Myrmecofauna (Hymenoptera: Formicidae) Present in Vineyards Infested with Eurhizococcus brasiliensis (Hemiptera: Margarodidae) in Southern Brazil

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Myrmecofauna (Hymenoptera: Formicidae) present in vineyards infested with *Eurhizococcus brasiliensis* (Hemiptera: Margarodidae) in southern Brazil

Aline Nondillo¹ *, Cristiano João Arioli², Alexander Wild³, Odair Correa Bueno⁴, and Marcos Botton¹

Abstract

*Eurhizococcus brasiliensis* (Wille) (Hemiptera: Margarodidae) is among the principal pests of Brazilian vineyards. Establishment and spread of this species in vineyards are associated with ants (Hymenoptera: Formicidae). This study determined the diversity of the ant fauna in *E. brasiliensis* infested vineyards in Rio Grande do Sul and Santa Catarina, the main grape-producing states in Brazil. Underground pitfall traps baited with a honey–water solution and sardines were used to sample ants in the spring–summer and fall–winter periods. Twenty-eight species of ants were identified in Rio Grande do Sul and 15 in Santa Catarina. *Linepithema micans* (Forel) (Hymenoptera: Formicidae) was the most frequent and abundant species collected in vineyards in both states. Control strategies for ants associated with the spread of *E. brasiliensis* should focus on *L. micans* in vineyards.

Key Words: survey; ant; soil scale; *Linepithema micans*

Resumo


Palavras Chave: levantamento; formiga; cochonilha; *Linepithema micans*

The presence of ants in agroecosystems may cause indirect damage to plants as a result of their symbiotic interactions with honeydew-producing hemipterans (Way 1963; Hölldobler & Wilson 1990; Delabie 2001). The honeydew excreted by these hemipterans supplements the nutritional needs of ants, providing carbohydrates and amino acids (Way 1963). In this relationship, the hemipterans might receive protection against predators and parasitoids (Moreno et al. 1987; Martínez-Ferrer et al. 2003; Daane et al. 2007). In addition to protecting them, ants can also transport the hemipterans to new protected feeding places, and can clean and remove dead individuals (Buckley 1987; Vanek & Potter 2010).

The relationship of some hemipterans with ants is mutually beneficial and can aid both groups to increase in abundance (Way 1963; Abbott & Green 2007; Daane et al. 2007). In South Africa and California, this relationship has significantly increased populations of scales from the family Pseudococcidae, which can be important virus vectors in vineyards (Addison & Samways 2000; Daane et al. 2006; Daane et al. 2007). In these regions, the main ant species associated with the spread of scale insects is the Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae) (Addison & Samways 2000; Geiger et al. 2001; Daane et al. 2007). In southern Brazil, *Eurhizococcus brasiliensis* (Wille) (Hemiptera: Margarodidae) is the principal insect pest in vineyards (Gallotti 1976; Soria & Gallotti 1986; Botton et al. 2010; Efrem et al. 2012).

The relationship of *E. brasiliensis* to ants in Brazilian vineyards was first reported by Gallotti (1976), who observed workers of *L. humile* and *Nyländeria fulva* (Mayer) (Hymenoptera: Formicidae) carrying 1st instar nymphs of *E. brasiliensis*. Soria & Gallotti (1986), Hickel (1994), and Soria et al. (1997) also mentioned *L. humile* as the predominant ant in areas infested by *E. brasiliensis* in southern Brazil. However, more recent studies found that *Linepithema micans* (Forel) (Hymenoptera: Formicidae) was associated with *E. brasiliensis* in vineyards in the state of Rio Grande do Sul (Martins & Bueno 2009; Sacchett et al. 2009).

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Considering that efficient management of *E. brasiliensis* should include management of ants involved in *E. brasiliensis* dispersal, knowledge of the myrmecofauna of vineyards is important. This study assessed the diversity of ants in vineyards infested by *E. brasiliensis* in Rio Grande do Sul and Santa Catarina.

**Materials and Methods**

**STUDY AREAS**

The collections were conducted in vineyards infested with the *E. brasiliensis* in grape-producing municipalities in Rio Grande do Sul (on 55 farms in Antônio Prado, Caxias do Sul, Dois Lajeados, Farroupilha, Flores da Cunha, Garibaldi, Monte Belo do Sul, Nova Pádua, Pinto Bandeira, Sarandi, and Veranópolis) and Santa Catarina (on 9 farms in lômero, Pinheiro Preto, Tangará, and Videira). Two ant collections were made in each vineyard: one in spring–summer and the other in autumn–winter, from 2008 through 2011.

The ants were collected using underground pitfall traps. The traps consisted of a set of two plastic pipes (3.3 cm diameter × 5.0 cm height) connected by a 50 cm string, with a cap and lateral holes (3 mm) (Mori-ni et al. 2004), and two baits. One of the pipes contained a honey-water solution (70%) absorbed in cotton wool, and the other contained sardines in edible oil placed in the cap of the pipe.

One pitfall trap was placed at each sampling point in the vineyard, with 20 traps per farm in Rio Grande do Sul and 12 per farm in Santa Catarina. The pitfall traps were buried underground at a depth of 20 cm. The sampling depth was based on the study by Hickel et al. (2008), who found that *E. brasiliensis* was present at a depth up to 30 cm. The traps were spaced 10 m apart and distributed centrally in the vineyard to avoid edge effects, along the rows of grape plants, close to their roots. The traps remained in the field for 24 h, then were collected and taken to the laboratory. Once removed from the traps, the ants were stored in bottles containing 70% ethanol for later identification.

**SPECIES IDENTIFICATION**

The ant morphospecies were separated, and a sample of each was determined using the methods described by Longino (2000). The ants were identified to subfamily (Bolton 2003) and gender (Bolton 1994). The morphological identification was done in 2 stages: to the morphospecies level by comparing the samples to ones deposited in the collection of the Laboratory of Myrmecology of Alto Tietê, at the Universidade de Mogi das Cruzes, state of São Paulo and then to the species level by Alex Wild, Curator of Entomology at the University of Texas at Austin. Due to their morphological similarities, the species of *Linepithema* and *Solenopsis* (Hymenoptera: Myrmicidae) were also identified through molecular analyses of the cytochrome oxidase I mitochondrial gene. This analysis was conducted at the Centro de Estudos de Insetos Sociais, Rio Claro, Brazil (Campos 2012; Martins et al. 2012).

**FAUNAL ANALYSIS**

Species distribution and patterns of the collected ants were based on the indices of abundance, constancy, dominance, and frequency, which were calculated using the equations proposed by Silveira Neto et al. (1976).

In order to define the abundance, the confidence interval (CI) of the arithmetic mean was calculated at 1 and 5% probability, using the formula of Silveira Neto et al. (1976):

\[ CI = m \pm t \times S(m) \]

Where: CI = confidence interval; m = arithmetic mean; t = value of t at 5% and 1% levels; and S(m) = standard error of the mean.

The following abundance categories were established: rare (r) = number of individuals captured was lower than the lower CI limit at 1% probability; dispersed (d) = number of individuals captured was between the CI limits of 1% and 5% probability; common (c) = number of individuals captured was in the CI at 5% probability; abundant (a) = number of individuals captured exceeded the limits of the CI at 5% and 1% probability; and very abundant (ma) = number of individuals captured was higher than the upper CI limit at 1% probability.

Constancy was calculated using the formula:

\[ C = P \times 100 / N \]

Where: C = constancy; P = number of collections containing each species; and N = total number of collections.

According to Bodenheimer (1955), species occurrence can be classified as: constant (w): when the species is present in more than 50% of the collections; incidental (i): when the species is present in 25 to 50% of the collections; and accidental (z): when the species is present in less than 25% of the collections.

The Sakagami and Laroca method, as described by Fazolin (1991), was used to determine dominance. This method considers a species as dominant if their frequency of occurrence exceeds the dominance limit, which can be calculated with the formula:

\[ DL = 1 \times 100 / S \]

Where: DL = dominance limit; and S = total number of species.

The frequency was calculated with the formula:

\[ F = I \times 100 / T \]

Where: F = frequency (%); I = number of specimens of the species in the sampling location; and T = total number of specimens of the group in the sampling location.

A frequency category was established for each species, based on the CI at 5% probability (Fazolin 1991). The following categories were established: low frequency (lf) = when the percentage of captured individuals was lower than the lower CI limit at 5% probability; frequent (f) = when the percentage of captured individuals was within the CI at 5% probability; and very frequent (vf) = when the percentage of captured individuals was higher than the higher CI limit at 5% probability.

The relative frequency was calculated using the following formula:

\[ RF = AF \times 100 / \sum AF \]

Where: RF = relative frequency (%); and AF = absolute frequency (number of locations where the species occurred per total number of locations).

The faunal analysis calculations were based on the number of records for each species from each location. These records were calculated according to the presence or absence of ants, as proposed by Longino (2000), as the social characteristics of ants can affect these analyses if they are based on the absolute numbers of specimens collected.

**Results**

A total of 135,920 ants were collected in the 55 vineyards of Rio Grande do Sul. The ants in these samples represented 28 species, 15 genera, and 6 subfamilies (Table 1). In Santa Catarina, a total of 41,542 ants representing 15 species, 9 genera, and 5 subfamilies (Table 1) were collected.
In the collections from Rio Grande do Sul, the subfamily Myrmicinae showed the highest species richness, followed by Formicinae, Ectatomminae, Ponerinae and Dolichoderinae (Table 1). The collections from Santa Catarina contained the same proportion of subfamilies, except that no species of Ectatomminae were found (Table 1).

In the faunal analysis, L. micans was classified as abundant, dominant, and very frequent in all locations in Rio Grande do Sul and Santa Catarina. Its occurrence was constant in 18% and 25% of the locations in Rio Grande do Sul and Santa Catarina, respectively (Tables 2 and 3). This was also the species with the highest frequency found in all locations (Figs. 1 and 2).

The genus Pheidole (Hymenoptera: Myrmicinae) was the most species-rich in all locations in Rio Grande do Sul and Santa Catarina, where it was represented by 8 and 5 species, respectively (Tables 2 and 3). Also, it was among the genera that showed high rates in the faunal analysis (Tables 2 and 3; Figs. 1 and 2). *Pheidole subarmata* (Mayr) was very abundant, incidential, dominant, and very frequent in 81, 18, 100, and 81% of the locations in Rio Grande do Sul (Table 2; Fig. 1). In Santa Catarina, it was very abundant, dominant, and very frequent in 75% of the locations, and accidental in all municipalities (Table 3; Fig. 2). *Pheidole aberrans* (Santschi) was classified as abundant, accidental, dominant, and very frequent in 36, 81, 54, and 36% of the samples in Rio Grande do Sul; in Santa Catarina, it was very abundant, accidental, dominant, and very frequent in 50, 100, 75, and 50% of the municipalities.

*Solenopsis* (Hymenoptera: Myrmicinae) showed the highest species richness, with 4 species in Rio Grande do Sul (Table 2) and 2 other species in Santa Catarina (Table 3). Among these species, *Solenopsis invicta* (Buren) in Rio Grande do Sul was classified as very abundant, accidental, dominant, and very frequent in 36, 54, 36, and 36% of the collection locations. However, in Santa Catarina, it was classified as rare, accidental, not dominant, and of low frequency in 50, 75, 50, and 50% of the municipalities.

### Discussion

The subfamily Myrmicinae displayed the highest species richness, comprising 57% of the species found in vineyards infested by *Eurhizococcus brasiliensis* in Rio Grande do Sul (Table 1) and 53% in Santa Catarina (Table 1). Myrmicinae is the largest ant subfamily with the greatest numbers of genera and species (Fernández & Sendoya 2004; AntWeb 2012). Fernández (2003) noted that the myrmicines are among the most diversified groups in their feeding and nesting habits. The subfamily includes arboreal, soil-inhabiting, and leaf-litter species; some are associated with plants, fungi, or with other ants (Fernández 2003). Myrmicine ants also are dominant in vineyards in South Africa (Addison & Samways 2000), Australia (Chong et al. 2011) and Brazil (Sacchett 2006; Picelli 2011; Rosado et al. 2012).

The second most diverse taxon was the subfamily Formicinae, with 21% of the species collected in Rio Grande do Sul and 20% in Santa Catarina being from this subfamily (Table 1). This subfamily comprises 51 genera worldwide, with approximately 2,915 described species (AntWeb 2012). Fernández and Sendoya (2004) noted that there are about 15 genera known to inhabit the Neotropical region of the world. Formicine ants are mostly generalists, but they are strongly attracted to carbohydrates such as those in extrafloral nectaries (Fowler et al. 1991; Longino 1994). They may be arboreal, or live in soil, leaf litter or underground (Fowler et al. 1991).

The most species-rich subfamilies in Rio Grande do Sul were Ectatomminae, Ponerinae and Dolichoderinae, containing 7, 7, and 3% of the species collected (Table 1). In Santa Catarina, Ponerinae and Dolichoderinae each contained 6% of the species collected (Table 1).

The single species of the subfamily Dolichoderinae was the highest in numerical abundance in both locations (Table 1). Andersen (2000) described this subfamily as both numerically and functionally dominant. Most dolichoderine species are omnivorous and forage on the soil surface, typically feeding on dead arthropods and plant secretions. These ants inhabit various microhabitats, including soil with or without plant cover, dead or living wood, and tree canopies (Cuezzo 2003).

The species richness found in this study was low compared to other surveys in vineyards. Addison and Samways (2000) found 42 ant species in 22 vineyards in South Africa, using pitfall traps and sardine bait, while Chong et al. (2011) in a 50 vineyard survey in Australia found 148 species of ants using only pitfall traps. In Brazil, Sacchett (2006), using pitfall and underground traps, recorded 41 ant species in vineyards of the Serra Gaúcha region, and Rosado et al. (2012) found 72 species in Rio Grande do Sul.

The diversity of species found in the vineyards in our study was also low compared with other surveys carried out in monocultures with perennial plants in Brazil. For example, 124 species were recorded in coca plantations in Bahia (Delabie & Fowler 1995), and 143 species were found in eucalyptus plantations in Minas Gerais (Marinho et al. 2002). However, the low number of species is similar to other surveys carried out in southern Brazil. For example, in São Leopoldo, 20 species were documented (Haubert et al. 1998); in Torres, 33 species (Hameister et al. 2003); and in Capapava do Sul, 51 species (Diehl et al. 2004). Low species richness is expected for the subtropical region, where myrmecofauna is less diverse than in tropical zones due to climatic factors and the less complex habitats (Ketterl et al. 2003). The 72 species found in the region of Campanha do Rio Grande do Sul by Rosado et al. (2012) was an exception.

Ant species richness may also be influenced by habitat characteristics, such as the structure of the vegetation. A habitat with greater plant complexity provides more sites for nest building and more abun-
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<th>Antônio Prado</th>
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A = Abundance: ma = very abundant, a = abundant, c = common, d = dispersed, r = rare; C = Constancy: w = constant, y = incidental, z = accidental, D = Dominance: d = dominant, nd = not dominant; F = Frequency: vf = very frequent, f = frequent, lf = infrequent, - = absent.
In agroecosystems, insecticide application heavily impacts the invertebrate distribution in the environment. Pesticide impacts include reduction of species diversity, occurrence of secondary pests, and reduction of natural predators (Theiling & Croft 1988). Peck et al. (1998) showed meaningful effects of soil management and insecticide application on the ant assemblage structure for species that inhabit the plant litter-soil interface. In coffee plantations, Perfecto & Vandermeer (2002) found greater species richness in organic culture compared with traditional culture. Picelli (2011) also attributed low ant diversity found in vineyards of São Paulo to insecticide application. However, in a study done in vineyards in Australia, Chong et al. (2007) showed that the ant assemblage structure in these locations was insensi-

Table 3. Faunal analysis of ant species sampled in vineyards with the presence of *Eurhizococcus brasiliensis*, in cities from the state of Santa Catarina.

<table>
<thead>
<tr>
<th>Species</th>
<th>Iomerê</th>
<th>Pinheiro Preto</th>
<th>Tangará</th>
<th>Videira</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brachymyrmex aphidicola</td>
<td>C</td>
<td>A</td>
<td>D</td>
<td>F</td>
</tr>
<tr>
<td>Camponotus melanoticus</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>Labidus cuecos</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>Pachycondyla striata</td>
<td>A</td>
<td>A</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>Pheidole aberrans</td>
<td>C</td>
<td>C</td>
<td>z</td>
<td>nd</td>
</tr>
<tr>
<td>Pheidole sp. 17</td>
<td>C</td>
<td>C</td>
<td>z</td>
<td>nd</td>
</tr>
<tr>
<td>Pheidole subbarmata</td>
<td>C</td>
<td>C</td>
<td>z</td>
<td>nd</td>
</tr>
<tr>
<td>Solenopsis invicta</td>
<td>C</td>
<td>C</td>
<td>z</td>
<td>nd</td>
</tr>
<tr>
<td>Solenopsis saevissima</td>
<td>C</td>
<td>C</td>
<td>z</td>
<td>nd</td>
</tr>
</tbody>
</table>

A = Abundance; ma = very abundant, a = abundant, c = common, di = dispersed, r = rare; C = Constancy; w = constant, y = incidental, z = accidental, D = Dominance: d = dominant, nd = not dominant; F = Frequency: vf = very frequent, f = frequent, lf = infrequent, - = absent.
tive to the pesticide application. However, the authors highlighted that the presence of shelter in these vineyards probably influenced the ant assemblage structure. An organism’s sensitivity to a pesticide depends on its toxicity and degree of exposure (Thomson & Hoffmann 2006). This may explain the low ant diversity of the vineyards in southern Brazil, because in most vineyards there, the ground is permanently exposed, which facilitates increased ant–pesticide contact.

In relation to the low species richness found in our study, the use of underground pitfall traps, which was different from other studies could have influenced the final species richness, making standardization of these methods necessary for a more valid comparison. According to Bestelmeyer et al. (2000), the use of pitfall traps is more effective, because they remain longer in the field, resulting in an increased number of species being collected.

The most representative genera in number of species in the 2 states were Pheidole and Solenopsis. In surveys carried out in vineyards of Australia (Chong et al. 2011) and in the Serra Gaúcha (Sacchetto 2006), the genus Pheidole also had the highest species richness. This genus is comparatively widely distributed, with about 900 species known worldwide (Hölldobler & Wilson 1990), with 620 species occurring in the Americas (Wilson 2003).

Among the ant species collected in the 2 states, L. micans was found in most of the vineyards infested by E. brasiliensis, and it was the dominant ant species where it occurred. This species was also one of the most important in vineyards in the Campanha do Rio Grande do Sul region, although in this region, E. brasiliensis was not detected. The presence of the ant in different locations shows its adaptability to different conditions and ecological roles. According to Wild (2009), this is due to its close phylogenetic relationship with L. humile, indicating that this species may also be invasive (Martins et al. 2012).

The information that L. micans is the predominant species in vineyards infested by E. brasiliensis is a new discovery, as until recently L. humile was considered the main dispersive agent of cochineal in Brazil (Gallotti 1976; Soria & Gallotti 1986; Hickel 1994; Botton et al. 2000). This potential misidentification in previous reports may be a result of the close similarity between the workers of both species, the notoriety of L. humile as a pest, and identification errors related to the morphological characteristics (Wild 2007). Because L. micans is the most frequently found species in vineyards infested by E. brasiliensis, L. micans must be considered the principal species related to E. brasiliensis, and should be an important target of control actions to reduce the spread of E. brasiliensis.

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