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Ecotoxicology

Control of *Linepithema micans* (Hymenoptera: Formicidae) and *Eurhizococcus brasiliensis* (Hemiptera: Margarodidae) in Vineyards Using Toxic Baits

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Abstract

Linepithema micans (Forel) (Hymenoptera: Formicidae) is the main ant species responsible for dispersal of *Eurhizococcus brasiliensis* (Wille) (Hemiptera: Margarodidae), a root scale that damages grapevines in southern Brazil. The effects of different formulations of toxic baits based on boric acid and hydramethylnon to control *L. micans* and *E. brasiliensis* were evaluated. Toxic baits with boric acid (1.0%) mixed in different concentrations of inverted sugar (20%, 30%, and 40%), and hydramethylnon, mixed with sardines (paste), cassava flour and peanut, brown sugar (sucrose), or sardine oil-based gel, were evaluated in a greenhouse and in the field. In the greenhouse experiment, the number of foraging ants was significantly reduced in the pots where the hydramethylnon in sardine paste (Solid S), sardine oil-brown sugar-based gel (GEL SAM), and peanut oil-brown-sugar gel (GEL AM) formulations were applied. The GEL SAM toxic bait effectively reduced the infestation of *L. micans*, and could be used for indirect control of *E. brasiliensis* on young grapevines.

Key words: hydramethylnon, ant, scale, control

One of the main limitations on the expansion of viticulture in Brazil is the damage and death of grapevines from insect pests and diseases (Almança et al. 2013, Cavalcanti et al. 2013). One of the main insect pests is the root scale *Eurhizococcus brasiliensis* (Wille) (Hemiptera: Margarodidae), native to southern Brazil (Hickel et al. 2008, Botton et al. 2010).

Eurhizococcus brasiliensis has a complex biological cycle (Soria and Gallotti 1986). Parthenogenetic eggs are laid inside mature cysts, and the first-instar nymphs emerge from the ruptured cysts. In this mobile phase, which occurs from November through March, the nymphs have little self-dispersal capacity. They move close to a root and remain feeding until full development; this phase usually lasts for one year (Gallotti 1976, Soria and Gallotti 1986, Botton et al. 2000, Soria and Dal Conte 2000, Foldi 2005). The parthenogenetic females appear and remain inside the cysts until they lay their eggs, after which they die (asexual reproduction). The scale can also reproduce sexually. In this case, the cyst becomes a mobile female that, at the time of mating, emerges to copulate with the winged male, and later returns to the ground to lay eggs (Soria and Dal Conte 2000).

To control this scale on grapevines, winegrowers apply the neonicotinoid insecticides thiamethoxam and imidacloprid, primarily through drenching (Botton et al. 2013). This practice has been effective for many years, although with some limitations, particularly the presence of toxic residues in the fruits and the risk of environmental contamination.

One of the important aspects of the survival of *E. brasiliensis* is its interaction with ants that harvest the expelled sugar excretions ("honeydew") in a mutualistic association, in which both the ant and hemipteran pests are benefited (Sacchett et al. 2009, Nondillo et al. 2013). *Linepithema micans* (Forel) (Hymenoptera: Formicidae) is the ant dispersant of the scale more frequent and abundant in vineyards in southern Brazil, the main grape-producing region in the country (Martins and Bueno 2009, Sacchett et al. 2009, Nondillo 2013).

One alternative to reduce scale infestation in vineyards is to control the dispersive ants (Nondillo et al. 2014). In vineyards of South Africa and California, the Argentine ant *Linepithema humile* (Mayr) has been the main ant species associated with scales of the family Pseudococcidae (Addison 2002, Daane et al. 2006). In these situations, infestation by hemipterans is significantly reduced when the ants are excluded from the plants (Geiger et al. 2001, Daane et al. 2008). The dispersant ants have been controlled primarily with insecticides applied to the soil or the trunk of the vine (Phillips and

Sherek 1991, Addison 2002, Klotz et al. 2003, Daane et al. 2006) or with the use of toxic baits (Nelson and Daane 2007, Daane et al. 2008, Nyamukondiwa 2008, Nyamukondiwa and Addison 2011, Bligth et al. 2011, Buczkowski et al. 2014b).

The toxicants most commonly tested for control of *L. humile* include boric acid, fipronil, hydramethylnon, imidacloprid, sulfluramide, and thiamethoxam (Klotz et al. 1998, Hooper-Bui and Rust 2001, Rust et al. 2004, Nelson and Daane 2007, Daane et al. 2008, Nyamukondiwa 2008, Nyamukondiwa and Addison 2011, Blight et al. 2011, Buczkowski et al. 2014a). These active toxicants must be administered in liquid, solid, granular, or gel form (Silverman and Brightwell 2008).

In Brazil, a similar strategy has been developed to manage *L. micans* in order to reduce *E. brasiliensis* infestations in vineyards, in a strategy of integrated management (Nondillo et al. 2014). The effects of different insecticides applied as toxic baits have been evaluated for the control of *L. micans* in greenhouses (Nondillo et al. 2014). In these experiments, hydramethylnon (0.5%) incorporated into sardine paste has shown promise for the control of *L micans*. However, this formulation is highly perishable, and therefore unsuitable for ant control in the field.

Many studies have obtained satisfactory results in the reduction of populations of *L. humile*, using hydramethylnon in liquid, solid, granular, or gel form (Knight and Rust 1991, Hooper-Bui and Rust 2000, Forschler and Evans 1994). Forschler and Evans (1994) evaluated hydramethylnon (0.9%) for the control of *L. humile* in the field, and observed a reduction in foraging activity 6 wk postapplication. Knight and Rust (1991) and Hooper-Bui and Rust (2000), in laboratory experiments with hydramethylnon (0.5–1.0%) mixed in a liquid solution, observed suppression of colonies of *L. humile* after 24 h, however, with no effect on the queens.

This study evaluated the effect of toxic bait formulations with hydramethylnon and boric acid for the control of *L. micans* in a greenhouse. After the most effective formulations were determined, a field study was conducted to assess the effect of these toxic baits on *L. micans* and *E. brasiliensis* in newly planted grapevines.

Materials and Methods

Effect of Toxic Baits for the Control of *L. micans* in a Greenhouse

The experiment was conducted in a greenhouse located at Embrapa Uva e Vinho, Bento Gonçalves, Rio Grande do Sul, Brazil. Paulsen 1103 rootstock seedlings (*Vitis berlandieri* × *Vitis rupestris*) planted in individual 5-liter pots were used.

The vine seedlings were grown for ~2 mo in the pots, and were then infested with nests of ants. Nests of *L. micans* of similar size, with ~10 queens and 1,500 workers, were transferred to each pot. All the nests contained eggs, larvae, and pupae. The ants were collected from vineyards infested with *E. brasiliensis* and *L. micans*. The ant nests were removed together with soil, transported to the laboratory in plastic bags, and transferred to plastic trays. To capture the ants, each tray received two tiles (10 by 10 cm) with the abrasive faces toward each other, and with wooden sticks (2 mm thick) between them. The sticks were placed with a space between their tips, for the ants to enter. Cotton moistened with inverted sugar solution (25%) was placed between the tiles to stimulate the ants to enter the set of tiles (Nondillo et al. 2012). Inverted sugar is produced commercially by the hydrolysis of sucrose to obtain a mixture of glucose and fructose. After the colonies established themselves between the tiles, a pair of tiles was placed on the surface of each pot, thus enabling the ants to transfer the colony themselves (Nondillo et al. 2013).

The pots were placed in trays filled with talcum powder, with the edges covered with Teflon-30 (Dupont), to prevent the ants from escaping.

After infestation, the initial ant population foraging in each pot was counted. Colonies were fasted for 24 h, with only water supplied. Next, an aqueous solution of inverted sugar (70%) was offered in the center of a white board (3 by 3 cm), and the number of workers on the food source was recorded every 10 min for 1 h (Fig. 1).

After the first evaluation, pots with fewer than 20 ants were reinfested and again monitored. After this second evaluation, the pots were grouped according to the number of workers foraging, in the following categories:

1—Pots with 20–50 ants foraging;
2—Pots with 50–100 ants foraging;
3—Pots with 150–200 ants foraging;
4—Pots with >200 ants foraging.

The pots were then grouped in the treatments according to the level of infestation in each pot.

After the infestation, the ants were fed three times a week with larvae of *Tenebrio molitor*, adults of *Gryllus* sp., and inverted sugar (25%). Water was supplied ad libitum through a sample tube with a cotton tuft on its free edge.

The toxic baits were evaluated in liquid, solid paste, or gel form, using boric acid (Sigma Aldrich) and hydramethylnon (Anhui Fuerpont Chemical Co.) as active toxicants (Table 1).

The liquid baits consisted of an aqueous solution of inverted sugar (20, 30, and 40%) and 1% boric acid. The solid baits were formulated with hydramethylnon (0.5%) mixed with sardine paste or cassava flour, or in gel form. The abbreviations used for each formulation are given in Table 1. The toxic baits were prepared in the Centro de Estudos de Insetos Sociais (CEIS), UNESP, Rio Claro, São Paulo.

Both the liquid and solid toxic baits were offered to the ants ad libitum in bait holders, and were replaced weekly. The bait holder is a rectangular box (8 by 5 cm), with a convex inner surface to hold the bait. The box is closed with a cover and has small lateral openings to allow ants to enter (Fig. 2).

After the products and baits were applied, evaluations were conducted weekly over a 12-wk period, by counting the number of ants foraging every 10 min for 1 h, on a food source (aqueous solution of 70% inverted sugar) as described above. Before each evaluation the colonies were fasted for 24 h, with only water available. The experiment was conducted in a fully randomized experimental design, with nine repetitions (one pot per repetition) per treatment.

Statistical Analysis

The maximum number of ants foraging in each hour was used as a response variable in the data analysis. This number was converted to a percentage of the maximum number of ants observed in each pot over the entire experiment.

The data were evaluated separately for each experiment. For each treatment, a curve of the percentage of ants foraging as a function of time was plotted, adjusting a modified decreasing exponential function:

$$Y = A.e^{-B.x.(x>0)} + C$$

This model represents a function that is constant before the application of the treatments (when $x \leq 0$) and follows a decreasing

A B

Fig 1. (**A**) An aqueous solution of inverted sugar (70%) in the center of a white board (3 by 3 cm) with *L. micans* workers on the food source. (**B**) Detail of ants feeding on food source during the evaluation period.

Table 1. Toxic baits evaluated in a greenhouse for control of *L. micans* on grapevines

Treatment	Active ingredient	Concentration (%) /kg of bait of the a.i	Bait
GEL SAM	Hydramethylnon	0.5	Gel formulation (sardine oil + brown sugar)
GEL AM	Hydramethylnon	0.5	Gel formulation (peanut oil + brown sugar)
GEL S	Hydramethylnon	0.5	Gel formulation (sardine oil)
GEL A	Hydramethylnon	0.5	Gel formulation (peanut oil)
GEL AC	Hydramethylnon	0.5	Gel formulation (sugar)
SOLID S	Hydramethylnon	0.5	Sardine paste
CEREALS	Hydramethylnon	0.5	Cereals
B.A. 20%	Boric Acid	1.0%	Inverted sugar (20%)
B.A. 30%	Boric Acid	1.0%	Inverted sugar (30%)
B.A. 40%	Boric Acid	1.0%	Inverted sugar (40%)

exponential curve after the application. A, B, and C are parameters of the model, where C represents the final percentage of ants after the end of the treatment, A represents the decrease in percentage of ants due to the treatment, and B is the rate of the decrease in number of ants. In some cases, where B approaches ∞, the model reduces to a simple step function.

In order to compare the entire response curves with each other (not just the means at one specific point), a hierarchical classification procedure was used. Treatments were grouped in pairs, and an F test was used to compare the result of fitting both treatments in a group using a single curve against fitting two separate curves, one for each treatment. The combination with the two most similar treatments (the one with the smallest *P* value) was accepted and the treatments of this combination were joined into a group. The entire process was repeated until all treatments were joined into a single group, and the results were expressed as a dendrogram. Thus, treatments were grouped hierarchically by similarity, using the F test of contrasts to compare the different treatment groups. All analyses used the R program (R Development Core Team 2013)

Effects of Toxic-Bait Formulations for Control of *L. micans* in the Field

The experiment was installed in two sites naturally infested with *E. brasiliensis* and *L. micans*, located in Caxias do Sul (29° 14′923″ S, 051° 14′376″ W) and Pinto Bandeira (29° 03′232″ S, 051° 27′871″ W), Rio Grande do Sul, Brazil.

Each site was divided into two areas, with a 30-m space between them. In Caxias do Sul, 154 seedlings on Paulsen 1103 (*Vitis berlandieri* × *Vitis rupestris*) rootstock were planted (77 seedlings in each area) with a spacing of 0.5 by 0.5 m. In Pinto Bandeira, 110 seedlings were planted (55 seedlings in each area) with a similar spacing. The rooted seedlings were planted in July, before the period of *E. brasiliensis* infestation, following recommended cultivation practices.

At each site, weekly, the first area received the hydramethylnon-based toxic bait selected in the greenhouse experiment (GEL SAM), keeping the second area as a control.

Before the application of toxic baits, premonitoring was done in order to quantify the initial population of *L. micans* in the area, using ground pitfall traps (20 per area). The traps consisted of a set of two plastic pipes (3.3 cm diameter by 5.0 cm height) connected by a 50-cm string, with a cap and lateral holes (3 mm; Morini et al. 2004). Two food attractants were used as baits to attract the ants into the traps. One of the pipes contained a honey-water solution (70%) absorbed in cotton wool, and the other contained sardines conserved in oil. The attractants were placed inside the cap. The traps were collected after 24 h in the field, and the ants were transferred to individual jars with 80% ethanol and counted in the laboratory (Nondillo et al. 2014).

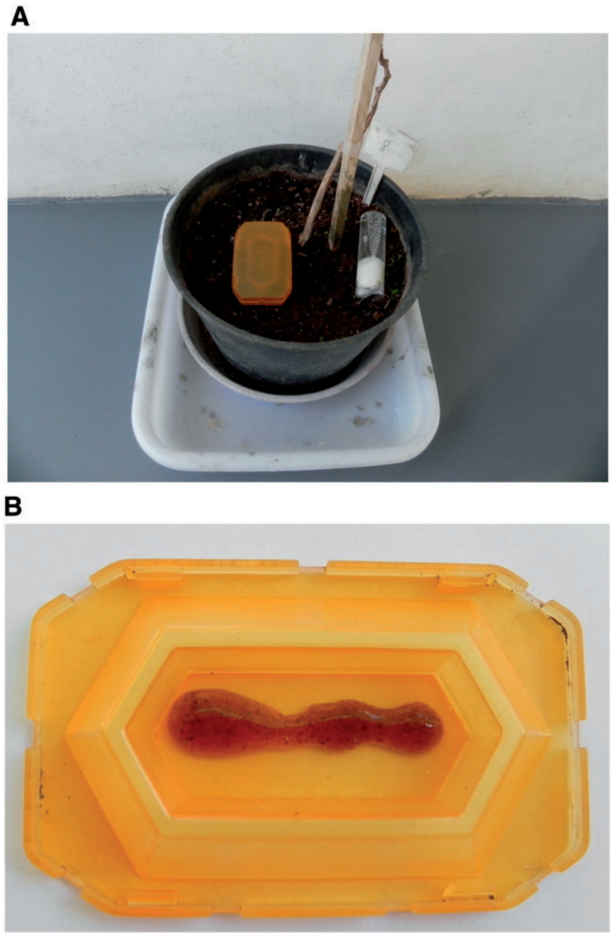

Fig. 2. (**A**) Paulsen 1103 rootstock seedlings (*Vitis berlandieri* × *Vitis rupestris*) planted in individual 5-liter pots infested with *L. micans* nests, and bait holders. (**B**) Detail of a bait holder with solid toxic bait (GEL SAM).

The toxic baits were arranged randomly in the bait holders (one per plant), replaced weekly, and applied beginning in November (when the infestation of *E. brasiliensis* starts). One gram of bait was placed in each bait holder, totaling 23 kg/ha in Caxias do Sul and 12 kg/ha in Pinto Bandeira during the experimental period.

The bait effect on the ant population was monitored weekly for 28 wk, using in-ground pitfall traps as described above.

In July, all the plants were pulled in order to count the number of scales per plant (Botton et al. 2013). A block of soil (25 cm diameter and 40 cm deep) was also collected from around each plant, with a shovel. The soil and roots were placed on a white tray, and the number of scale cysts was counted.

Statistical Analysis

The data for number of cysts of *E. brasiliensis* in the area treated with bait and the control area were compared by Student's *t* test, using the R program.

Results and Discussion

Effects of Toxic Baits on Control of *L. micans* in a Greenhouse

The nests were established in all the infested pots, which made it possible to count the initial population of foraging ants. The ants were counted immediately after the infestations were completed.

The major diference observed between the treatments (toxic baits) evaluated in the greenhouse was between the group of toxic baits comprising GEL AM, GEL SAM, and SOLID S, and the other treatments (GEL AC, CONTROL, 40% B.A., 20% B.A., CEREALS, GEL A, GEL S and 30% B.A.) as shown in the dendrogram ($P < 0.0001$). The first group was clearly more effective in reducing the ant population (Fig. 3).

Among the three most-effective treatments (GEL AM, GEL SAM, and SOLID S), the hydramethylnon-sardine paste bait (SOLID S) was significantly more effective in reducing the number of foraging ants than the gel formulations (GEL SAM and GEL AM; $P = 0.0036$; Fig. 3). The SOLID S bait produced a larger and more rapid reduction in the number of ants.

Although the formulations GEL SAM and GEL AM were less effective than SOLID S, they caused mortality above 80% (Fig. 3). In all three treatments, the colonies did not reestablish during the 12-wk evaluation period.

The pots where GEL AC was applied showed no significant difference from the control ($P = 0.22$; Fig. 3). The boric acid-based bait (40 and 20% B.A.) reduced the ant population compared with the control ($P < 0.0001$), but was less effective than (GEL AM, GEL SAM, and SOLID S), which controlled >80% of the population. This same pattern was recorded for other baits (CEREALS, GEL, GEL S, B.A. 30%; Fig. 3).

Studies on control have mainly focused on *L. humile* because it is a major pest worldwide, and most have evaluated the use of contact insecticides or toxic baits (Klotz et al. 2002, 2003; Silverman and Brightwell 2008; Nelson and Daane 2007; Daane et al. 2008; Buczkowski et al. 2014a). Liquid-sugar baits containing boric acid (0.5–1.0%) have proved effective at reducing populations of *L. humile* in both urban and agricultural areas (Klotz et al. 1998, Hooper-Bui and Rust 2000, Klotz et al. 2007). This is one of the main active toxicants used to control *L. humile* in Californian and South African vineyards (Daane et al. 2006, 2007, 2008; Nyamukondiwa and Addison 2011). However, boric acid in different concentrations was ineffective in controlling colonies of *L. micans*, (Nondillo et al. 2014), probably because of the relatively high sucrose concentrations used (50 and 70%).

For this reason, in the present experiment, boric acid was evaluated in lower concentrations of inverted sugar (20, 30, and 40%), close to those used for other members of Dolichoderinae (Klotz et al. 1998, 2007; Hooper-Bui and Rust 2000). However, even in solutions with lower sugar concentrations, boric acid remained ineffective in controlling *L. micans*. This can be explained by the different foraging behavior of this species from *L. humile*. *L. micans* has a diffuse nest and underground foraging habits, while *L. humile* forages on aerial trails to collect honeydew from phloem-feeding homopterans (Daane et al. 2008).

Hydramethylnon effectively controlled *Solenopsis invicta* colonies and *L. humile* workers, when it was mixed in sugar solutions (Hooper-Bui and Rust 2001, Stanley 2004). In protein baits, hydramethylnon provided satisfactory control of *L. humile*, especially in field conditions (Forschler and Evans 1994, Klotz et al. 2000, Krushelnycky and Reimer 1998).

The hydramethylnon-sardine paste (Solid S) gave the best results in reducing the *L. micans* population, in agreement with findings of Nondillo et al. (2014). However, this formulation is too perishable for field use. Hydramethylnon (0.5%) in the formulation GEL SAM also effectively reduced the population of *L. micans* in the

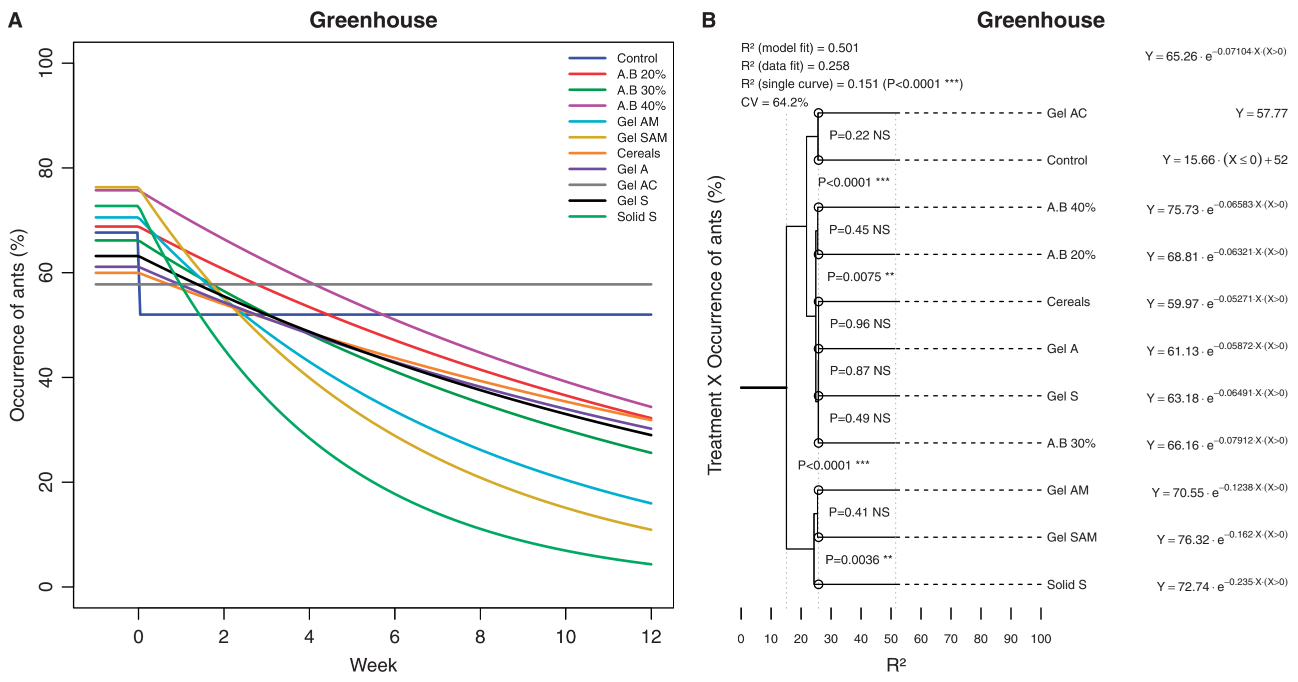

Fig. 3. (**A**) Percentage of *L. micans* workers foraging after treatment with toxic baits in a greenhouse. Curves for each treatment represent the fitted response using the modified decreasing exponential model for that treatment. (**B**) Graphical representation of the statistical comparison of treatments. Treatments are grouped by similarity of model curves. P values were computed using the F test to compare a model with data for two groups fitted as separate curves against a model that uses a common curve for both groups. NS = not significant; * significant at 5% probability; ** significant at 1% probability; *** significant at 0.1% probability.

greenhouse, where the gel can easily be applied. This formulation was selected for the field experiments.

Effectiveness of Toxic Baits for Control of *L. micans* in the Field

In the field experiment, the number of ants foraging in the area where the hydramethylnon gel bait (GEL SAM) was applied was significantly reduced compared to the control (Figs. 4 and 5), in both Caxias do Sul ($P < 0.0001$) and Pinto Bandeira ($P < 0.0001$). Colonies failed to reestablish during the 28-wk evaluation period in all of the areas where the baits were used.

The results from the field experiments using the gel formulation (GEL SAM) concorded with the results of the greenhouse experiment, showing that the bait is efficient in reducing populations of *L. micans* in vineyards.

For *E. brasiliensis* on roots of vines in Caxias do Sul, in the plot where the toxic bait was applied, the mean number of cysts per plant (54.15 ± 11.07) was significantly smaller ($t = 4.724$; df = 76; $P \leq 0.001$) than in the control area (4.13 ± 0.81; Fig. 6). In the parallel experiment in Pinto Bandeira, the mean of 20.11 ± 8.51 cysts per plant in the control area differed significantly ($t = 2.338$; df = 54; $P \leq 0.023$) from the 3.16 ± 1.10 cysts found in the treatment area where *L. micans* population was controlled (Fig. 6).

These results indicate that *E. brasiliensis* cannot colonize new grapevine seedlings when the *L. micans* population is controlled. Thus, the use of toxic bait in new vineyards would be an alternative for managing scale insects, as due to lack of mobility, with no support from the ants, they would not reach the roots. In established vineyards where the scale insects are already present on the roots, eliminating the ant populations and applying approved insecticides for viticulture would reduce the scale population.

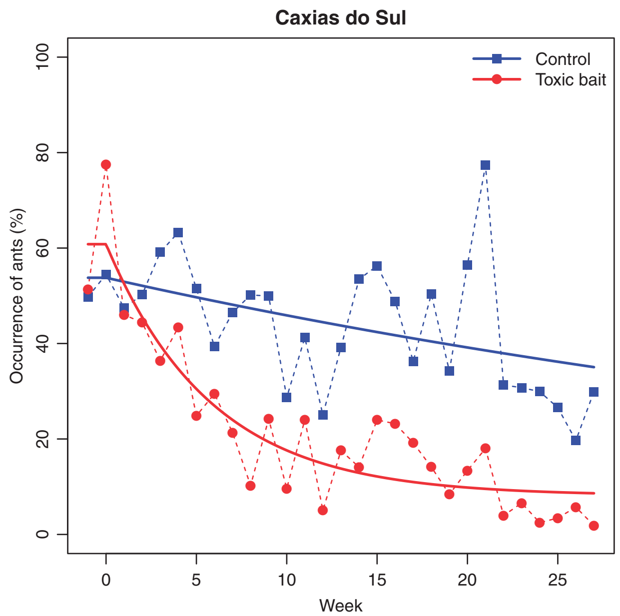

Fig. 4. Mean number of *L. micans* workers per trap after treatment with toxic baits in a vineyard in Caxias do Sul.

Similar results have been obtained in the control of *L. humile* in vineyards in California, USA, and in South Africa (Addison and Samways 2000; Daane et al. 2006, 2007). In these countries, the ant is especially associated with scale insects of the family Pseudococcidae, which are virus vectors. Infestations of these scales were reduced when the ants were excluded from the plants by the use of toxic baits (Daane et al. 2007).

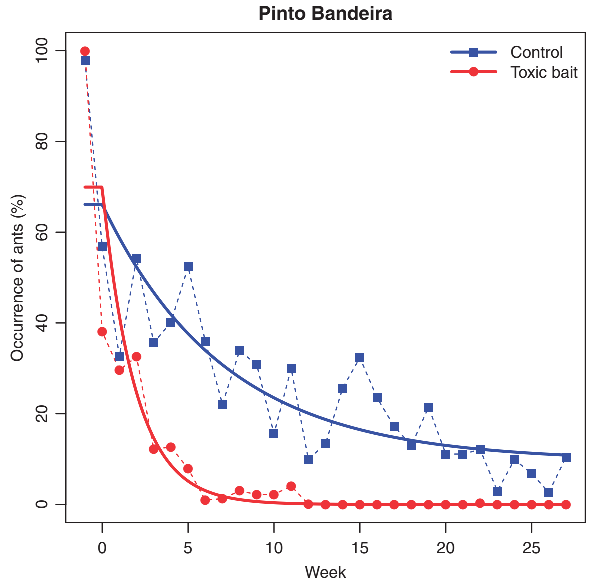

Fig. 5. Mean number of *L. micans* workers per trap after treatment with toxic baits in a vineyard in Pinto Bandeira.

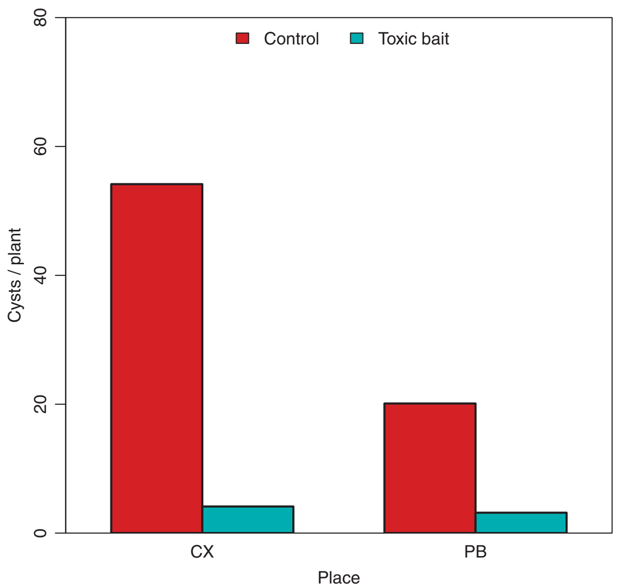

Fig. 6. Mean number (± SE) of cysts of *E. brasiliensis* per plant in vineyards located in Caxias do Sul (CX) and Pinto Bandeira (PB), Rio Grande do Sul.

Taking into consideration that *L. micans* is the predominant species of ant in areas where *E. brasiliensis* is present (Nondillo 2013) and that it affects the population dynamics of *E. brasiliensis* in vineyards, implementation of a management program for scale must also include control of the ants. The use of hydramethylnon-based toxic baits is promising as a measure to reduce ant infestations, and as a consequence the populations of *E. brasiliensis*.

Studies are needed on the behavior of *L. micans* foraging in vineyards at different times of year. This will provide information about the optimum number of baits per area in the different seasons.

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