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Source: Florida Entomologist, 104(1) : 56-57

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.104.0110

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Using sticky card traps to evaluate entomopathogen fungi occurrence in insect populations: a cautionary tale

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Tea brewed from the leaves of yerba mate or Paraguay tea (*Ilex paraguariensis* St. Hil.; Aquifoliaceae) is a very popular beverage in Argentina, Paraguay, and Brazil. The leaves of this plant also are used as a raw material for medicines and cosmetics (Cardozo & Morand 2016). Immatures of the psyllid, *Gyropsylla spegazziniana* Lizer & Treles (Hemiptera: Aphalaridae) cause galling (ampules) and disfiguration in the leaves of *I. paraguariensis* (Formentini et al. 2015). For this reason, the psyllid's common name is 'ampola-da-erva-mate' (yerba mate ampule). Infestation by *G. spegazziniana* leads to significant loss of leaves for harvest and weakens the plants. Production losses of up to 54% and 35% have occurred in Brazil (Chiaradia et al. 2000; Leite 2001) and Argentina (Fernández Diaz 1997), respectively. However, control of the psyllid is limited to manual picking and pruning because few insecticides are registered for use in yerba mate and consumers prefer tea grown "in natura." Therefore, alternatives to insecticides are needed to control this psyllid in yerba mate orchards.

A biocontrol strategy using entomopathogenic fungi to control *G. spegazziniana* in commercial yerba mate orchards is under development. An initial study showed that *G. spegazziniana* is susceptible to *Beauveria bassiana* (Blas.-Criv.) (Hypocreales: Cordycipitaceae) (Alves et al. 2013). Further screening identified a certain strain, *B. bassiana* (*sensu lato*) strain Unioeste 44, as being highly toxic to *G. spegazziniana* (Formentini et al. 2015). Since then, efforts have focused on developing spore formulations and adjuvants to improve conidiospore performance and designing an "attract and infect" device (autodisseminator) to efficiently dispense the spores in yerba mate orchards (Loeblein 2019).

Because *G. spegazziniana* is attracted to yellow (Loeblein et al. 2018), prototype autodisseminators were colored yellow to promote visitation to the devices by *G. spegazziniana* (Loeblein 2019). The prototypes were exposed to psyllids in screened cages and the psyllids were recovered on yellow sticky card traps placed inside the cages. The psyllids were held in humid chambers and later examined for mycosis to determine the proportions of individuals infected by each prototype device (Loblein 2019). These cage tests showed that the psyllids could be effectively infected by autodisseminator prototypes dispensing formulations of *B. bassiana* Unioeste 44 conidiospores (Loeblein 2019). The results also demonstrated that infected psyllids could be recovered on yellow sticky card traps, and that these psyllids mycosed when held in a humid chamber (Loeblein 2019). This result is important because it showed that yellow sticky card traps can be used to estimate the level of *B. bassiana* contagion within *G. spegazziniana* populations. This, in turn, will facilitate evaluation of the efficacy of different autodisseminator designs and fungal strains in yerba mate orchards.

Two different types of sticky card traps were used in the cage tests: a ready-to-use sticky trap (Colortrap, ISCA Technologies, Ijuí, Rio Grande do Sul, Brazil) and a yellow non-sticky cardboard card, also from ISCA Technologies, that we coated with a fine layer of insect glue (Colly Química Indústria e Comércio Ltda., Mombuca, São Paulo, Brazil). Even though the 2 traps caught similar numbers of psyllids, the level of mycosis developing on the psyllids differed significantly between the 2 traps (Loeblein 2019). There was no difference in the attractiveness of the traps to healthy psyllids and those infected with Unioeste 44.

Removal of individual psyllids from the surface of sticky traps during infection level studies is tedious and time consuming. To save time and effort, it is desirable to incubate an entire trap with its contingency of trapped insects. To develop a standardized method for incubating whole traps returned from field tests, as well as to confirm that the type of glue used with the trap can negatively affect mycosis, the following tests were performed. *Beauveria bassiana* (*sensu lato*) Unioeste 44 was grown on conidia production medium (KH_2PO_4 0.36 g, $NA_2HPO_47H_2O$ 1.05 g, $MgSO_47H_2O$ 0.60 g, KCl 1.0 g, glucose 10.0 g, $NaNO_3$ 1.58 g, yeast extract 5.0 g, agar 20.0 g, 1,000 mL distilled water) in Petri dishes at 26 ± 1 °C; 12:12 h (L:D) photophase for 7 to 10 d. Conidia were collected by scrapping the culture media surface; then they were dried by placing them in a desiccator with silica gel for 7 d. The material was sieved (32 mesh) and then stored at −20 °C (hermetic containers; 1.1×10^{11} conidia g^{-1}; minimum viability of 90%). Both types of traps measured 5 × 7 cm.

Twenty *G. spegazziniana* adults were confined for 30 minutes in a plastic cup (Copaza Descartáveis Plásticos, Içara, Santa Catarina, Brazil) containing dry conidia of *B. bassiana* Unioeste 44. Afterwards, the psyllids were transferred to screened cylindrical cages (30 cm h × 12 cm d), each containing a yerba mate seedling (Loeblein et al. 2019). The cages were maintained in a controlled environment room (26 ± 2 °C; 60 ± 10% RH; 12:12 h [L:D] photoperiod). After 2 d, 1 adhesive trap was placed inside the cages near the top of the yerba mate seedling. Five cages were provided with the commercial trap from ISCA Technologies and 5 cages were provided with the trap coated with Colly™ insect glue. The traps were replaced every 2 d, for a period lasting 21 d. After removal, the traps were transferred to plastic vessels (Copaza Descartáveis Plásticos, Içara, Santa Catarina, Brazil) whose bottoms were covered with a layer of polyurethane foam moistened with distilled water. The vessels were closed and then placed in an incubator at 26 ± 2 °C, 12 h (L:D) photoperiod to permit the development of mycosis on the

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cadavers. The development of mycosis confirmed that mortality was caused by Unioeste 44 infection. Five cages that held untreated psyllids and were not subjected to the sticky card traps were used as a control. Attraction and mortality data were analyzed by ANOVA and compared with Tukey's test ($p < 0.05$).

As observed in previous studies, both traps were equally attractive to the psyllids. Capture levels of 89 and 93% were observed, respectively, on the traps coated with Colly™ insect glue and on the commercial traps from ISCA Technologies (Table 1). Also, the percentage of cadavers that mycosed was significantly higher in the ISCA traps (mean = 78%) than in the traps we had coated with Colly™ insect glue (mean = 2.0%) (Table 1). These results confirmed that care must be taken to use an insect glue that does not interfere with mycosis when selecting or designing traps for use in evaluating entomopathogen infection levels in target insect populations.

The results also showed that individual sticky traps can be collected and then incubated for the purpose of evaluating the level of mycosis in *G. spegazziniana* or other small, delicate insects captured on the trap surface. Incubating an entire trap, rather than individuals extracted from its surface, will be a time-saving measure that will facilitate determination of entomopathogen infection levels in populations of *G. spegazziniana* in yerba mate orchards. This, in turn, will help to develop and validate measures using entomopathogens to control *G. spegazziniana* in yerba mate orchards.

The authors wish to thank "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)" and ISCA Tecnologias Ltda. for financial support.

Summary

This study confirmed that some glues used for trapping insects may inhibit mycosis; therefore, care should be taken to select "mycosis-friendly" glues when selecting sticky traps for monitoring entomopathogen infection levels in target pest populations. The results also showed that, rather than attempting to extract small, delicate insects from the glue for further incubation, the entire sticky trap simply can be incubated for the purpose of evaluating mycosis levels.

Key Words: field trial; fungus infection; natural occurrence; incidence; epizootiology

Table 1. Attraction of *Gyropsylla spegazziniana* adults to and mortality caused by *Beauveria bassiana* Unioeste 44 using 2 types of yellow card traps.

Trap	*n*	Trapped (%)	Confirmed Mortality (%)
Control (without trap)	5	–	–*
Adhesive trap with Colly™ glue layer	5	89.0 ± 11.4 a	2.0 ± 4.5 b
Yellow Sticky Trap™ ISCA	5	93.0 ± 9.7 a	78.0 ± 1.5 a
VC (%)		15,23	26,74
p-value		< 0.0001	< 0.0001

Means (± MSE) followed by the same letter in the column do not differ significantly (Tukey's test, $p < 0.05$); *not observed.

Sumario

Este estudo confirmou que algumas colas usadas para capturar insetos podem inibir a micose; portanto, deve-se tomar cuidado para selecionar colas "compatíveis com o fungo" ao selecionar armadilhas adesivas para monitorar os níveis de infecção de entomopatógenos em populações de pragas-alvo. Os resultados também mostraram que, em vez de tentar extrair insetos pequenos e delicados da cola para incubação posterior, toda a armadilha adesiva pode ser simplesmente incubada com o objetivo de avaliar os níveis de micose.

Palavras Chaves: experimento em campo; infecção fúngica; ocorrência natural; incidência; epizootiologia

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