

## Bioassay Technique and Maintenance of *Gyropsylla spegazziniana* (Lizer, 1719) (Hemiptera: Psylloidea: Aphalaridae) *in vitro* Conditions

Jaqueline Suelen Loeblein<sup>1</sup> , Luis Francisco Angeli Alves<sup>1</sup> ,  
Miryan Denise Araujo Coracini<sup>1</sup>, Maria Elena Schapovaloff<sup>2</sup>

<sup>1</sup>Universidade Estadual do Oeste do Paraná – UNIOESTE, Cascavel/PR, Brasil

<sup>2</sup>Instituto Nacional de Tecnología Agropecuária – INTA, Montecarlo/Misiones, Argentina

### ABSTRACT

*Gyropsylla spegazziniana* is one of the most important pest of yerba mate by sap-sucking that lead to leaves deformation and there are no insecticides registered for its control. The present study describes a new technique to be used in bioassays with the insect. Galls were collected from infested yerba mate plants. In the laboratory, the galls were partially opened and distributed in plastic containers. The emerged adults were transferred to plastic containers with the perforated bottom and a central opening in the lid, and subjected to spraying. After that, insects were transferred to yerba mate seedlings in plastic cages and maintained in laboratory to daily assessment. The use of a lid with central opening prevented the insects escaping, and avoiding the previous anesthetic to spraying. The mortality was less than 10% after 10 days. The technique was considered adequate for maintenance and performance of laboratory bioassays.

**Keywords:** yerba mate, insect, pest, control, Paraguay tea ampule.

## 1. DEVELOPMENT

*Ilex paraguariensis* St. Hil. (Aquifoliaceae), popularly known as 'yerba mate', occurs predominantly in southern Brazil. It is cultivated as a source of raw material for the production of beverages, or as a source of active ingredients for the pharmaceutical industry (Chiaradia et al., 2000; Heck & Mejia, 2007). Among the main pest that attack yerba mate, the ampule of yerba mate *Gyropsylla spgazziniana* (Lizer) (Hemiptera: Aphalaridae) damages both yerba-mate plant and seedlings (Chiaradia et al., 2002).

These insects cause deformities in the new leaves, which grow up as a bivalve-shaped galls in which the nymphs develop (Isaias et al., 2013). Consequently, the deformed leaves usually fall, reducing the leaf area and hindering the growth and formation of healthy leaves (Chiaradia et al., 2000, 2002).

There are no insecticides registered for controlling ampule of yerba mate in Brazil, and their use in the crops is not allowed (Brasil, 2018). In this way, alternative control strategies become necessary. So, bioassays should be conducted with alternative products as vegetal extracts or entomopathogenic fungus under controlled conditions. Descriptions of bioassay methods for ampule of yerba mate are scarce. There is one method for studying their biology (Leite & Zanol, 2001) and other where last-instar nymphs of the ampule were collected in a crop and transferred directly to yerba mate seedlings with a fine damp brush (Formentini et al., 2015, 2016).

Aiming to assess the efficiency of different treatments on adult of the ampule, bioassays were conducted adapting the methods cited above. Highlight the need to maintain the insects for a minimum of 10 days, mainly in tests with entomopathogenic fungi. On the other hand, in these previous bioassays it was observed high mortality of insects, especially in the first days, indicating problems in the methodology. Probably, the results have been affected by stress or mechanical damages of the immature individuals by manipulating them to select the individual to bioassay. Another difficulty was to keep the adult in the containers during the application of the treatments, requiring the use of anesthetic methods.

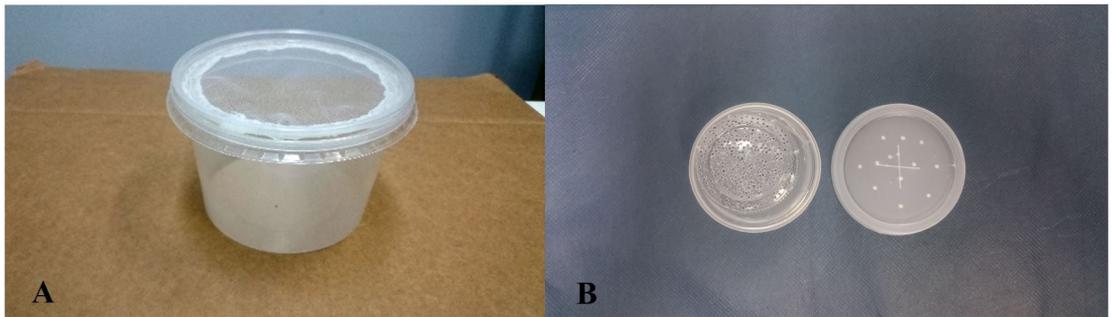
Historically, CO<sub>2</sub> and cold have been long-known anesthetic methods for handling insects in the

laboratory (Rayl & Wratten, 2016). Regarding the use in psyllids, there are reports of the exposure of adults of *Diaphorina citri* Kuwayama 1908 (Hemiptera: Liviidae) for 30 minutes at 4 °C, without causing negative side effects (Moran et al., 2011). Also, Arenas et al. (2014) used CO<sub>2</sub> as an anesthetic method for handling adults of *D. citri*, keeping the insects for two minutes under the effect of the gas.

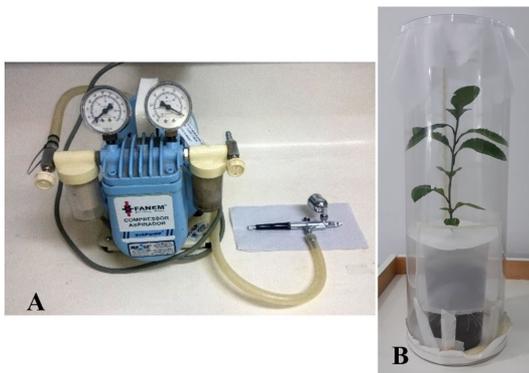
Thus, we tested adults of *G. spgazziniana* exposing them to cold (-10 °C) and CO<sub>2</sub> as a way to reduce the activity for manipulation and spraying treatment. Different exposure times were tested for each of the methods; however, both were unsatisfactory due to the high mortality rate observed after the treatments (minimum of 40%), affecting the bioassay results.

So we collected galls from infested plant branches. In the laboratory, the galls were partially opened, placing those with fourth- and fifth-instar individuals in plastic mesh containers (7 cm in diameter × 12 cm in height) (Figure 1A). The containers were kept in an air-conditioned room (26 ± 1 °C, 12-h photophase, 60 ± 10% RH) for approximately 24 hours until the emergence of adult individuals, which were transferred into plastic containers (5 cm in height × 6 cm in diameter) with a lid using a fine damp brush. The plastic containers used had numerous perforations at the bottom (approximately 1 mm in diameter) and a cross-shaped central opening in the lid (Figure 1B). Twenty adults were transferred into each container, composing one replicate, and using five replicates per treatment, according to Formentini et al. (2015). After that, 0.2 ml of 0.01% Tween solution in distilled water were sprayed onto the insects with an airbrush sprayer coupled to an air compressor (Figure 2A), with outlet pressure of 0.5 kgf/cm<sup>2</sup>. The airbrush was inserted into the central opening of the lid of the closed containers.

After spraying, each group of insects was immediately transferred to a yerba mate seedling in cages of colorless polyvinyl chloride (PVC) (13 cm in diameter × 40 cm in height) (Figure 2B) with a side opening and the top covered with voile fabric, as described by Alves et al. (2013). The insects were kept in a controlled room under the same conditions (26 ± 1 °C, 12-h photophase, 60 ± 10% RH) and assessed on a daily basis for 10 days. Every two days the seedlings were irrigated with 20 ml of tap water. A similar group was placed in cages under



**Figure 1.** Plastic mesh container (A) used to store partially opened galls until emergence of adult individuals. Plastic container (B) used for grouping of insects, with perforated bottom and lid containing the central cross-shaped opening to insert the airbrush.



**Figure 2.** Airbrush coupled to an air compressor (A) used to spray the suspension onto the insects. Yerba mate seedling (*Ilex paraguariensis* St. Hil.) (B) used to perform each replicate of the treatments.

the same conditions and assessed on a daily basis during the same period, thus constituting the control group.

It was observed that the mortality in both the control and the treated group was less than 10% over the 10 days of assessment. We also observed the formation of new galls in most of the seedlings as a result of mating and oviposition in laboratory conditions.

The maintenance and spraying technique proved to be efficient for conducting bioassays. Given that the insects remained inside the galls until they emerged, the damages caused by excess manipulation were avoided. In addition, as the insects had been transferred into spray containers shortly after becoming adults, the insect escapade was reduced.

Regarding the spraying method of the treatments, the fact that the containers had numerous perforations at the bottom, and an opening in the central part of the lid allowed the air to escape and, consequently,

reduced escapes and damages caused by spraying. In addition, this method does not require the use of an anesthetic procedure (low temperature or CO<sub>2</sub>). This way, stress of individuals is reduced. It was also observed that the maintenance of the insects in the seedlings after the application of the treatments also favored the assessment through direct observation, allowing the removal of dead individuals.

The galls resulting from mating in seedlings infested with the insects after pulverization were collected and we observed the presence of insects in different nymphal instars. This shows the possibility of future experiments aiming to evaluate the effects of some treatment on the insect development under laboratory conditions. This method for maintaining *G. spegazziniana*, and the bioassays proved to be efficient and can be recommended for laboratory studies aimed at the development of strategies for control this insect. .

## ACKNOWLEDGEMENTS

Indústria Mate Laranjeiras Ltda., CAPES, CNPq.

## SUBMISSION STATUS

Received: 1 nov., 2018

Accepted: 9 apr., 2019

## CORRESPONDENCE TO

**Luis Francisco Angeli Alves**

Universidade Estadual do Oeste do Paraná

– UNIOESTE, Rua Universitária, 2069,

CEP 85819-110, Cascavel, PR, Brasil

e-mail: luis.alves@unioeste.br

## REFERENCES

- Alves LFA, Formentini MA, Fanti ALP, Schapovaloff ME, Barzotto ILM. Susceptibility of *Gyropsylla spegazziniana* (Lizer & Trelles) (Hemiptera: Psyllidae) to *Beauveria bassiana* (Bals.) Vuill. *Arquivos do Instituto Biológico* 2013; 80(3): 363-366. <http://dx.doi.org/10.1590/S1808-16572013000300016>.
- Arenas LDO, Mendoza-García EE, García GAL, Ruíz VGA. Toxicidad de extractos de “venadillo” (*Swietenia humilis* ZUCC.) en adultos y ninfas de *Diaphorina citri* (HEMIPTERA: LIVIIDAE). *Entomologia Mexicana* 2014; 1(1): 746-749.
- Brasil. Ministério da Agricultura, Pecuária e Abastecimento. *Agrofit: sistema de agrotóxicos fitossanitários* [online]. Brasília; 2018 [cited 2018 Sept 20]. Available from: [http://agrofit.agricultura.gov.br/agrofit\\_cons/principal\\_agrofit\\_cons](http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons)
- Chiaradia LA, Milanez JM, Sabedot SM. Caracterização e danos da ampola-da-erva-mate. *Revista Agropecuária Catarinense* 2000; 13(1): 50-53.
- Chiaradia LA, Milanez JM, Zidko A. Estimativa das gerações anuais de *Gyropsylla spegazziniana* (Lizer, 1917) em função de sua exigência térmica. *Ciência Rural* 2002; 32(3): 385-391. <http://dx.doi.org/10.1590/S0103-84782002000300004>.
- Formentini MA, Alves LFA, Schapovaloff ME, Mamprim AP, Bonini AK, Pinto FGS. Characterization and activity of entomopathogenic fungi isolates against “Paraguay tea ampul” (*Gyropsylla spegazziniana*) (Lizer & Trelles) (Hemiptera: psyllidae). *Semina: Ciências Agrárias* 2015; 36(6): 3553-3566. <http://dx.doi.org/10.5433/1679-0359.2015v36n6p3553>.
- Formentini MA, Alves LFA, Schapovaloff ME. Insecticidal activity of neem oil against *Gyropsylla spegazziniana* (Hemiptera: Psyllidae) nymphs on Paraguay tea seedlings. *Brazilian Journal of Biology = Revista Brasileira de Biologia* 2016; 76(4): 951-954. <http://dx.doi.org/10.1590/1519-6984.04915>. PMID:27143053.
- Heck CI, Mejia EG. Yerba mate mea (*Ilex paraguariensis*): a comprehensive review on chemistry, health implications, and technological considerations. *Journal of Food Science* 2007; 72(9): 138-151. <http://dx.doi.org/10.1111/j.1750-3841.2007.00535.x>. PMID:18034743.
- Isaias RMS, Carneiro RGS, Oliveira DC, Santos JC. Illustrated and Annotated Checklist of Brazilian gall morphotypes. *Neotropical Entomology* 2013; 42(3): 230-239. <http://dx.doi.org/10.1007/s13744-013-0115-7>. PMID:23949804.
- Leite MSP, Zanol KMR. Biologia e morfologia de *Gyropsylla spegazziniana* (Lizer y Trelles) (Hemiptera, Psyllidae). *Acta Biológica Paranaense* 2001; 30(10): 19-34. <http://dx.doi.org/10.5380/abpr.v30i0.598>.
- Moran PJ, Patt JM, Cabanillas HE, Adamczyk JL, Jackson MA, Dunlap CA et al. Localized Autoinoculation and dissemination of *Isaria fumosorosea* for control of the Asian citrus psyllid in south Texas. *Yaredai Zhiwu Kexue* 2011; 63(1): 23-25.
- Rayl RJ, Wratten DAS. Comparison of anesthesia techniques for entomological experimentation: longevity of the leaf-mining fly pest *Scaptomyza flava* Fallén (Drosophilidae). *Peer Journal Preprints* 2016; 1(5): 2874-2903.