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## Bioassay

# Indirect control of the ground-pearl *Eurhizococcus brasiliensis* (Wille, 1992) (Hemiptera: Coccomorpha: Margarodidae) using ants toxic baits

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**Abstract.** *Linepithema micans* (Forel, 1908) (Hymenoptera: Formicidae) is the main ant species responsible for spreading the ground pearl, *Eurhizococcus brasiliensis* (Wille, 1992) (Hemiptera: Margarodidae). The effect of different formulations of hydramethylnon toxic baits (TB) was evaluated on the control of *L. micans* and *E. brasiliensis*. Four formulations of TB were evaluated in a greenhouse to control *L. micans.* Based on the greenhouse experiment results, the small pellet formulation of the TB was selected to be evaluated, through weekly applications, in two vineyards naturally infested by *L. micans* and *E. brasiliensis*. Later, in order to establish the number of applications, 1, 3, 6 and 10 applications in the season were evaluated. The bait treatments were compared with the number of ants and ground pearls counted on one area free of TB and one area with the drench application of thiamethoxam. Hydramethylnon (0.5%) TB in the small pellet (SP) and large pellet (LP) formulations were the most efficient in *L. micans* control. The SP TB, applied six times during the vine cycle, effectively control the number of *L. micans*, reducing 100% of the ant population, as well as the *E. brasiliensis*, reducing 99.9% of the scale population on plants.

**Keywords:** *Linepithema micans*, ground pearl, dispersal, hydramethylnon, pest management.

*Linepithema micans* (Forel, 1908) (Hymenoptera: Formicidae) is the primary agent responsible for the spreading of *Eurhizococcus brasiliensis* (Wille, 1992) (Hemiptera: Margarodidae) (Nondillo et al. 2013), a neotropical ground pearl, cyst-like scale insects, usually present on roots of grapevines that is believed to be associated with the decline and death of grapevine plants in southern Brazil (Zart et al. 2014).

The invasive Argentine ant *Linepithema humile* (Mayr, 1868) is the main species associated with honeydew-producing scale insects in worldwide grapevine production regions (Daane et al. 2006). However, in Brazil, the sister species *L. micans* was the main species recorded in grapevines in the Southern region. These ants are associated with the transport and attachment of first-instar nymphs of *E. brasiliensis* on grapevine roots (Nondillo et al. 2013).

Ants control may be a recommended practice for the management of scale insects on grapevines. Toxic baits (TB) are especially interesting due to the low doses of insecticides used and their slow effect, not only killing the ants that directly came into contact with the toxicant but acting on the whole nest through trophallaxis (Nondillo et al. 2016). Unlike *L. humile* which is often controlled using sugar-based baits (Daane et al. 2006), it was observed that toxicants incorporated in protein-based solid baits are more effective in controlling *L. micans* (Nondillo et al. 2016). Previous studies have evaluated the effect of hydramethylnon on *L. micans* control, with positive results (Nondillo et al. 2016). However, the formulations previously studied are inadequate for application in the field due to the high perishability or high handling by ants, killing the workers before trophallaxis occurs. Granular baits may be an alternative to improve the use of baits, which showed a satisfactory control of *L. humile* (Klotz et al. 2000; Krushelnycky et al. 2004) and can be adapted to *L. micans.* In this work, the effect of a hydramethylnon-based granular TB on the control of *L. micans* and indirectly control of *E. brasiliensis* in grapevines was evaluated.

To evaluate the effect of solid TB on the control of *L. micans* an experiment was conducted in a greenhouse at Embrapa Uva e Vinho, RS, Brazil. Nests of *L. micans* were collected in Flores da Cunha, RS, and used to infest 5-L pots following the protocol proposed by Nondillo et al. (2013). Each pot received approximately 10 queens and 1,500 workers, and all nests contained eggs, larvae, and pupae. The pots were placed in trays coated with Teflon-30 (Dupont) and talcum powder to prevent the escape of the ants and fed with larvae of *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) or adults of *Gryllus* sp. (Orthoptera: Gryllidae) and inverted sugar (25%), three times per week. After 15 days, to evaluated the mean number of ants per pot, colonies were fasted for 24 hours and only water was provided. After this period, an aqueous solution of inverted sugar (70%) was offered in the center of a whiteboard (3 × 3 cm). Then, every 10 minutes, for one hour, the number of workers foraging on the food supply was counted for each pot in an image taken by a digital camera. The pots were grouped in the treatments according to their infestation levels.

Toxic baits containing hydramethylnon (0.5%) in (i) gel, (ii) large pellet (0.8 mm ⌀) (LP), (iii) small pellet (0.6 mm ⌀) (SP), and (iv) cereal forms were evaluated, in contrast with the insecticide thiamethoxam (Actara 250 WG®) at 125 g of a.i./ha used as a standard treatment. Baits were prepared in the Research Center on Social Insects - UNESP - Rio Claro, SP. Except for cereal form that was prepared using cassava flour and soybean oil as food attractants the other solid formulations were prepared using cereal flour, vegetable oil and preservatives.

The TBs were provided *ad libitum* in bait holders (Nondillo et al. 2016), with baits being replaced weekly. Thiamethoxam was applied on the soil surface of the pots using a motorized backpack sprayer. Evaluations were carried out weekly for a period of 10 weeks, counting the mean number of ants per pot as described above. The experiment was conducted in a completely randomized design with nine replicates, being each pot considered as an experimental unit.

To validate the results obtained in the greenhouse, an experiment was conducted under field conditions, applying SP TB in two commercial

vineyards with a history of *L. micans* and *E. brasiliensis* infestation, both located in Flores da Cunha, RS. About 150 seedlings of Paulsen 1103 rootstock were planted in each vineyards (corresponding to a spacing of 450 seedlings/ha). Each vineyard was divided into two plots that were 30 m apart, each with 72 vine seedlings, with one plot receiving the toxic bait weekly, for 19 weeks, and the other kept as a control (without bait). Before the toxic bait was applied, the initial population of ants was monitored twice, 7 days apart.

To quantify the number of ants, in-ground pitfall traps were used, which consisted of a set of two plastic tubes (3.3 cm diameter × 5.0 cm height), connected with a 50 cm string, with a cap and side holes (3 mm) (Morini et al. 2004). A honey and water solution (70%), absorbed in cotton wool, was used as food attractant in one tube, whereas sardine conserved in oil was used in the other. In each plot, 24 in-ground traps were equidistantly buried, 20 cm deep, remaining in the field for a period of 24 hours. After this period, the traps were collected in plastic bags and frozen for later evaluation.

The toxic baits were placed in bait holders (one per plant, totaling 72 per plot), and applied three months after planting, when *E. brasiliensis* infestation should begin. One gram of bait was placed in each bait holder, corresponding to 450 g/ha per week (2.25 g of a.i.), and 8.5 kg/ha throughout the experimental period. The bait effect on the ant population was evaluated weekly using in-ground pitfall traps. For the assessment of the number of the ground pearl per plant, after 19 weeks, all the planted seedlings have been uprooted, along with a block of soil (25 cm diameter × 40 cm depth), and placed in a white tray where the number of ground-pearls were counted.

In order to reduce the number of bait applications, a third experiment was conducted to evaluate the frequency of application required to control both species populations. About 180 seedlings of Paulsen 1103 were planted in a vineyard (Caxias do Sul, RS). Before the experiment, the population of *L. micans* was evaluated. The vineyard was divided into six plots, 30 m apart, based on the application frequency of small pellet (SP) bait, that were: (i) one (450 g/ha); (ii) three (1.35 kg/ha); (iii) six (2.7 kg/ha); and (iv) ten applications (4.5 kg/ha); also (v) one application of the thiamethoxam (0.25 g of i.a/plant) used as the standard treatment; and (vi) control (without baits). The TBs were placed in bait holders (one per plant) and replaced weekly, except the treatment with a single application frequency. As in the second experiment, one gram of bait was placed in each bait holder. Thiamethoxam was applied via drench directed to the surface of the soil in the plant using a motorized backpack sprayer. The effect of the treatments was measured weekly for 23 weeks. For each treatment, 15 in-ground pitfall traps were used, that remained in soil for 24 hours, after this period, the traps were collected for subsequent screening. The final evaluation consisted of counting the number of ground pearls present in the roots of the planted vines and soil, as explained before.

For both, greenhouse and field experiments, the treatments were hierarchically grouped by similarity, with the F test of contrasts to compare the different treatment groups (Nondillo et al. 2016). For field experiments, the data for the number of *E. brasiliensis* cysts in the control and treated area was compared by Student's t test (to evaluate the effect of the TB in the field) and Tukey's (to evaluate the number of applications). All analyses were performed using R (R Development Core Team 2013).

In the greenhouse experiment hydramethylnon (0.5%) TB in the formulation SP and LP were the most efficient in ant control, causing 100% mortality of workers in the first 5 weeks of application and not allowing the colony's re-establishment (Fig. 1). The results are similar to those observed by Mothapo & Wossler (2016) for the control of *L. humile*, where it was found that granular baits containing hydramethylnon caused 85-100% mortality of workers and 63-75% of queens in 24 h.

The gel formulation of the hydramethylnon TB also provided 100% mortality, however, was slower when compared with the other two formulations (Fig. 1). The cereal formulation did not differ from the control (P=0.97). Although the results for formulations SP and LP were equivalent (P=0.75), the formulation SP was selected for the field experiments due to its better handling by the ants. In pots treated with thiamethoxam, a significant reduction was observed in the population of ants in the first week after the treatment, differing significantly from the control (P<0,0001) however, the colonies re-established as the experiment progressed (Fig. 1). The direct application of insecticides on the ground often kills or repels active workers with less effect on queens and offspring (Bueno & Bueno 2007). In addition, can increase ant infestation by the fragmentation of the colony that might promote the rise of infestation level in the area or the dispersal of ants to other areas (Bueno & Bueno 2007).

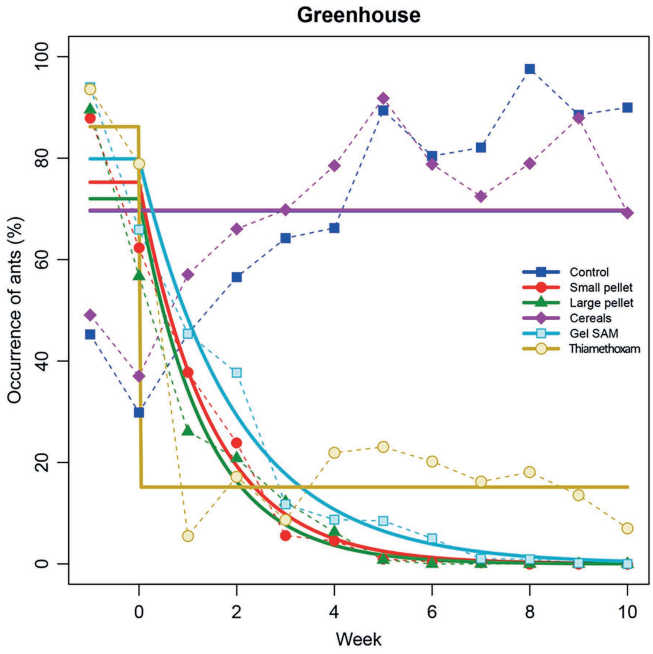


**Figure 1.** Percentage of *Linepithema micans* workers foraging after treatment with toxic baits in a greenhouse. Curves for each treatment represent the response fitted using the exponential model adapted for the treatment.

In the field, hydramethylnon-based TB applied in the SP formulation caused 100% reduction in the number of ants foraging in the two evaluated areas (Fig. 2) differing significantly from the control (P < 0.0001) without re-establishment of the colonies during the 19 weeks of the TB use. This result corroborates the obtained in the greenhouse, where 100% mortality of the *L. micans* workers was observed in the first weeks (Fig. 1). Baits with protein containing hydramethylnon also have shown satisfactory control of *L. humile* in the field (Klotz et al. 2000).

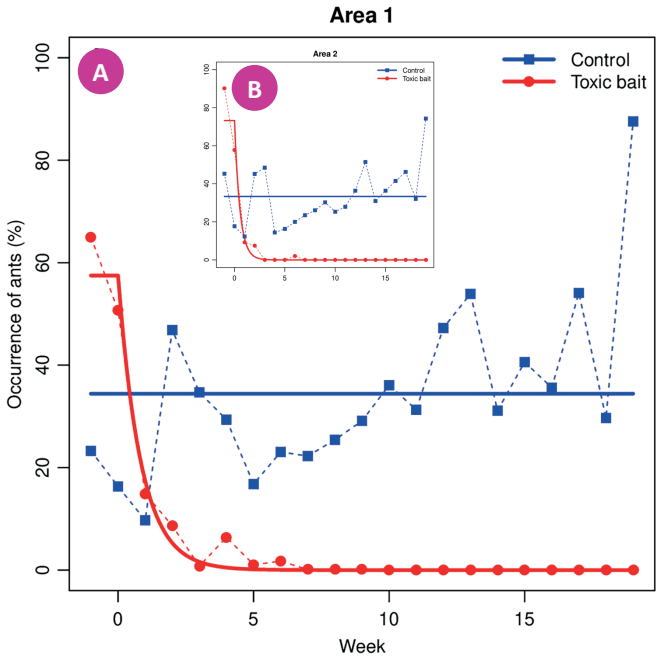


**Figure 2.** Effect of toxic baits on the mean number of *Linepithema micans* workers per trap in vineyards. **(A)** Area 1. **(B)** Area 2.

The mean number of *E. brasiliensis* cysts/plant in the roots of the rootstock where the TB was applied was 0.07 ± 0.003, which was significantly lower (P<0.0001) when compared to the control (32.65 ± 1.46). The same was observed for the second area, which presented a mean of 0.18 ± 0.14 cysts/plant and differed significantly (P<0.0001) from the control with 36 ± 0.91 cysts/plant. These results reinforce the findings of Nondillo et al. (2016), demonstrating the importance of the ant *L. micans* in the establishment of *E. brasiliensis* in new grapevine plants as well as the indirect effect of TBs on reducing the scale insect population.

Regarding the number of applications of TB to control *L. micans* in the field, plots that received six and ten applications of the hydramethylnon TB showed a significant reduction (P <0.0001) in the number of ants when compared to the plot that received the lowest number of applications (Fig. 3). No significant difference was observed between treatments with one application of thiamethoxam and three applications of TB (P=0.44). Furthermore, it was observed in treatment with thiamethoxam an initial decrease in the number of foraging ants, however, the population re-establish after the first weeks. This result supports what was observed in the greenhouse experiment (Fig. 1).

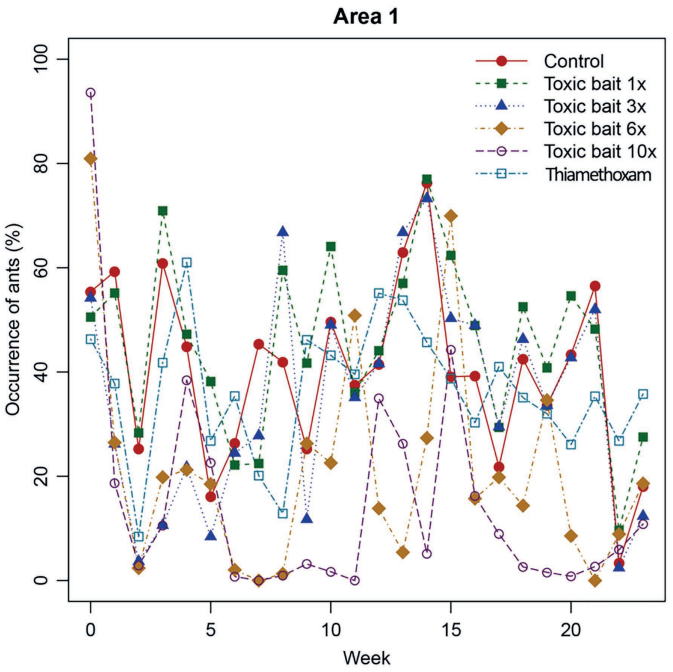


**Figure 3.** Effect of thiamethoxam and weekly applications of toxic baits on the mean number of workers of *Linepithema micans* per trap on a vineyard.

Regarding the number of cysts, the treatments that received one, three, six and ten TB applications had an average number of 15.36 ± 3.4, 6.7 ± 2.60, 0.35 ± 0.22 and 0.95 ± 0.49 cysts/plant, respectively, while the control showed an average number of 25.03 ± 4.73 cysts/plant. Positive control with one thiamethoxam application showed an average number of 4.59 ± 1.52 cysts/plant not differing from the treatments with three or more applications (Fig. 4).

Soil applications of insecticides are of great risk to beneficial organisms, including pollinators, such as neonicotinoid insecticides (Cresswell et al. 2012). The use of TB with small doses of insecticide is the most considered option for the management of ants associated with scale insects in vineyards (Rust et al. 2003), which can be taken to the nest and distributed to larvae and queens via grooming, trophyallaxis, necrophagia and necrophoresis (Soeprono & Rust 2004). In addition to the ecological advantage, since they have less impact on non-target organisms, TBs are safer to the applicator and less harmful to the environment due to the small doses of insecticide used (Rust et al. 2003; Daane et al. 2006). In this work, it was observed that six applications of granulated toxic baits based on hydramethylnon in the vine cycle are efficient in controlling *L. micans* and consequently cause a reduction in *E. brasiliensis* infestation in grapevine plants. More studies are needed to know the foraging behavior of *L. micans* in vineyards at different times of the year, thus allowing to define the number of bait stations needed per area.

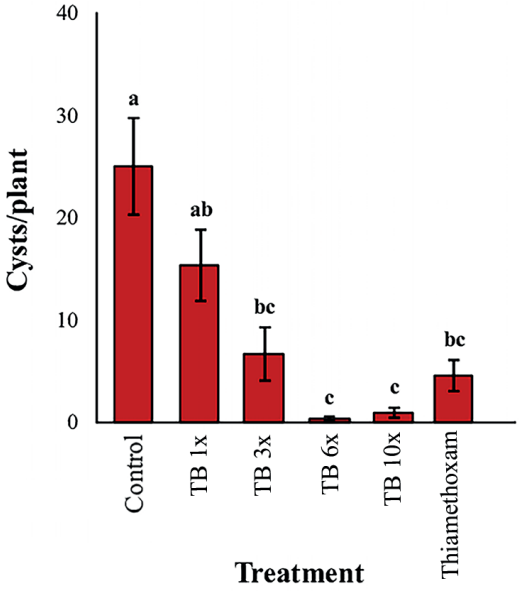


**Figure 4.** Effect of thiamethoxam and weekly applications of toxic baits on the mean number (± SE) of *Eurhizococcus brasiliensis* cysts per plant on vineyards.

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## Authors' Contributions

Conceptualization: AN, AEL, OCB, MB; Data collection: SA, AN; Data analysis: SA, AN, FBF; Writing and editing: SA, AN, VCPS, MB; Final manuscript correction: SA, AN, VCPS.

## Conflict of Interest Statement

There is no conflict of interest.

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