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Evaluation of Insecticides for the Control of *Linepithema micans* (Hymenoptera: Formicidae)

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ABSTRACT *Linepithema micans* (Forel) (Hymenoptera: Formicidae) is the main ant species responsible for the spreading of *Eurhizococcus brasiliensis* (Wille) (Hemiptera: Margarodidae), a soil scale that damages grapevine plants in southern Brazil. The effect of contact and ingestion of insecticides on the control of *L. micans* was evaluated in a greenhouse using grapevines (*Vitis* spp.) infested by *L. micans.* The insecticides thiamethoxam (250, 187.5, and 125 g/ha), fipronil (4, 5, and 50 ml/ha), and imidacloprid (650 g/ha) were sprayed on the ground, whereas toxic baits containing boric acid (0.5, 1.0, and 1.2%), pyriproxyfen (0.3 and 0.5%), and hydramethylnon (0.5%) were evaluated in different formulations. Hydramethylnon (toxic bait) and thiamethoxam (chemical barrier) were the most efficient active ingredients for the control of *L. micans.*

KEY WORDS *Eurhizococcus brasiliensis*, vineyard, ant, control

Linepithema micans (Forel) (Hymenoptera: Formicidae) is the predominant ant species in vineyards in southern Brazil (Martins and Bueno 2009, Sacchett et al. 2009, Nondillo 2013), where it acts as a dispersive agent of *Eurhizococcus brasiliensis* (Wille) (Hemiptera: Margarodidae) (Nondillo et al. 2013), the main pest of grapes in Brazil (Gallotti 1976, Soria and Gallotti 1986, Botton et al. 2010).

E. brasiliensis has been controlled through the application of neonicotinoid insecticides (imidacloprid and thiamethoxam) to the soil (Botton et al. 2000, Teixeira et al. 2002). This strategy, in addition to being expensive, is difficult to perform and frequently yields unsatisfactory results (Botton et al. 2000, Teixeira et al. 2002).

An alternative strategy to reduce scale infestation in vineyards may be the control of dispersive ants. Because of the predominance of *L. micans* in infested areas and its potential as a dispersive agent, implementation of a management program for *E. brasiliensis* would also involve the control of the scale by reducing ant populations in vineyards.

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 such situations, infestation by Hemiptera is significantly reduced when the ants are excluded from the plants (Daane et al. 2007). The control of *L. humile* in vineyards has been mainly carried out by applying insecticides on the ground or the trunks of the vines (Phillips and Sherk 1991, Addison 2002, Klotz et al. 2003, Daane et al. 2006), or with toxic baits (Nelson and Daane 2007, Daane et al. 2008, Nyamukondiwa 2008, Blight et al. 2011, Nyamukondiwa and Addison 2011). In these cases, the insecticides that are most often used as chemical barriers are chlorpyrifos, bifenthrin, and fipronil (Phillips and Sherk 1991, Addison and Samways 2000, Klotz et al. 2003), and as toxic baits are boric acid, fipronil, hydramethylnon, imidacloprid, sulfluramid, and thiamethoxam (Klotz et al. 1998; Hooper-Bui and Rust 2000, 2001; Rust et al. 2004; Nelson and Daane 2007; Daane et al. 2008; Nyamukondiwa 2008; Nyamukondiwa and Addison 2011; Blight et al. 2011).

This study evaluated the effect of insecticides applied as a chemical barrier or as toxic bait for the control of *L. micans.*

Materials and Methods

The experiment was carried out from January 2011 to April 2012 in a greenhouse located at Embrapa Uva e Vinho, Bento Gonçalves, Rio Grande do Sul, Brazil. Seedlings rooted on Paulsen 1103 vine rootstocks (*Vitis berlandieri* × *Vitis rupestris*) and planted in individual 5-liter pots were used.

After they were planted, the vine seedlings remained in the pots for ≈2 mo, after which they were infested with colonies of *L. micans,* each containing ≈10 queens, as well as several pupae and workers. The colonies were collected according to methods described by Nondillo et al. (2012, 2013).

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After infestation, the initial ant population foraging in each pot was counted. Colonies were kept fasting for 24 h, with only water supplied, after which 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. After the first evaluation, pots with <20 ants were reinfested.

Pots were distributed among treatments based on the number of ants foraging during the second evaluation, so that all treatments started with a similar level of infestation.

Application of Insecticides. Insecticides used for soil spraying were as follows: 1) thiamethoxam (Actara 250 WG; Syngenta Proteção de Cultivos Ltda., São Paulo, Brazil), 2) fipronil (Klapp; BASF, Nelspruit, South Africa), and 3) imidacloprid (Provado 200 SC, Bayer CropScience, Isando, South Africa). These insecticides were selected because they are used in the control of leaf-cutter ants (fipronil) or of *E. brasiliensis* (imidacloprid and thiamethoxam), as recommended by Agrofit (2012).

The insecticides were applied to the pots using a back sprayer (500 liters/ha) directed to the surface of the soil in the pots.

Because of the lack of information on the use of active ingredients for the control of *L. micans*, the same toxic baits recommended for *L. humile* were used (Klotz et al. 1998; Hooper-Bui and Rust 2000, 2001; Rust et al. 2004; Nelson and Daane 2007; Daane et al. 2008; Nyamukondiwa 2008; Nyamukondiwa and Addison 2011): boric acid, pyriproxyfen, and hydramethylnon.

Toxic baits were evaluated in liquid form, as an aqueous solution of inverted sugar (50 and 70%), and in solid, paste, or gel form. Boric acid (0.5, 1.0, and 12%), was used as the liquid bait. Solid baits were formulated using pyriproxyfen (0.3 and 0.5%) and hydramethylnon (0.5%). Both the liquid and solid toxic baits were offered to the ants ad libitum in bait-holders, and were replaced weekly.

The effects of insecticides and toxic baits on the control of *L. micans* were assessed in three experiments (Tables 1 and 2) in a completely randomized experimental design with 10 repetitions (one vase per repetition) per treatment.

Evaluation of the Effect of the Baits. After the products and baits were applied, weekly evaluations were made using the same method as in the presampling phase, that is, by counting the number of ants foraging every 10 min for 1 h, on a food source (aqueous solution of 70% inverted sugar).

Evaluations lasted 15 wk in the first experiment, 7 wk in the second experiment, and 13 wk in the third experiment.

Statistical Analysis. The maximum number of ants foraging per 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 vase over the entire experiment.

The data were evaluated separately for each experiment. For each treatment, a curve of the percentage

Table 1. Sprayed insecticides used for *L. micans* control

Active ingredient	Commercial brand	Concn. of the a.i. (g/kg or)	First experiment: Dose (g or ml/ha)[a] c.p.	First experiment: Dose a.i.	First experiment: Dates of applications[b]	Second experiment: Dose[a] c.p.	Second experiment: Dose a.i.	Second experiment: Date of application	Third experiment: Dose[a] c.p.	Third experiment: Dose a.i.	Third experiment: Date of application
Thiamethoxam	Actara 250 WG	250	1000	250	25 Jan. 2011 25 Mar. 2011	750	187.5	19 Aug. 2011	500	125	02 Feb. 2012 21 Mar. 2012
Fipronil	Klapp	200	20	4	25 Jan. 2011 25 Mar. 2011	25	5	19 Aug. 2011	250	50	02 Feb. 2012 21 Mar. 2012
Imidacloprid	Provado 200SC	200	—	—	—	—	—	—	625	125	02 Feb. 2012 21 Mar. 2012

[a] Refers to the dose applied in the tests for a solution vol of 500 liters/ha.
[b] Two applications were made in this phase, one at the beginning and another 9 wk after the first.
a.i., active ingredient; c.p., commercial product.

Table 2. Toxic bait insecticides used for *L. micans* control

Active ingredient	First experiment			Second experiment			Third experiment		
	Concentration (%)/kg of bait of the a.i.	Feed attractant	Date of application	Concen. (%)/kg of bait of the a.i.	Feed attractant	Date of application	Concen. (%)/kg of bait of the a.i.	Feed attractant	Date of application
Boric acid	1.0	Inverted sugar (70%)	25 Jan. 2011	1.2	Inverted sugar (70%)	19 Aug 2011	0.5 and 1.0	Inverted sugar (50%)	02 Feb. 2012
Hydramethylnon	0.5	Paste	25 Jan. 2011	0.5	Gel	19 Aug 2011	0.5	Paste	02 Feb. 2012
Pyriproxyfen	0.3	Paste	25 Jan. 2011	0.5	Gel	19 Aug 2011	0.5	Paste	02 Feb. 2012

of ants foraging as a function of time was plotted, adjusting the function using the following formula:

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

This model represents a function that is constant before the application of the treatments (when $x \leq 0$), and follows a decreasing 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 as a result of the treatment, and B is related to the rate of decrease in number of ants. In some cases, where B approaches ∞, the model reduces to a simple step function.

To compare the different treatment groups, treatments were grouped hierarchically based on similarity using the *F*-test of contrasts. All analyses used the R software (R Foundation for Statistical Computing, version 3.0.2, 2013; R Foundation for Statistical Computing, Vienna, Austria).

Results

In the first experiment, the number of ants foraging in the pots sprayed with thiamethoxam or hydramethylnon bait decreased significantly ($P < 0.0001$) compared with the control and the other treatments (Fig. 1A and B). In both types of treatments (spray and toxic bait), the colony did not reestablish during the 15 wk of evaluation. Pots treated with pyriproxyfen-based toxic baits (0.5%) did not differ ($P < 0.30$) from the control (Fig. 1A and B), while pots treated with 1.0% boric acid differed slightly ($P = 0.021$). Even though spraying with fipronil differed ($P < 0.0001$) compared with the control and other spray treatments, colonies still survived in the pot (Fig. 1A and B). The results of the first experiment indicated that *L. micans* can be controlled either through spraying (chemical barrier) or through the use of toxic baits; however, the effectiveness of the treatment depends on the active ingredient used.

In the second experiment, there was a significant ($P < 0.0001$) reduction in the population of ants in the treatment with thiamethoxam 1 wk after application, compared with the control and other treatments (Fig. 2A and B). In the other treatments, although in some cases the population of ants foraging decreased in some cases, other populations increased, with no significant control of the ant population (Fig. 2A and B). These results confirm those obtained in the first treatment, thereby demonstrating the efficiency of thiamethoxam applied in spray form for the control of *L. micans.*

In the third experiment, in the pots where the boric acid-based toxic bait (0.5%) was used, there was no significant difference from the control (Fig. 3A and B). Although the curves for fipronil (50 g/ha), imidacloprid, and the toxic baits based on pyriproxyfen and 1.0% boric acid were slightly different from the control, the change was small compared with the treatments using hydramethylnon and thiamethoxam, which controlled >90% of the population (Fig. 3A and

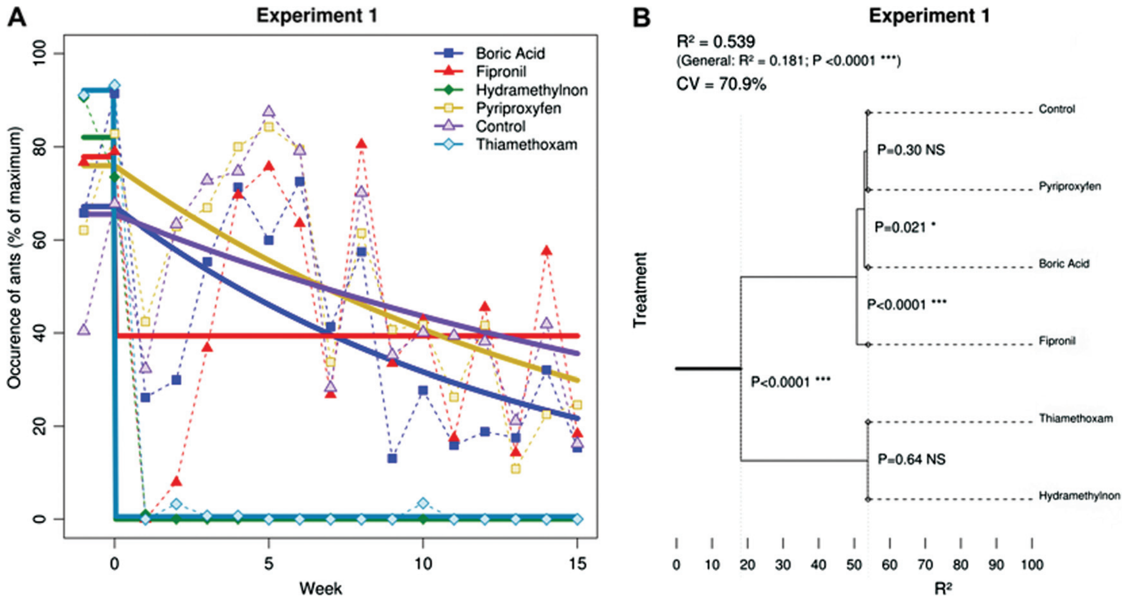

Fig. 1. Results of experiment 1 (January to May 2011): A) Percentage of workers of *L. micans* foraging after the treatment with insecticides and toxic baits; narrow dotted lines and symbols represent the actual measurements; wide solid lines represent the fitted functions, according to the model $Y = A \cdot e\hat{}(-B \cdot x \cdot (x > 0)) + C$. B) Graphical representation of the analysis of variance; the equations to the right of each treatment name represent the respective function, as estimated by the model; note that for fipronil, thiamethoxam, and hydramethylnon, the simplified step function version of the model ($Y = A \cdot (x \leq 0) + C$) was used, given that B tends to infinity; the equation at the top right represents the general function, ignoring all treatment effects; the dendrogram represents the hierarchical grouping of similar treatments, as measured by the *F*-test, with more-similar treatments being grouped first; the probabilities that two groups are the same, according to the *F*-test, are shown (NS = not significant; * = significant at 5% probability; ** = significant at 1% probability; *** = significant at 0.1% probability). (Online figure in color.)

B). In the case of thiamethoxam, although the concentration of the insecticide was 50% lower than in the first experiment, the active ingredient was still effective in controlling *L. micans,* with no reinfestation observed. Same results were recorded for treatment using hydramethylnon in the form of paste (Fig. 3A and B).

Discussion

Because of sparse information about other species of the genus *Linepithema* and the importance of *L. humile* as an invader, studies on infestation control have targeted this species, mainly evaluating the use of contact insecticides or toxic baits (Klotz et al. 2002, 2003; Silverman and Brightwell 2008).

Most studies evaluating control methods for *L. humile* have been directed to urban areas (Silverman and Brightwell 2008); however, a few studies have evaluated control methods in citrus and grapevine crops (Phillips and Serk 1991; Addison 2002; Klotz et al. 2003; Tollerup et al. 2004; Daane et al. 2006, 2008; Nelson and Daane 2007; Nyamukondiwa 2008; Nyamukondiwa and Addison 2011). In these crops, the main insecticides used as a chemical barrier are chlorpyrifos, bifenthrin, and fipronil, all of which have been shown to be effective for a period of ≈60 d, after which they must be reapplied (Phillips and Sherk 1991, Addison and Samways 2000, Klotz et al. 2003).

Fipronil can reduce the populations of *L. humile* by >90% in urban areas (Klotz et al. 2007, 2008). The efficiency of fipronil has been attributed partly to the absence of repellency and to the ability to transfer the active ingredient between individuals in the colony (Rust et al. 1996, Scharf et al. 2004, Soeprono and Rust 2004, Klotz et al. 2007, Wiltz et al. 2009). In spite of the transfer ability of fipronil, which makes it one of the most efficient insecticides in controlling many species of ants (Choe and Rust 2008), it was not effective in this study. Inferences regarding the inefficiency of an insecticide on *L. micans* must be made with caution, because factors such as the toxicity of the insecticide, type of substrate, concentration, and exposure time of the insect in the treated area, among others, affect the transfer process (Rust and Saran 2006).

Fipronil is poorly soluble in water (between 1.9 and 2.4 mg L^{-1} 25°C) and has limited soil mobility (Tingle et al. 2003). Bobé et al. (1997) showed that when sprayed on the surface of the ground, fipronil can penetrate only 10 cm into the soil. Hypothetically, because the nests of *L. micans* are underneath the ground, the insecticide's low mobility could explain its relatively small effect on populations of this species.

In this study, imidacloprid did not provide a satisfactory reduction in the number of workers foraging. As with fipronil, the lower effectiveness of this insecticide compared with thiamethoxam was attributed to

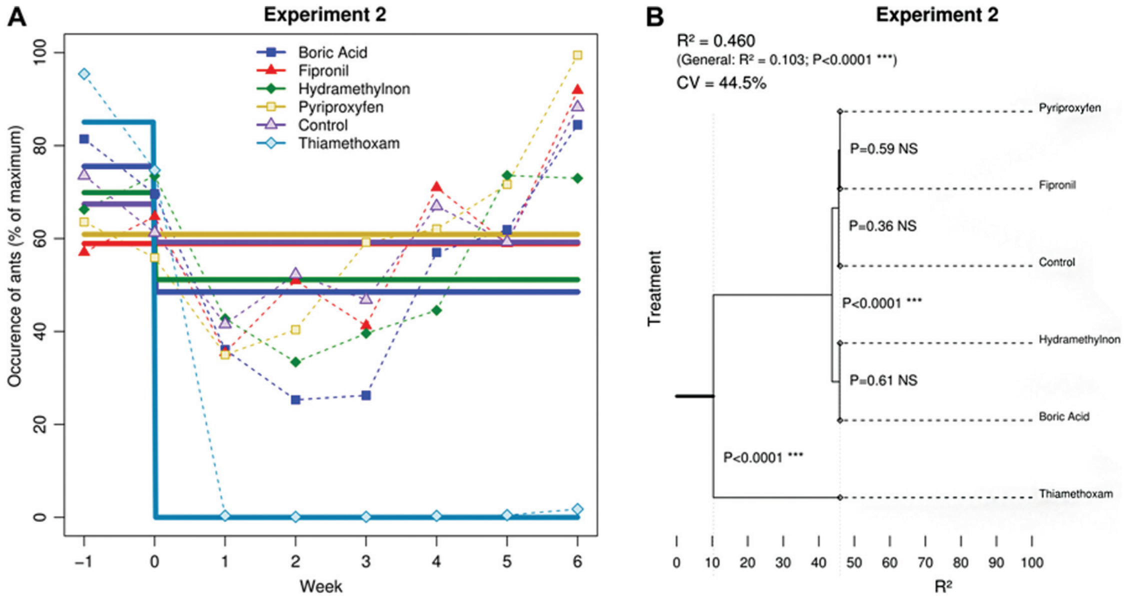

Fig. 2. Results of experiment 2 (August to September 2011): A) Percentage of workers of *L. micans* foraging after the treatment with insecticides and toxic baits; narrow dotted lines and symbols represent the actual measurements; wide solid lines represent the fitted functions, according to the model $Y = A \cdot e\hat{}(-B \cdot x \cdot (x > 0)) + C$. B) Graphical representation of the analysis of variance; the equations to the right of each treatment name represent the respective function, as estimated by the model; note that, for all treatments, the simplified step function version of the model ($Y = A \cdot (x \leq 0) + C$) was used, given that B tends to infinity; the equation at the top right represents the general function, ignoring all treatment effects; the dendrogram represents the hierarchical grouping of similar treatments, as measured by the *F*-test, with the most-similar treatments being grouped first; the probabilities that two groups are the same, according to the *F*-test, are shown (NS = not significant; * = significant at 5% probability; ** = significant at 1% probability; *** = significant at 0.1% probability). (Online figure in color.)

its lower solubility (0.6 g litres^{-1}), which made contact with the underground nests of *L. micans* more difficult.

In all three experiments, thiamethoxam was efficient when used as a chemical barrier. Unlike imidacloprid and fipronil, thiamethoxam is highly soluble (4.1 g litres^{-1}), which increases its mobility in the soil. Júnior and Regitano (2009) observed that this compound can reach a soil depth of 50 cm, because of its low degree of adsorption. Therefore, this factor may have facilitated control by creating a chemical barrier around the nests of *L. micans,* which are underground.

All evaluated doses of thiamethoxam eliminated the population of *L. micans.* Application of insecticides may kill or repel only the workers that come out to forage, and have little effect on the queens and pupas (Bueno and Bueno 2007). Moreover, the problem may be worsened by the fragmentation of the colony, which in the medium term will increase the level of infestation in the area or the dispersal of ants to other areas (Bueno and Bueno 2007). This is the case for *L. humile,* which abandons its nest under any unfavorable conditions (Newell and Barber 1913).

Insecticides sprayed on the soil may reach nontarget species, especially pollinators (Pinheiro and Freitas 2010). According to Pereira (2010) and Cresswell et al. (2012), depending on the crop and the application method, fipronil and the neonicotinoids are related to high mortality of *Apis mellifera.* Honeybees can come in contact with these chemical agents through their drinking water, plant resins, pollen, and nectar-harvesting activities, mainly at the time of flowering of crops (spring–summer) (Pinheiro and Freitas 2010). During this period, the number of *L. micans* foraging and spreading in the vineyards increases, because of the rise in temperature. This seasonality must be taken into consideration in a management program involving *L. micans* and the spraying of insecticides.

Chemicals that act rapidly and through contact can be applied more efficiently after the colonies have been located. This is an exceptional situation for ants, because most species form large populations that are established over large areas. In this case, the use of toxic baits can provide better results in reducing infestation (Bueno and Bueno 2007).

Several studies have shown satisfactory results in reducing the population of *L. humile* using boric acid (0.5 to 1.0%) embedded in sugar water-based liquid baits, in both urban and rural areas (Klotz et al. 1998, 2007; Hooper-Bui and Rust 2000). Boric acid is one of the main active ingredients used to control *L. humile* in vineyards in California and South Africa (Daane et al. 2006, 2007, 2008; Nyamukondiwa and Addison 2011).

In this study, we expected that boric acid would control *L. micans* similarly to other dolichoderine ants (Klotz et al. 1998, 2007; Hooper-Bui and Rust 2000). However, this did not prove to be the case.

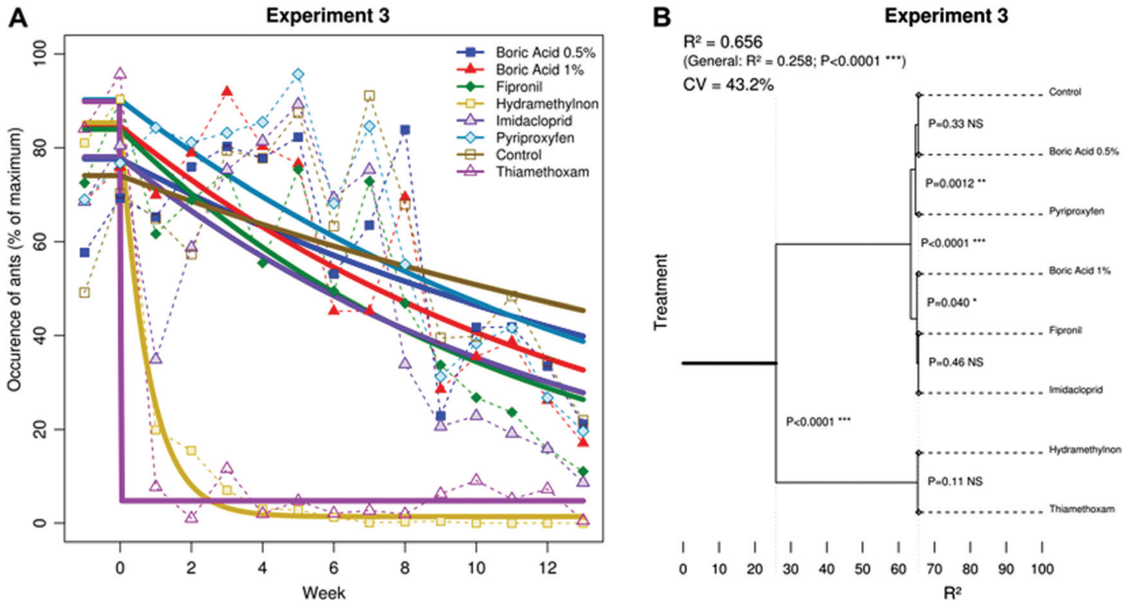

Fig. 3. Results of experiment 3 (January to April 2012): A) Percentage of workers of *L. micans* foraging after the treatment with insecticides and toxic baits; narrow dotted lines and symbols represent the actual measurements; wide solid lines represent the fitted functions, according to the model $Y = A \cdot e\hat{}(-B \cdot x \cdot (x > 0)) + C$. B) Graphical representation of the analysis of variance; the equations to the right of each treatment name represent the respective function, as estimated by the model; note that, for thiamethoxam, the simplified step function version of the model ($Y = A \cdot (x \leq 0) + C$) was used, given that B tends to infinity; the equation at the top right represents the general function, ignoring all treatment effects; the dendrogram represents the hierarchical grouping of similar treatments, as measured by the *F*-test, with more-similar treatments being grouped first; the probabilities that two groups are the same, according to the *F*-test, are shown (NS = not significant; * = significant at 5% probability; ** = significant at 1% probability; *** = significant at 0.1% probability). (Online figure in color.)

Hydramethylnon has been effective in controlling colonies of *Solenopsis invicta* and workers of *L. humile,* when incorporated into sugar solutions; however, it has no effect on the queens of these species (Hooper-Bui and Rust 2001, Stanley 2004). Another problem with this active ingredient is that it has no delayed action (Knight and Rust 1991, Davis and Van Schagen 1993). However, when incorporated into protein-based solid baits, this insecticide has provided satisfactory results in the control of *L. humile,* mainly in field conditions (Forschler and Evans 1994; Krushelnycky and Reimer 1998a,b; Klotz et al. 2000). In the case of *L. micans,* hydramethylnon (0.5%) in paste bait was efficient 1 wk after its application. For a formulation of toxic bait to be successful in controlling ants, it must be sufficiently slow-acting to be brought to the nest and shared with the workers of the colony (Rust et al. 2000). Stringer et al. (1964), working with *Solenopsis invicta,* considered that a bait has slow toxicity when it causes <15% mortality up to 24 h after application, and >89% after 20 d. Other studies have considered the formulation of toxic bait to be efficient if the colony is eliminated after 10–18 d of application (Vander Meer et al. 1985, Knight and Rust 1991, Klotz et al. 2004).

The results of the present research showed that ≈90% mortality of colonies of *L. micans* occurred before the 10- to 18-d period. For this species, the amount of active ingredient and the rate of transfer were sufficient to reach not only the workers of the colony but also the queens, determined by confirming the death of the queens at the end of each of the experiments.

Hydramethylnon has also been offered in gel form to workers of *L. micans;* however, in this form it did not show the same control efficiency, probably, due to the lack of attractiveness of the matrix. Silverman and Roulston (2001) tested the attractiveness of liquid and gel matrices to *L. humile,* and found that this species prefers liquid and sugar solutions, ingesting larger amounts of liquids than gel.

The results of this study indicate that the use of thiamethoxam as a chemical barrier, and hydramethylnon as a bait are efficient in the control of *L. micans.*

Acknowledgments

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