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Insecticidal activity of neem oil against *Gyropsylla spegazziniana* (Hemiptera: Psyllidae) nymphs on Paraguay tea seedlings

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Abstract

Gyropsylla spegazziniana (Paraguay tea ampul) is one of the most important pests of Paraguay tea plants, and prohibition of synthetic insecticide use for control of this pest has led to the search for alternative methods. This laboratory study aimed to compare different control strategies for *G. spegazziniana*, utilizing a commercial neem seed oil product. Paraguay tea seedlings were treated with neem oil solution both pre- and post-infestation with 5th instar nymphs. The systemic action of neem oil was also evaluated by treating plant soil with the neem oil solution, followed by transfer of the insects to plants 24 h post-treatment. Spray treatments were effective against the pest, especially post-infestation (80% mortality), demonstrating the potential of neem oil for control of the Paraguay tea ampul. No significant effects were observed with respect to systemic activity.

Keywords: Gyropsylla spegazziniana, botanical insecticide, alternative control.

Atividade inseticida de óleo de nim contra ninfas de *Gyropsylla spegazziniana* (Hemiptera: Psyllidae) em mudas de erva-mate

Resumo

Gyropsylla spegazziniana (ampola da erva-mate) é uma das principais pragas da erva-mate, tanto em áreas de plantio como em viveiros. A proibição do uso de inseticidas para o controle de pragas da cultura tem levado à busca de alternativas. Este trabalho visou avaliar comparativamente, em laboratório, estratégias de aplicação de um produto comercial à base de óleo de nim sobre *G. spegazziniana*, em solução a 5%. Os testes foram realizados em mudas de erva-mate, tratadas pré e pós-infestação com ninfas de 5º instar do inseto, para avaliação de contato. Também, avaliou-se a ação sistêmica do óleo de nim, aplicando-se a solução do produto no solo da muda de erva-mate, seguido da transferência dos insetos após 24 h. Verificou-se que os tratamentos via pulverização foram eficientes contra a praga, principalmente na aplicação pós-infestação (80% de mortalidade), comprovando o potencial inseticida do óleo de nim contra a ampola-da-erva-mate em mudas. Entretanto, em relação à atividade sistêmica, não foram observados efeitos consideráveis sobre a praga.

Palavras-chave: ampola da erva-mate, planta inseticida, controle alternativo.

1. Introduction

Paraguay tea (*Ilex paraguariensis* St. Hill) is a forest species cultivated primarily in monoculture systems, with great socio-economic importance to producing regions of Brazil, Argentina and Paraguay. The leaves are used to prepare beverages and the plant is commonly used in pharmacological studies, making it an excellent crop choice for farmers.

The current form of exploitation resulting from agricultural expansion in the 1970's favored abundant populations of insects and mites, many of which are crop pests, which damaged Paraguay tea production (Gazeta de Comunicações, 2000; Chiaradia et al., 2000, 2002; Borges et al., 2003). *Gyropsylla spegazziniana* (Lizer & Trelles) (Hemiptera: Psyllidae), the Paraguay tea ampul, is considered one of the main pests of this crop. It is found associated with the shoots in seedlings (in the greenhouse and field) and in mature trees, on which it feeds and oviposits. Feeding by this insect leads to the development of galls, or ampuls, due to the inoculation of toxic saliva into developing leaves. The nymphs reside in

these ampuls, continuously feeding on sap until near-adult stage. The deformed leaves fail to grow and then fall off, after which the plant responds by producing new lateral shoots, leading to reduced productivity.

Estimated crop losses from this pest are about 35% in Argentina and 54% in Brazil (Rivera Flores, 1983; Penteado, 1995; Diaz, 1997; Chiaradia et al., 2000; 2002; Leite, 2002). These pests may also infest seedlings in nurseries, sometimes leading to damage, after which plants may be discarded due to negative impacts on development and the possibility of death; alternatively, if infested nursery seedlings are planted then the pests may be dispersed among the crop site (Chiaradia, 2000).

There are currently no products in Brazil indicated for control of this pest (Brasil, 2015). A review by Barzotto and Alves (2013) highlighted some strategies used to limit the increase of insect populations, such as the selection of resistant plants, soil preparation and fertilization, management of plant density and species diversity, proper pruning, biological control, and use of plant extracts. Botanical insecticides are safer than chemical insecticides, and certifying agencies endorse their use in organic farming systems (Machado et al., 2007; Koul and Walia, 2009; Brasil, 2014).

Neem oil is rich in tetranortriterpenoid-limonoid complex (Mordue and Nisbet, 2000), and a 10% solution applied to branches and leaves showed activity against *G. spegazziniana* in laboratory and field studies (Haas et al., 2010; Barzotto, 2010). However, its effectiveness against nymphs in Paraguay tea seedlings is unknown. This study aimed to evaluate the effectiveness of neem oil and mineral oil against *G. spegazziniana* nymphs on Paraguay tea seedlings, both in nurseries and on newly planted crops, via different application strategies.

2. Material and Methods

Paraguay tea seedlings ~15 cm in height and containing 6 to 8 leaves were obtained from a commercial nursery and grown in mixture of soil and organic matter. The seedlings were kept in a greenhouse (26 ± 1 °C, RH 60 ± 10%), and watered daily.

Insects were collected from infested Paraguay tea branches (with many closed ampuls) from a commercial crop in Cascavel, Paraná, Brazil. The branches were kept in glass vessels with water, inside of cages (60 × 40 × 40 cm) in a climatized room (26 ± 1 °C, 12:12 L:D photoperiod, RH 60 ± 10%). For experiments, fifth-instar nymphs were collected using a soft paintbrush (Barzotto, 2010). We chose this developmental stage because *G. spegazziniana* fifth-instar nymphs naturally leave the galls, and are less sensitive to handling (Leite, 2002).

We used the commercial oil-based product Organic Neem® (94% neem oil and 6% organic adjuvants, according to manufacturer (Dalquim Industria e Comércio Ltda., 2015) in 5% water solution. This product was previously used by Haas et al. (2010) and Barzotto (2010) against *G. spegazziniana* adults, but in 10% water solution.

2.1. Evaluation of contact action

We evaluated the effects of application both pre- and post-infestation. In the pre-infestation treatment (residual effect), seedlings were sprayed with neem oil solution from a distance of 15 cm to the point of run-off (~2 mL/seedling), using an airbrush apparatus coupled to a continuous airflow compressor (output of constant pressure 0.7 kgf/cm^2). After drying in the shade, 23 fifth-instar *G. spegazziniana* nymphs were transferred to the seedlings, which were then individually placed in cylindrical, colorless PVC cages (diameter 13 × 40 cm), and maintained under previously described laboratory conditions. For the post-infestation treatment, seedlings were infested with 23 fifth-instar *G. spegazziniana* and subsequently treated with neem oil solution, as previously described (direct contact effect). We also evaluated effects of commercial mineral oil in aqueous solution (0.1%) both pre- and post-infestation following the same procedures.

For all experiments, control treatments consisted of seedlings infested with *G. spegazziniana* fifth-instar nymphs. Observations were performed daily for 10 days by counting the number of dead insects on each plant. Five seedling replicates were used for all treatments (pre- and post-infestation treatment with neem oil or mineral oil, and controls).

2.2. Evaluation of irrigation treatment (systemic action)

Paraguay tea seedlings were maintained under laboratory conditions for 48 hours without irrigation, after which 50 mL of 5% neem oil solution in distilled water was applied directly to the soil using an automatic pipette. *G. spegazziniana* fifth-instar nymphs were transferred 24 h after application of the product, as previously described. Control plants received distilled water only. Five seedling replicates were used for all treatments and controls. Observations were performed daily by counting number of dead insects on each plant, and continued for 10 days. Water was also applied directly to the soil.

Experiments were repeated twice to confirm results. Data were checked for normality using the Shapiro-Wilk test. Data following a normal distribution were analyzed using one-way ANOVA and Tukey test ($p < 0.05$) using SISVAR software (Ferreira, 2011).

3. Results

Neem oil showed significant insecticidal activity (Table 1). Spraying was the most efficient application method. The post-infestation spray treatment caused 80% mortality. Pre-infestation application of neem oil yielded mortality of 43%. Neem oil showed rapid insecticidal action in both pre- and post-infestation treatments, withg 65% and 38% mortality, respectively, within 48 hours of application. Insect mortality in soil-treated plants was only 19.9% (Table 1). It is noteworthy that in all treatments, dead insects were found lying on the bottom of the cage, and there were no visible signs of phytotoxicity in treated plants. Application of mineral oil yielded results similar to controls.

Table 1. Mean mortality (± SE) of *Gyropsylla spegazziniana* nymphs on Paraguay tea (*Ilex paraguariensis*) 10 days after pre- and post-infestation treatment with neem oil and mineral oil (26 ± 1 °C; 12:12 h L:D photoperiod, R.H. = 60 ± 10%).

Treatment	Nymph Mortality (%)
Control	8.7 ± 1.2 d
Mineral Oil - Spraying Pre-infestation	5.2 ± 0.3 d
Mineral Oil - Spraying Post-infestation	9.6 ± 1.7 d
Neem Oil - Spraying Pre-infestation	49.6 ± 5.7 c
Neem Oil - Spraying Post-infestation	81.7 ± 5.6 a
Neem Oil - Irrigation treatment	26.9 ± 4.6 b
CV (%) = 28.75	

Different letters indicate significant differences between treatment groups (Tukey test, $p < 0.05$).

4. Discussion

Neem oil efficiency in control of psyllids has been demonstrated in laboratory, causing both mortality and developmental changes in nymphs of *Diaphorina citri* (Kuwayama) (Weathersbee and Mckenzie, 2005) and *Bactericera cockerelli* (Sulc) (Flores-Dávila et al., 2011). In contrast with Weathersbee and Mckenzie (2005), we did not find dead insects on the Paraguay tea leaves, possibly indicating that death occurred after contact with the product without necessarily having ingested it.

Laboratory studies showed higher efficiency of 10% neem oil solution against *G. spegazzinianna* nymphs when the product was sprayed directly on insects (60% mortality) compared to spraying plants after infestation (45% mortality) (Barzotto, 2010). Neem oil solution was found to be effective against *G. spegazziniana,* either due to translaminar action, or due to newly emerged nymphs from the gall contacting the concentrated product (10%) on the leaf surfaces (Haas et al., 2010).

The systemic action of neem has been demonstrated in studies conducted with other hemipterans, including the black aphid *Aphis craccivora* (Koch) (Hemiptera: Aphididae) in cowpea (*Vigna unguiculta* (L.) Walp) (Gonçalves and Bleicher, 2006a), and *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in melon and tomato (Souza and Vendramim, 2005; Gonçalves and Bleicher, 2006b).

It is It is noteworthy that in those studies the nymphs were in early developmental instars and azadirachtin (the main active ingredient of neem oil) acts on both ecdysteroid and juvenile hormone, interfering with growth and molting (Mordue and Nisbet, 2000). In our study only 5th instar nymphs were used, which may have resulted in a short period of insect contact with the product. Thus, the amount of active ingredient ingested or absorbed may have been insufficient to cause physiological changes in the insect at the end of the nymphal stage, which could explain the low insect mortality in the irrigation treatment compared to direct contact with the active ingredient.

The low insecticidal activity in the irrigation treatment may also be attributed to the concentration of active ingredient in the plant, which may have been insufficient to trigger a reaction in the insect either due to delay in translocation of the product through phloem to the younger plant tissues (where ampuls are formed), or due to product degradation in the soil prior to plant absorption (Bernardi et al., 2013; Botton et al., 2013).

Our results indicate that neem oil is an effective product for control of *G. spegazziniana* nymphs on Paraguay tea seedlings. They also indicate that direct contact (post-infestation application) provides promising results for pest control on seedlings, either in the nursery or in newly planted areas. To better evaluate insecticidal activity, developmental effects and possible behavioral changes, further studies are needed using products derived from neem applied in different concentrations, and with different (i.e., longer) exposure times.

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References

BARZOTTO, I.L.M., 2010. *Atividade inseticida de extratos vegetais sobre Gyropsylla spegazziniana (Lizer & Trelles, 1917) (Hemiptera: Psyllidae)*. Cascavel: Universidade Estadual do Oeste do Paraná, 47 p. Masters Dissertation.

BARZOTTO, I.L.M. and ALVES, L.F.A., 2013. Bioecologia e manejo de *Gyropsylla spegazziniana* em erva-mate. *Arquivos do Instituto Biológico*, vol. 80, no. 4, pp. 457-464. http://dx.doi.org/10.1590/S1808-16572013000400015.

BERNARDI, D., BOTTON, M., CUNHA, U.S., BERNARDI, O., MALAUSA, T., GARCIA, M.S. and NAVA, D.E., 2013. Effects of azadirachtin on *Tetranychus urticae* (Acari: Tetranychidae) and its compatibility with predatory mites (Acari: Phytoseiidae) on strawberry. *Pest Management Science*, vol. 69, no. 1, pp. 75-80. http://dx.doi.org/10.1002/ps.3364. PMid:22807305.

BORGES, L.R., LÁZZARI, S.M.N. and LÁZZARI, F.A., 2003. Comparação dos sistemas de cultivo nativo e adensado de erva mate, *Ilex paraguariensis* St. Hil., quanto à ocorrência e flutuação populacional de insetos. *Revista Brasileira de Entomologia*, vol. 47, no. 4, pp. 563-568. http://dx.doi.org/10.1590/S0085-56262003000400005.

BOTTON, M., BERNARDI, D., EFROM, C.F.S. and BARONIO, C.A., 2013. Eficiência de inseticidas no controle de Eurhizococcus brasiliensis (Hemiptera: Margarodidae) na cultura da videira. *BioAssay*, vol. 8, pp. 1-5. http://dx.doi.org/10.14295/BA.v8.0.120.

BRASIL, 2014 [viewed 24 March 2015]. *Instrução normativa conjunta SDA/SDC nº 2. Especificações de referência de produtos fitossanitários com uso aprovado para a agricultura orgânica*. Diário Oficial da União [online], Brasília, 15 jul. Seção 1, pp. 6-8. Available from: www.aenda.org.br/fito/MAPA_IN02-13.pdf

BRASIL, Ministério da Agricultura, Pecuária e Abastecimento, Coordenação-Geral de Agrotóxicos e Afins, 2015 [viewed 24 March 2015]. *AGROFIT: sistemas de agrotóxicos fitossanitários* [online]. Brasília. Available from: http://www.agricultura.gov.br/servicos-e-sistemas/sistemas/agrofit

CHIARADIA, L.A., 2000. Aspectos da ecologia de *Gyropsylla spegazziniana* (Lizer, 1971) (Hemiptera, Psyllidae) em erva-mate. In: *Anais do 2º Congresso Sul-Americano da Erva-mate; 3ª Reunião Técnica da Erva-Mate*, 2000, Encantado, RS. Porto Alegre: URGS/Fundação Estadual de Pesquisa Agropecuária, pp. 50-53.

CHIARADIA, L.A., MILANEZ, J.M. and SABEDOT, S.M., 2000. Caracterização e danos da ampola-da-erva-mate. *Revista Agropecuária Catarinense*, vol. 13, pp. 50-53.

CHIARADIA, L.A., MILANEZ, J.M. and ZIDKO, A., 2002. Estimativa das gerações anuais de *Gyropsylla spegazziniana* (LIZER, 1917) em função de sua exigência térmica. *Ciência Rural*, vol. 32, no. 3, pp. 385-391. http://dx.doi.org/10.1590/S0103-84782002000300004.

DALQUIM INDÚSTRIA E COMÉRCIO LTDA., 2015 [viewed 24 March 2015]. [online]. Available from: http://www.dalquim.com.br

DIAZ, D.Y.F. 1997. Perspectivas del manejo integrado de plagas em yerba mate. In: *Anais do 1º Congresso Sul-Americano da Erva-mate; 2ª Reunião Técnica do Cone Sul sobre a Cultura da Erva-mate*, 1997, Curitiba. Curitiba: Edição dos Organizadores, pp. 371-390.

FERREIRA, D.F., 2011. Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia*, vol. 35, pp. 1039-1042. http://dx.doi.org/10.1590/S1413-70542011000600001.

FLORES-DÁVILA, M., GONZÁLEZ-VILLEGAS, R., GUERRERO-RODRÍGUEZ, E., MENDOZA-VILLARREAL, R., CÁRDENAS-ELIZONDO, A., CERNA-CHAVEZ, E. and AGUIRRE-URIBE, L., 2011. Insecticidal effect of plant extracts on *Bactericera cockrelli* (Hemiptera: Psyllidae) nymphs. *The Southwestern Entomologist*, vol. 36, no. 2, pp. 137-144. http://dx.doi.org/10.3958/059.036.0203.

GAZETA DE COMUNICAÇÕES, 2000. *Anuário brasileiro da erva-mate*. Santa Cruz do Sul: Gazeta de Comunicações. 80 p.

GONÇALVES, M.E.C. and BLEICHER, E., 2006a. Atividade sistêmica de azadiractina e extratos aquosos de sementes de nim sobre o pulgão-preto em feijão-de-corda. *Revista Ciência Agronômica*, vol. 37, no. 2, pp. 177-181.

GONÇALVES, M.E.C. and BLEICHER, E. 2006b. Uso de extratos aquosos de nim e azadiractina via sistema radicular para o controle de mosca-branca em meloeiro. *Revista Ciência Agronômica*, vol. 37, no. 2, pp. 182-187.

HAAS, J., TOMKIEL, M.V., ALVES, L.F.A. and FANTI, A.L.P., 2010. Efeito de óleo de sementes de nim (Azadirachta indica A. Juss.) sobre Gyropsylla spegazziniana (Lizer & Trelles), na cultura da erva-mate (Ilex paraguariensis St. Hil.). *Revista Brasileira de Agroecologia*, vol. 5, no. 2, pp. 194-199.

KOUL, O. and WALIA, S., 2009. Comparing impacts of plant extracts and pure allelochemicals and implications for pest control. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, vol. 4, no. 49, pp. 1-30.

LEITE, M.S.P., 2002. *Biologia e determinação do dano de Gyropsylla spegazziniana (Lizer y Trelles, 1919) (Hemiptera, Psyllidae) na cultura da erva-mate (Ilex paraguariensis, St. Hil.)*. Curitiba: Universidade Federal do Paraná, 84 p. PhD Thesis.

MACHADO, L.A.,BARBOZA E SILVA, V. and OLIVEIRA, M.M., 2007. Uso de extratos vegetais no controle de pragas em horticultura. *Instituto Biológico*, vol. 69, no. 2, pp. 103-106.

MORDUE, A.J. and NISBET, A.J., 2000. Azadirachtin from the neem tree *Azadirachta indica*: its action against insects. *Anais da Sociedade Entomológica do Brasil*, vol. 29, no. 4, pp. 615-632. http://dx.doi.org/10.1590/S0301-80592000000400001.

PENTEADO, S.R.C., 1995. Principais pragas da erva-mate e medidas alternativas para o seu controle. In: H. WINGE, A.G. FERREIRA, J.F.A. MARIATH and L.C. TARASCONI, eds. *Erva-mate: biologia e cultura no Cone Sul*. Porto Alegre: UFRGS.

RIVERA FLORES, S.E., 1983. *Control del psilido de la yerba mate (Gyropsilla spegazziniana Liz.)*. Cerro Azul: INTA. 12 p. Informe Técnico, no. 39.

SOUZA, A.P. and VENDRAMIM, J.D., 2005. Efeito translaminar, sistêmico e de contato de extrato aquoso de sementes de nim sob*re Bemisia tabaci* (Genn.) biótipo B em tomateiro. *Neotropical Entomology*, vol. 34, no. 1, pp. 83-87. http://dx.doi.org/10.1590/S1519-566X2005000100012.

WEATHERSBEE, A.A. and MCKENZIE, C.L., 2005. Effect of a neem biopesticide on repellency, mortality, oviposition, and development of *Diaphorina citri* (Homoptera: Psyllidae). *The Florida Entomologist*, vol. 88, no. 4, pp. 401-407. http://dx.doi.org/10.1653/0015-4040(2005)88[401:EOANBO]2.0.CO;2.