27, 28].

We also used Lugol’s solution to verify the presence of starch granules in the leaf’s cells in the three stages [25]. After the staining, we mounted the material on temporary slides for viewing under light microscopy (Olympus AX70) and photographed the images (Axio Cam HRc Zeiss) for further analyses.

**2.4. Description of the ontogeny of psyllid on guava leaves**

Along with the procedure described above to registers the morphological changes in guava leaves, we also observed and registered on photographs the behaviour and development of the psyllids from eggs to adults on a daily basis. These procedures started just after transferring the guava plants with recently laid eggs to the greenhouse, and we recorded it daily. From the 2nd instar on, the psyllids nymphs were inside the rolled edges of the leaves. To access the nymphs and make the necessary observations and registers, we opened these structures according to what was described by AA Semeao, JC Martins, MC Picanço, CH Bruckner, L Bacci and JF Rosado [10].

The species *T. limbata* is from temperate and neotropical regions. The adults are about 2 mm long, with a black stripe on the fore wings. Immature individuals are elongate, dorsoventrally flattened with short, curved antennae and have conspicuous spines laterally on the fore margin of the head [8, 29]. *T. limbata* pass through an egg and five nymphal instars before becoming an adult. The individuals are bisexual, and the male is the heterogametic sex. By the fifth instar, male and female nymphs are morphologically distinct. Their generations are continuous throughout the year in tropical areas, but their growth rates are determined by climatic factors, especially ambient temperature and the availability of water, besides host-plant conditions [30, 31]. The eggs and nymphs are highly susceptible to desiccation. *T. limbata* adults have low ability to fly distances, and its dispersion is mainly by wind.

**2.5. Biochemical investigations on leaves attacked by psyllids referring to some hormones concentrations**
We choose guava plants from the psyllid free greenhouse and transferred them to the Ecology Laboratory (UFV) for acclimation for ten days. After this time, we selected one leaf belonging to the first pair below the apical bud from five plants, cut them off wrapped them individually in aluminium foil and placed them into a Styrofoam box (96 x 80 x 55 mm) where we add liquid nitrogen. Then we stored this material in the ultra-freezer -80C (Thermo Scientific, Forma TM 8600 Series). These procedures determined the first treatment of the experiment, named Time 0. After that, we discarded the plants.

On this very same day, we placed five psyllid nymphs of the 3rd instar on the adaxial side of the first pair of leaves below the apical bud of another 30 guava plants. To do so, we used a small brush and monitored the nymphs for about 1 hour to make sure they stayed on the leaves. From this day on until six days after, every twenty-four hours, we choose randomly five plants from the thirty-first ones and repeated the procedures of cutting the leaves, place them in liquid nitrogen and store them in the ultra-freezer. Before storing them, we brushed off the nymphs from the leaves. Therefore, we ran an experiment with seven treatments with five replicates each.

We set the experimental time in 6 days because we observed that this is the time needed to get the leaf edge completely rolled as a response to the psyllids feeding. Besides, we choose to place the nymphs of the 3rd instar on the adaxial side of the first pair of leaves below the apical bud because 1) nymphs of the 1rd and 2rd were too tiny to manipulate ending up hurt; 2) we observed from previous experiments that psyllid females prefer to lay eggs on these leaves.

At the end of the experimental time, we crushed the leaves individually according to their respective treatment in a mortar with liquid nitrogen, measured their he massed in an analytical balance (Denver Instrument, A-250, 0.1 mg accuracy) and transferred each material to separated 1.5 mL microtubes. Then, we added 400 μl of extractive solution (methanol: isopropanol: acetic acid in the ratio of 20: 79: 1) to each microtube, that was shaken for 20 seconds four times in vortex (Fisher Vortex Genie). We transferred the microtubes to a sonicator (Branson®, 2510) where they remained for 8 minutes at 4 °C and then transferred to a styrofoam box containing ice for 30 minutes. Afterwards, the samples were centrifuged (Eppendorf 5424 R) at 14,000 g for 30 minutes at 4 °C and then we transferred the supernatant new 1.5 mL microtubes, maintaining its respective treatment identities. We centrifuged the remaining pellets again under the same rotation and collected the supernatants that were added to the respective microtubes. The microtubes were centrifuged at 14,000 g once again for 10 minutes at 4 ° C. From the supernatant of each sample was transferred 300 μL to a 2 mL vial [32, 33].

We injected the samples individually in a chronograph (chromatographic column Agilent Eclipse Plus, RRHD, 1.8 μm, 2.1x50 mm; flow rate of 0.3 mL /minute) coupled online with a triple quadrupole mass spectrometer QqQ (Agilent). The salicylic acid, abscisic acid, methionine, auxin, methyl jasmonate, and gibberellin hormones were quantified on five replicates from each treatment. These hormones are known to affect many aspects of development and growth in plants, and they are classic hormones studied to detect plant reaction to herbivores.
Afterwards, we used the MassHunter software to process the generated mass spectra and the Skyline software to make corrections of the areas and to determine concentration values in nanogram/gram (ng / g) [32, 33]. We did all these procedures at the Núcleo de Análise de Biomoléculas (NuBioMol, CCB, UFV).

We submitted the data obtained to an analysis of variance (α = 0.05) and then. We submitted the data of those hormones that presented a significant difference in concentration over the experimental time (P <0.05) to regression analysis. The regression models were selected based on the following criteria: significance (α = 0.05), simplicity, biological significance, and higher coefficient of determination (R²) [34].

3. Results

3.1. Morpho-anatomical investigations on leaves attacked by the psyllids and the ontogeny of these insects.

We found that the guava leaves attacked by the psyllids shown several changes in its morphology (Figure 2) and anatomy (Figure 3 and 4). Concerning the morphological changes, we separate it into three stages according to the shape of the leaves and its colours. Stage one started on the first day of infestation by the insects, and the third ended 24 days after when we observed no more alterations in the leaves' shape. The first stage lasted seven days, beginning with a slightly folding in one part of the edge of the leaves. As the insects carry on feeding on them, their edges kept folding upward, creating a kind of tunnel, and the psyllid nymphs at the second and third instar lodged inside them. Stage two lasted six days, during which we observed the edge still folding upward in most of the leaves and its colour changing to a pale green. At the end of stage two, the leaves presented a yellowish colour more evident in its rolled edges. At this stage, the nymphs were in the third and fourth instar. During the next and last stage, the rolled edges of the leaves started to become amberish, and them it was changing slowly to a purplish-red colour. Also, the leaves edge became dehydrated, and at the end of stage three, after 24 days under herbivory by the psyllid nymphs, the rolled edges were parched and brittle, showing symptoms of tissue necrosis. At this point, the nymphs were in its fifth and last instar (Figure 2).

From the second stage of the ontogeny of the psyllid nymphs, we observed white wax wires inside the rolled edges of the leaves. The nymphs were underneath the tangle of wires. Come to our attention that in the first instar the T. limbata nymphs were on the abaxial surface of the guava leaves which had a similar appearance to the non-attacked leaves. In the second instar the nymphs moved to the adaxial surface, and then the morphological changes of the leaves started to show.

Comparing anatomical sections between leaves attacker and non-attacked by T. limbata nymphs, we observed differentiation of the mesophyll tissues evidenced by the identification of palisade and spongy parenchyma in the later ones (Figure 3 and 4). At the same time, the damages leave remained with the tissues undifferentiated in the first seven days after attacked by T. limbata. Also, in these leaves, the mesophylls cells were bigger and increased in number per area unit. Besides, only in the attacked leaves,
the epidemics cells were stained by safranin. Although the adaxial layer was reddish darker than the abaxial, in both was evidenced the presence of lignin. Despite these anatomical changes in the mesophyll tissue, we do not observe any change in the vascular system due to the psyllid attack (Figure 3). Inside the cells of guava leaves forming the winding tunnel, we observed concentrations of starch granules (Figure and Figure 5).

3.2. Biochemical investigations on leaves attacked by psyllids referring to hormones' concentrations

After the statistical analysis of biochemical investigation on the hormone we found enhancement statistically significant in the concentrations of jasmonic acid (F 6; 28 = 4.00; P = 0.0051) and zeatin (F 6; 28 = 3.69; P = 0.0080) for guava leaves attacked by *T. limbata*.

The investigations of the other hormone concentrations did not show significant differences between attacked and non-attacked guava leaves (salicylic acid (\( \bar{x} = 10.51 \pm 4.84 \text{ ng/g, F}_{6,28} = 2.06, P = 0.0902 \)); abscisic acid (\( \bar{x} = 41.55 \pm 5.65 \text{ ng/g, F}_{6} = 28 = 1.50, P = 0.2139 \)); methionine (\( \bar{x} = 660.54 \pm 78.01 \text{ ng/g, P = 0.0526} \)); auxin (\( \bar{x} = 2.69 \pm 0.20 \text{ ng/g, F}_{6} = 28 = 0.9150, P = 0.5 \)); methyl jasmonate (\( \bar{x} = 31.11 \pm 4.53 \text{ ng/g, F}_{6,28} = 1.65, P = 0.1695 \)) and gibberellin (\( \bar{x} = 0.82 \pm 0.06 \text{ ng/g, F}_{6} = 28 = 1.04, P = 0.5800 \)).

The regression model for the jasmonic acid concentration in the leaves as a function of time after *T. limbata* attack was a simple linear regression until this curve reached a maximum (plateau). From this point on the concentration of jasmonic acid in the leaves remained constant. We estimated that the increase of the jasmonic acid concentration in the leaves started immediately after the beginning of the psyllid attack and that the high concentration of JA was reached about four hours after, remaining at 0,76 ng/g, in average, until the end of the experimental time (Figure 6A). For zeatin, we found that its concentration started to decrease immediately after the beginning of the nymphs attack and diminished until three days after that, remaining constant and low until the end of the experiment (Figure 6B).

4. Discussion

Based on the morpho-anatomical and biochemical changes and the observations we made in the guava leaves under the *T. limbata* nymphs attack, we stated that, despite the plant is fighting back, the insects are overcoming its defenses and are successful in feeding and sheltering on them.

Our observations confirmed that the changes in the leaves shape are following RMdS Isaias, R Carneiro, D Oliveira and JC Santos [35] description of a leaf rolling or margin roll gall formed by the rolling movement of one or both leaf margins. These galls are open tube-shaped structures like tunnels that house the psyllid nymphs from the 2nd to the 5th stage of development, giving them protection and food access, like the typically closed galls [36, 37].
As we can see at Fig 3A and 3B, the hyperplasia and hypertrophy of the guava leave tissue under psyllid attack cause uneven growth of the abaxial and adaxial foliar tissues occurred, which leading the margins of the guava leaves to wind upwards. Substances in the psyllid nymphs saliva possibly caused the hyperplasia and hypertrophy of the guava leave cells. Interestingly, although there is little doubt that salivary injection is the primary stimulus for gall formation [30, 38] even for guava leaves [39, 40], as we know so far there is no scientific report of the nature of the salivary component-inducing gall formation by the *T. limbata*.

The double stain of the slices of guava leaves with safranin and Astra blue showed lignin concentration in the epidermic cells of the mesophyll [28], where the psyllid nymphs were feeding. Lignin is a phenolic polymer with high molecular weight, complex composition, and structure mainly present at the secondary cell wall, which is the first plant barrier against external injuries [41]. Therefore, one of the reactions of the plants to insect resistance is the enhancement of lignin synthesis [42]. Y Wang, L Sheng, H Zhang, X Du, C An, X Xia, F Chen, J Jiang and S Chen [43] reported enhancement of lignin gene expression and lignin accumulation in the cell wall leaves of chrysanthemum after the plants are attacked by the aphid *Macrosiphoniella sanborni*, limiting the aphids feeding and increasing the plant's tolerance. Thus, the concentration of lignin in the feeding spots of the psyllid nymphs shows evidence of the guava resistance to the insects [44].

Indeed, L Denness, JF McKenna, C Segonzac, A Worrin, P Madhou, M Bennett, J Mansfield, C Zipfel and T Hamann [45] identified two stages that regulate the synthesis of lignin triggered by the plant cell wall damage, stating with calcium and reactive oxygen species (ROS) production inducing jasmonic acid (JA) synthesis and accumulation. During the second stage, a negative feedback loop between JA and ROS regulates lignin production in the damage cells of Arabidopsis seedlings.

JA, its methyl ester (MeJA) and isoleucine conjugate (JA-Ile) are derivatives of a class of fatty acids collectively known as jasmonates (JAs) which are related with abiotic and biotic plant stress and involved in the regulation of plant growth and development [46] including promotion xylem development [47]. Thus, JAs is associated with plant defense against mechanical damage caused by insects [46, 48] which can cause rapid and transient accumulation of JA and JA-Ile at the site of injury, thereby activating the expression of defense genes and producing a local defense response [46]. Also, JAs are precursor of volatile organic compounds (VOCs) which attract insect’s herbivore natural enemies [49].

Previous studies showed that crosstalk between JA and other hormones modulates plant defense and development. This way, JA may inhibit the production of zeatin [36, 47, 50], which is the most prevalent naturally occurring cytokinins [51].

Cytokinins are involved in the plant growth and development control in many levels, including increasing of cell number and cell expansion in line with increased sugar content and turgor pressure, development of vascular tissue as a negative regulator of xylem development, stomata and chloroplast biogenesis and photosynthesis process [47, 51, 52]. According to J Skalák, L Vercruyssen, H Claeyts, J Hradilová, M Černý, O Novák, L Plačková, I Saiz-Fernández, P Skaláková and F Coppens [52], cytokinin in excess during the
cell proliferation phase resulted in leaves with few chloroplasts and irregular photosynthetic activity. By the other hand, excess of cytokinin in the cell expansion phase stimulates chlorophyll biosynthesis indicating chloroplast biogenesis and that the onset of photosynthesis seems to be tightly linked to cell expansion These authors concluded that the timing of cytokinin content fluctuations is a key factor mediating transitions from cell proliferation to cell expansion in leaves.

Regarding our results, we suggest that the psyllid nymphs interfere with the hormone concentration balance in the guava leaves, specially JAs and Cytokinins, in its early stages of development. In doing so, the outcomes are increasing in chloroplast number and photosynthesis rate along with the stimulation of xylem cell development. Hence, our results show that the psyllids' nymphs feeding behaviour triggers the guava plant's resistance and indicates the existence of a complicated relationship between these sap-sucking insects and guava plants [36, 53].

Although the anatomical changes and lignin concentration were more evident in the adaxial epidermic cells, corresponding to the internal part of the roll gall where the nymphs from the 2nd to 5th stage fed, we also observed these changes in the abaxial epidermic cell indicating that even the nymphs at the first stage can trigger the plant reaction. Despite that, it is not possible to affirm that the substances released by the saliva of the psyllid have its action restricted to the insect feeding site.

The feeding of psyllid nymphs also caused the accumulation of starch in the leaves cells. The starch granules may have a nutritional role for galls tissue maintenance or the insects' nutrition [54]. Nevertheless, the explanation for this is still incomplete.

Concerning to the psyllids feeding behaviour, we collected electropenetrography data from *T. limbata* and observed that the nymphs feed on both xylem and phloem vessels in the smallest vein (Mayara Moledo Picanço, unpublished data). As the psyllids are tiny insects and guava leaves have brochidodromous secondary venation [55, 56], we suggest that feeding on the small quaternary and or quinternary vein in the leaves edge allow them to avoid the high pressure of the phloem and xylem in the primary and secondary larger vessels.

Galls protect the insects against weather and natural enemies [57, 58]. A Semeão, J Martins, M Picanço, M Chediak, E da Silva and G Silva [11] found that the mortality of *T. limbata* by natural enemies is about 50% higher when nymphs are out of the galls during their 1st instar than when they are inside the galls, from the 2nd to 5th instars. At these stages, rainfall kills twice more psyllids outside galls. Therefore, inducing the leaf edge to wing upwards by manipulating is metabolism and development is an evolutionary strategy to gain shelter and protection while feeding during most fragile stages of the insects' development.

The leaf colour alteration, necrosis, and leaf galls, observed in this work, due to the *T. limbata* attack should contribute to the occurrence of damages in the guava plants. In this context, M Moreira [20] reports that the attack of the *T. limbata* nymphs can cause up to 55% of productivity losses in guava plants. The leaf colour alterations were due to the chlorophyll degradation (yellowing-leaf chlorosis) and
accumulation of anthocyanins (purplish-red leaf colour) [59]. Morpho-anatomical changes in guava leaves caused by *T. limbata* reduces plant productivity, possibly due to the reduction of photosynthesis and an increase of photoassimilates consumption [60, 61]. The chlorosis caused the decrease of leaf area, formation of roll galls, and necrosis of the leaves observed in this work. On the other hand, the feeding of sap-sucking insects like the psyllid consumes photoassimilates of the plants [60, 61].

The fact that the eggs, first instar nymphs, and adults of *T. limbata* are on the leaf surface while the 2nd, 3rd, 4th and 5th instar nymphs are inside the galls has implications in the planning of control strategies for this pest. In this context, biological and chemical control will more easily reach pest stages that are on the leaf surface than those sheltered inside the galls. To control the nymphs that are inside the gall (2\textsuperscript{nd}, 3\textsuperscript{rd}, 4\textsuperscript{th}, and 5\textsuperscript{th} instar), it has needed to reach the gall internally. For this purpose, systemic insecticides must be applied to the soil, or adjuvants can be added to the insecticidal sprays. Among the adjuvants that increase the penetration rate of insecticides in plant tissues are the oils [62, 63]. Thus, from the results from *T. limbata* bioecology, it is possible to plan efficient strategies for the control of this psyllid in guava orchards.

In commercial guava orchard, the control of *T. limbata* nymphs has been difficult. Thus, the understanding of the composition of this protective layer can help to find the most effective and efficient control solution for this pest. This efficiency can be obtained by the use of adjuvants in insecticidal syrups that allow the penetration of these products through this protective layer. Also, MR Dsouza and B Ravishankar [64] observed that the galls made by the *Pauropsylla depressa* Crawf (Hemiptera: Psyllidae) psyllid in *Ficus glomerata* plants, there is an increase in the concentration of starch.

### 5. Conclusions

We conclude that the attack of this psyllid triggers the plant defense. Despite the evidence that the plant has its immune system activated, the insect attack does not stop, and they complete their development feeding on the guava leaves.

### Abbreviations

UFV: Universidade Federal de Viçosa

JA: Jasmonic Acid

ROS: Reactive oxygen species

JAs: Jasmonates

JA-Ile: Jasmonoyl isoleucine

### Declarations
Ethics approval and consent to participate

Not Applicable

Consent for publication

Not Applicable

Availability of data and material

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

FMSC, AAA, HJOR, OFFS and MCP. conceived and planned the experiments. MMP, LLL and RSS contributed to the rearing of the plants and the psyllids. MMP, RSS, FMSC, and AAA, contributed to the morpho-anatomical investigations. HJOR. and LLL contributed to the biochemical investigations. MMP, FMSC, OFFC and MCP. analyzed the data. MMP wrote the manuscript. RSS, MCP, FMSC and OFFS contributed to the editing and revising of the final version of the manuscript. The authors read and approved the final manuscript.

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**Figures**
Figure 1

(A) Guava plants growing in pots, (B) roll galls, (C) Triozoida limbata female and eggs, and (D) nymphs of this insect.

| Stages of the life cycle of *T. limbata* on guava leaves: |
|----------------------------------------------------------|
| **Egg** | **1st instar** | **2nd instar** | **3rd instar** | **4th instar** | **5th instar** | **Adult** |
| **Leaf before the attack** | **Stages of morphological changes in leaves attacked by *T. limbata*** | **Leaf after the attack** |
| ![Leaf before the attack](image) | ![Stage 1:](image) beginning (7 days) | ![Stage 2:](image) beginning (6 days) | ![Stage 3:](image) beginning (11 days) | ![Adult](image) |
| ![Leaf after the attack](image) | ![End](image) | ![End](image) | ![End](image) | |

Figure 2

Life cycle stages of Triozoida limbata and morphological changes in the guava leaves attacked by this insect.
Figure 3

Anatomical sections of guava leaf (A) attacked and (B) non-attacked by the Triozoida limbata psyllid. PP = Palisade parenchyma, LP = spongy parenchyma, VS = vascular system, and SG = secretory gland.
Figure 4

Anatomical sections of guava leaf attacked by the Triozoida limbata: (A) tunnel formed by the leaf winding, (B) the inner layer of the tunnel and (C) the outer layer of the tunnel.
Figure 5

Anatomical section of guava leaf attacked by the Trioza limbata psyllid stained with lugol to evidence the starch granules.
Figure 6

Concentrations of (A) jasmonic acid and (B) zeatin on guava leaves as a function of time after the Triozoida limbata attack. = Mean ± standard error. Leaves at time zero were not attacked by the insect.