Ant Species Associated With the Dispersal of *Eurhizococcus brasiliensis* (Hempel in Wille) (Hemiptera: Margarodidae) in Vineyards of the Serra Gaúcha, Rio Grande do Sul, Brazil

by

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ABSTRACT

The ground-pearl, *Eurhizococcus brasiliensis* (Hempel in Wille), is a hypogeic hemipteran that feeds on plant roots, being one of the major pests of vineyards. Ant species may account for the dispersal of scale insects, since the ants tend first instar nymphs for honeydew. This research was conducted to investigate, under experimental conditions, whether the ants also recognize and carry *E. brasiliensis* cysts. Therefore, choice experiments were conducted in two vineyards naturally infested with the ground-pearl, in the municipality of Bento Gonçalves, RS. From the 11 ant species observed visiting the cysts only two were actively dispersing them, *Linepithema micans* (Forel 1908) and an unidentified *Pheidole* species. This *Pheidole* species and the *L. micans* removed and carried *E. brasiliensis* cysts. The workers of *L. micans* visited the greatest amount of test plates, but the *Pheidole* sp.11 workers removed more cysts. Although both species were more active to disperse the hemipteran's cysts, *L. micans* workers were dominant in reference to the workers of *Pheidole* sp.11. Thus, in the vineyards where they occurred together; if the first species is controlled, the second species can become the main *E. brasiliensis* disperser.

Keywords: Formicidae, Hemiptera, *Linepithema*, *Pheidole*, vine-growing areas.

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INTRODUCTION

Trophobiosis is a mutualistic relationship between ants and hemipterans (Fischer & Shingleton 2001), which can be facultative or obligatory (Way 1963, Schultz & McGlynn 2000). Ants associate with hemipterans to get honeydew, rich in carbohydrates and amino acids, ecologically equivalent to extrafloral nectaries (Bristow 1991). Most species that visit hemipterans are regarded as opportunistic, and they show feeding characteristics similar to those seen in extrafloral nectaries' visiting ants, generally, the same species (Carrol & Janzen 1973, Delabie & Fernández 2003). One explanation for the ant-hemipteran interaction is the chemical composition of honeydew, which varies according to the host plant from which the hemipteran obtains the sap, seeing that the excreted substances are the ones which would attract the ants (Bristow 1991; Fischer *et al.* 2001, Fischer & Shingleton 2001).

Most ant species which associate with hemipterans belong to the Dolichoderinae, Formicinae, and Myrmicinae subfamilies, but some belong to the Ponerinae (Way 1963, Schultz & McGlyn 2000, Delabie 2001, Delabie & Fernández 2003). Some species of Dolichoderinae and Formicinae seem to be more adjusted since they have well-developed structures to collect the fluid later transferred to the other colony's individuals through trophallaxis (Hölldobler & Wilson 1990). In general, hemipterans are myrmecophilous. As they depend on ants to eliminate honeydew, they tend to make their tender easy; if the hemipteran fails to get rid of it, fungi can grow and cause its death (Way 1963, Fischer *et al.* 2001). The hemipteran's size is also a major characteristic for the ant, since it must be carried in case there is food scarcity in the host plant or it has to be protected against natural enemies (Delabie 2001, Delabie & Fernández 2003).

Margarodidae is a Hemiptera family in which the individuals stand out for their size. From that family, *Eurhizococcus brasiliensis* (Hempel in Wille 1922), due to its subterranean life and cyst shape, is known as the ground-pearl (Gallotti 1976). Its facultative parthenogenetic reproduction gives forth a generation per year, with oviposition occurring inside the cysts. In their sexual phase, females leave the cysts and go to the surface to copulate, soon returning to the ground for oviposition. Oviposition along the sexual phase happens from November to February, as well as cyst eclosion (Soria *et* *al.* 1990, Soria & Dal Conte 2000), corresponding to the hot and dry season in Southern Brazil.

Since adults lack a mouth apparatus, only the ground-pearl nymphs suck sap. Nymphs have legs and antennae, but their mobility is poor (Gallotti 1976, Soria *et al.* 1990, Soria & Braghini 1999). When ants look for honeydew, in their turn, they associate with the hemipterans and, in exchange for food, they disperse the nymphs to other sites of the host plant or farther, besides offering protection against natural enemies (Hickel 1994, Soria & Dal Conte 2000, Botton *et al.* 2000, 2003).

In Rio Grande do Sul, vineyards are the greatly affected by this activity. Vineyard growth is a major revenue source of the Serra Gaúcha, which accounts for nearly 60% of vineyard production in Brazil (Mello 2001). When hemipterans suck the sap, it triggers a gradual decrease of plant vitality, followed by a decrease in grape production, and eventually the vineyard's death (Soria & Braghini 1999, Botton *et al.* 2003).

From the Formicinae subfamily, *Linepithema humile* (Mayr) is considered the main species associated with ground-pearl nymph dispersion (Hickel 1994, Botton *et al.* 2000, 2003, Soria & Dal Conte 2000), but other ants and/or hemipterans can be involved. The objective of this work was to identify, under experimental conditions, the ant species which recognize and carry *E. brasiliensis* cysts in vineyard cultivation areas, naturally infested by that hemipteran, in the Serra Gaúcha.

MATERIAL AND METHODS

Study area

Bento Gonçalves municipality (29°56'S; 51°33'W) is located in the Encosta Superior do Nordeste of the Serra Gaúcha, in the Depressão Central of Rio Grande do Sul, 645 meters above sea level. According to Köppen's classification, the climate there is subtropical and wet, with temperature ranging from −4°C to 36°C. Annual rainfall is about 1,700 mm, and the relative air humidity is approximately 76% (www.bentogoncalves.com.br). The regional native vegetation is mixed ombrophilous forest, of the montane type, where clay latosoils are prevalent, from basic basalt outpourings (Teixeira *et al.* 1986).

Tests were carried out in March 2005, in two vineyards of the *Vitis labrusca* cv. Isabel cultivar - Valê dos Vinhedos (VV) and Pinto Bandeira (PB) - about

20km apart from each other, both naturally infested by the ground pearl. The vineyards occupy a hectare, with 2.5-meter intervals between each two vines.

Experiments

To capture the ant species which disperse ground-pearl cysts, ten cardboard plates (18cm x 10cm) were randomly distributed on the ground, divided into twelve-15cm^2 sectors. In six sectors, sorted out onto each plate, ground-pearl cysts were placed (1/sector), with the same amount of sweet pellets (ø = 3 mm) in the other sectors. The plates, the cysts, and the pellets were used only once to avoid markings made with pheromones by the ants. Thirty minutes was the observation time per plate, with the time taken by the ants to arrive at the test plates, and the number of cysts and pellets dislocated and removed by the ants being recorded. A dislocation (D) occurred when the cysts and/or pellets were displaced from the sector, but not from the plate; a removal (R) was defined as completely moving the cysts and/or pellets out of the plate.

Ant identification

The workers of each species visiting the plates, dislodging and/or removing cysts and/or pellets were collected and placed into flasks with 70% alcohol, and collection data. Bolton's proposition (2003) was adopted for subfamily, and genera classification was made according to Bolton (1994). Species and morphospecies were separated through comparison with the Formicidae Collection, of the Laboratório de Insetos Sociais da UNISINOS (São Leopoldo, RS), where the vouchers are deposited.

Data Analysis

In order to test whether the differences between the occurrence frequencies of the two vineyards' ants and whether the differences between the numbers of the dislocated cysts and the removed cysts in each vineyard were significant, an Analysis of Variance (ANOVA) was carried out, and Tuckey's test was carried out for the significant results. The comparison of the mean arrival time of the ants at the plates, and the comparison of the mean numbers of the removed cysts from both vineyards were made through the Student's t-test. The SYSTAT 11 program (Wilkinson 2000) was used for all statistical analyses.

RESULTS

The mean time to begin plate visits to the VV vineyard was 2.8 ± 1.9 minutes (limits 1.0 – 8.0 minutes), whereas in PB it was 2.1 ± 1.2 (limits 1.0 – 5.0 minutes), a non-significant difference between those times (t=0.97, df=18, P<0.05).

A total of 11 ant species visited the plates in both vineyards (Fig. 1), from which seven (Table 1) were common to the two vineyards. Two taxa were found exclusively in the VV vineyard (*Camponotus* sp.5 and *Paratrechina* sp.), and two other taxa (*Hylomyrma* sp.1 and *Pheidole* sp.15) only in PB. The frequencies of the 11 species which visited the plates in both vineyards varied significantly ($F_{10,11}$ = 11.501 P<0.0001). *L. micans* was the most frequent species, followed by *Wasmannia* sp and *Camponotus rufipes* (Fig. 1).

Of the 60 sweet pellets alloted by vineyard, only two (3.3%) in VV, and four (6.7%) in PB were displaced by the ants, but none were removed. In both vineyards, seven species visited the *E. brasiliensis* cysts, from which four were common to both vineyards (Table 1). As for cysts, in the VV vineyard 40.0% were removed, whereas in PB the ants carried 58.3%. Despite the difference in the amount of removed cysts per each species in both vineyards (Fig. 2), a comparison of the total mean numbers of the removed cysts per vineyard (Fig. 3) showed no significant differences (t = 1.095; P > 0.005).

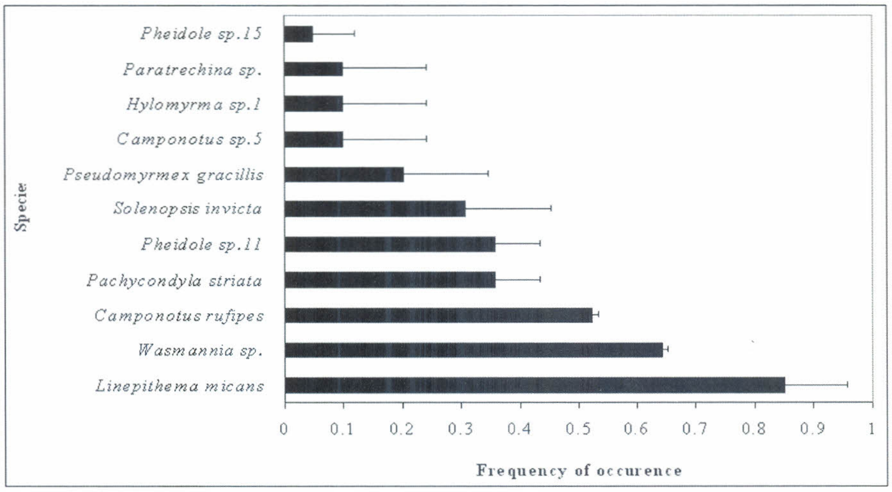

Fig. 1 – Mean (± SD) frequency of occurrence (in arcsin) of the ant species visiting the test-plates (N=20) in both vineyards in Bento Gonçalves, RS.

Table 1. Number of visited test-plates (N_V), number and frequency of *Eurhizococcus brasiliensis* dislocated cysts [N_D (%)], and number and frequency of removed cysts [N_R (%)] by each ant species in Vale dos Vinhedos (VV) and Pinto Bandeira (PB) vineyards, in Bento Gonçalves, RS.

Species	VV			PB		
	N_V	N_D (%)	N_R (%)	N_V	N_D (%)	N_R (%)
Camponotus rufipes	5	2 (3.3)	0 (-)	5	3 (5.0)	0 (-)
Linepithema micans	8	9 (15.0)	9 (15.0)	7	15 (25.0)	14 (23.3)
Pachycondyla striata	4	3 (5.0)	1 (1.7)	3	1 (1.7)	0 (-)
Paratrechina sp.	2	2 (3.3)	2 (3.3)	0	0 (-)	0 (-)
Pheidole sp.11	3	12 (20.0)	12 (20.0)	4	18 (30.0)	18 (30.0)
Pheidole sp.15	0	0 (-)	0 (-)	1	1 (1.7)	1 (17)
Solenopsis invicta	2	0 (-)	0 (-)	4	3 (5.0)	2 (3.3)
	24	28 (46.6)	24 (40.0)	24	41(68.3)	35 (58.3)

10 test-plates/vineyards; 6 cysts/plate

Only two species removed significantly (Tuckey P<0.005) more cysts than the other ones: *Pheidole* sp.11 (20.0% in VV, and 30.0% in PB), and *L. micans* (15.0% in VV, and 23.3% in PB). Nevertheless, the differences in the frequencies of the removed cysts, either by *Pheidole* sp. and *L. micans*, between VV and PB vineyards were not significant.

DISCUSSION

The mean time recorded when visits began was similar in both vineyards, and the first ants to arrive at the plates, in general, were *Camponotus rufipes*, *Camponotus* sp.5, *P. striata*, and *Wasmannia* sp.. Despite *C. rufipes* workers showing a high occurrence frequency, and their dislocation of some cysts on some plates, they did not carry them. Many species of the *Camponotus* genus show trophobiotic relationships with hemipterans (Way 1963, Buckley 1987a, b), but *C. rufipes* did not show any evidence which allowed us to connect it to cyst dispersion. But it must be emphasized that both *C. rufipes* workers and the *Camponotus* sp.5 workers were quickly expelled from test plates by *L. micans*. According to Andersen (2000), the main characteristic to include a *Camponotus* species into the functional group of the subordinated camponotinean is submission to the ants belonging to the Dolichoderinae subfamily.

A confrontation between *L. micans* workers and *Pheidole* sp.11 workers on a plate where both species had co-occurred was observed, where the *Pheidole* workers, despite being the first ones to arrive, were expelled by the *Linepithema* workers. According to Andersen (1997, 2000), ants of the *Pheidole* genus are generalists, and they frequently fight the dolichoderineans for food. Bond & Slingsby (1984) reported that *L. humile,* an invasive ant in South Africa, also expelled native species, despite taking longer to arrive at the test seeds.

Although *Wasmannia* sp. presented the second-highest visitation frequency to the plates, it neither displaced, nor removed the ground-pearl cysts, probably due to its very small size. The only behavior shown by the workers was antennation of the sweet pellets and the *E. brasiliensis* cysts.

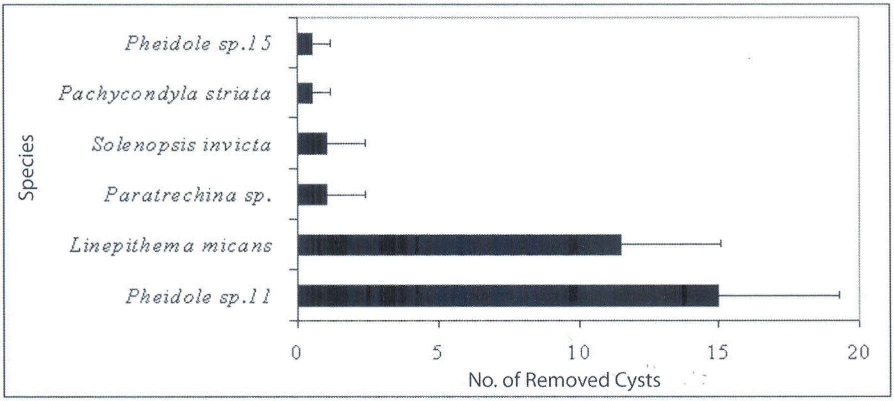

Fig. 2 – Mean (± SD) number of removed cysts of *Eurhizococcus brasiliensis* by each ant species in both vineyards in Bento Gonçalves, RS.

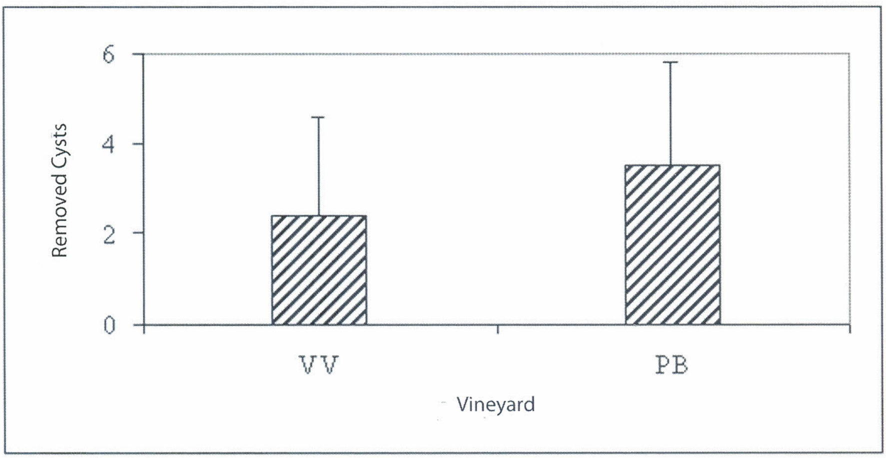

Fig. 3 – Mean (± SD) number of removed cysts of *Eurhizococcus brasiliensis* by the ants at Vale dos Vinhedos (VV) and Pinto Bandeira (PB) vineyards in Bento Gonçalves, RS.

On many plates, the ants only displaced some cysts and very few sweet pellets from one sector to the other. Only three species (*C. rufipes*, *Hylomyrma* sp.1, and *P. striata*) displaced the pellets, but they did not remove them, showing that, despite being sweet, they were not attractive.

From the six species of ants which removed ground-pearl cysts, *P. striata*, *Paratrechina* sp., *Pheidole* sp.15, and *S. invicta* only took the cysts out of the plates to leave them right after. Despite being a patrolling and generalistic predator species (Silvestre *et al*. 2003), *P. striata* did not show interest in the removed cysts, leaving them beside the plate. As to *S. invicta*, workers removing a few *E brasiliensis* cysts were noticed only in the PB vineyard. According to Buckley (1987a), *S. invicta* can show mutualistic interactions with hemipterans, but because they are rare, these interactions are seldom observed.

Two species were significantly different from the other species regarding the number of removed cysts: *Pheidole* sp.11 and *L. micans*. In both vineyards, despite visiting less plates, *Pheidole* sp.11 workers carried many more ground-pearl cysts than *L. micans* workers, which visited more plates, but the former workers removed fewer cysts. Ants from both species were seen carrying cysts as far as ground openings, possibly their nest openings. The recruiting system was also more active in *Pheidole* sp.11 than in *L. micans*, whose workers took longer to arrive at the plates and remove the cysts.

Some *Pheidole* species, such as *P. biconstricta*, show mutualistic interactions with hemipterans (Blütghen *et al*. 2000). It was reported by Campbell (1994) that *P. megacephala* is positively correlated with the presence of a pseudococcidae species, *Formicicoccus njalensis* Laing, in cocoa plantations in Ghana. This author also noticed that the hemipteran abundance increased with the presence of *P. megacephala*, but when another ant species (*Crematogaster clariventris* Mayr) regarded as dominant was present, the association was not so intense. In pineapple plantations in Hawaii, *P. megacephala* was regarded as the main disperser of the pseudococcid *Dysmicoccus brevipes* (Cockrell). In the absence of that ant, the *D. brevipes* population decreased dramatically because it was attacked by its natural enemies (Gonzáles-Hernández *et al*. 1999). In South-African vineyards, Addison & Samways (2000) observed two species of *Pheidole* tending hemipterans, but dominance indexes were low when compared with *L. humile*'s indexes. Similar results were observed in this study, because, despite the fact that *Pheidole* sp.11 removed more cysts, *L. micans* showed dominance and expelled that species' workers from plates with *E. brasiliensis* cysts.

The trophobiotic relationship between *L. humile* and hemipterans may be one of the most well-known relationships of this kind, referenced by many authors (Way 1963, Buckley 1987a, b, Bristow 1991, Buckley & Gullan 1991, Hollway *et al.* 2002). This species, prevalent in most ecosystems, is an invader in many world regions (Way *et al.* 1999). According to Hollway *et al.* (2002), invading species have a tendency to increase their abundance in areas where there are many hemipterans, and may be more efficient tenders than native ants. One feature which may give *L. humile* a greater success in its interactions with hemipterans is its aggressiveness, because, according to Buckcley & Gullan (1991), aggressive species protect the hemipterans they tend better. Way (1963) reported that the aggressiveness shown by *L. humile* increases when the honeydew supply gets scarce. Addison & Samways (2000) noted that in vineyards with less abundance of hemipterans *L. humile* was not prevalent. In that situation another ant species, *Technomyrmex albipes* (Smith), was the main disperser of *Planoccocus ficus* (Signoret).

The findings in both vineyards show that *Pheidole* sp.11 plays a major role in ground-pearl cyst dispersion. But, despite the large number of transported cysts, that ant can not be seen as the main disperser because it is subordinate to *L. micans*. More detailed studies could provide important information regarding the dominance degree between those two species. Concomitantly, besides first-instar nymphs being dispersed by *L. humile*, as previously reported (Hickel 1994, Botton *et al.* 2000, 2003), this paper showed that cysts are also transported by *L. micans*, as well as by a *Pheidole* species, thus favoring still more the dispersion of *E. brasiliensis*, and the infestation of more vineyards.

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