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Interaction Between *Linepithema micans* (Hymenoptera: Formicidae) and *Eurhizococcus brasiliensis* (Hemiptera: Margarodidae) in Vineyards

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ABSTRACT *Eurhizococcus brasiliensis* (Wille) (Hemiptera: Margarodidae) is a soil scale that is considered the main pest of vineyards in Brazil. The ant *Linepithema micans* (Forel) (Hymenoptera: Formicidae) is frequently found associated with this species of scale in infested areas. The effect of the presence of *L. micans* on the infestation and dispersal capacity of *E. brasiliensis* on vine roots was measured in a greenhouse, using Paulsen 1103 rootstock seedlings planted in simple and double "Gallotti Cages." Treatments measured were: infestation of roots with *E. brasiliensis* or *L. micans*, and infestation with both species together. In the experiment using simple Gallotti Cages, with *E. brasiliensis* associated with *L. micans*, higher mean numbers of cysts and ants per plant were recorded, a result significantly different from that found for infestation with scale only. When double Gallotti Cages were used, first-instar nymphs were transported between the cages. The results showed that *L. micans* transports and aids in the attachment of *E. brasiliensis* to vine plants.

KEY WORDS Brazil, Gallotti cage, scale density, grapevine root

In all wine-producing areas of the world, pests and diseases are a major impediment to the expansion of vine cultivation, affecting both the quantity and the quality of the final product (Kuhn and Nickel 1998). One of the most prominent insect pests that limit production in the different wine-producing regions of Brazil is the soil scale *Eurhizococcus brasiliensis* (Wille) (Hemiptera: Margarodidae), (Gallotti 1976; Soria and Gallotti 1986; Botton et al. 2004, 2010; Hickel et al. 2008). This scale is the main pest in vineyards, and attacks roots of both cultivated and wild plants (Soria and Gallotti 1986, Botton et al. 2004, Efrom et al. 2012).

The high population levels of *E. brasiliensis* found in infested areas and the continuous suction of sap in the vine roots causes progressive wasting of the plants, reducing production and eventually killing them (Botton et al. 2000).

This scale insect is found mainly in extreme southern Brazil, where it is believed to be native. High infestations also are found in the states of Santa Catarina, Paraná, and São Paulo and, more recently, in the region of the Valley of São Francisco, the main center in Brazil for growing and exporting table grapes (Lourenção et al. 1989, Hickel 1996, Haji et al. 2002).

Eurhizococcus brasiliensis has a complex biological cycle (Soria and Gallotti 1986). It starts with the parthenogenetic egg-laying inside mature cysts, rupture of the cysts, and emergence of first-instar nymphs. In this mobile phase the nymphs have little self-dispersal capacity. They move close to a root and remain feeding until full development, which usually lasts for 1 yr (Gallotti 1976, Soria and Gallotti 1986, Botton et al. 2000, Soria and Dal Conte 2000, Foldi 2005). The parthenogenetic females appear in this phase and remain inside the cysts until they lay their eggs, after which they die (asexual reproduction). *E. brasiliensis* can also reproduce sexually. In this case, the cyst becomes a mobile female that, at the time of mating, surfaces to copulate with the winged male, and later returns to the ground to lay eggs (Gallotti 1976, Soria and Gallotti 1986, Botton et al. 2000, Soria and Dal Conte 2000).

The long-distance dispersal of *E. brasiliensis* occurs through the transportation of infested seedlings or soil adhered to agricultural implements (Mariconi and Zamith 1973). In the case of *E. brasiliensis*, which excretes honeydew, its dispersal may be helped by ants that seek out scale insects in search of sugary excretions (Gallotti 1976, Hickel 1994, Soria and Dal Conte 2000, Botton et al. 2004).

Mutualistic relationships involving ants and honeydew-producing insects have been described as keystone interactions because of their extraordinary in-

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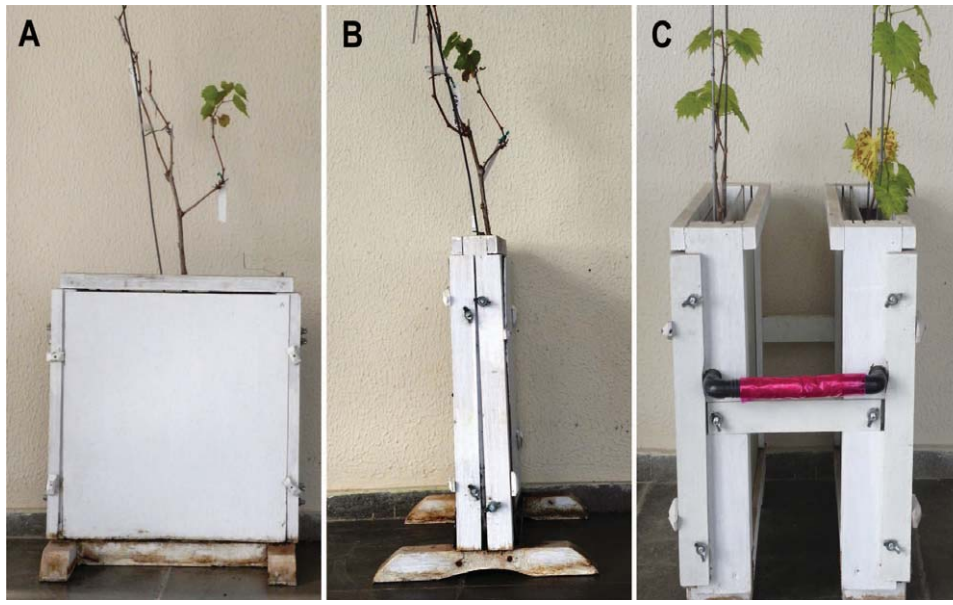

Fig. 1. Gallotti cage. (A) Front view of simple Gallotti cage with vine plant; (B) side view of simple Gallotti cage; (C) side view of double Gallotti cage with hose covered by red cellophane paper. Photograph: Aline Nondillo. (Online figure in color.).

fluence on the structure of a community (Eubanks and Strysky 2006, Brightwell and Silverman 2010). 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, Martinez-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 also cleaning and removing dead individuals (Buckley 1987, Vanek and Potter 2010). This relationship is called trophobiosis, and is usually beneficial to both organisms, resulting in increased density of hemipterans and ants in a certain area (Way 1963, Abbott and Green 2007, Daane et al. 2007, Delabie 2001).

Soil scale species of economic importance have been found in Chile: *Margarodes vitis* Philippi; California: *Margarodes meridionalis* Morrison; and South Africa: *Margarodes trimeni* Giard, *Margarodes capensis* Giard, *Margarodes greeni* Brian, and *Margarodes prieskaensis* (Jakubski) (Foldi 2005). However, there are no reports on the interaction of these species with ants.

In southern Brazil, for many years *Linepithema humile* (Mayr) (Hymenoptera: Formicidae) was reported as predominant in areas infested by *E. brasiliensis*, and was considered primarily responsible for spreading the scale to new locations in the same host or nearby plants (Gallotti 1976, Soria and Gallotti 1986, Hickel 1994, Soria and Dal Conte 2000, Botton et al. 2004). However, in a recent study using molecular techniques, Martins and Bueno (2009) recorded *Linepithema micans* (Forel) (Hymenoptera: Formicidae) in vineyards with *E. brasiliensis*, and did not record *L. humile.* In two vineyards in southern Brazil, Sacchett et al. (2009) reported several genera of ants in areas infested by *E. brasiliensis*, especially *L. micans* and *Pheidole* sp.

Information on the relationship of *L. micans* to the establishment and dispersal of scale has not been reported. This study was carried out to measure the existing relationship between *E. brasiliensis* and *L. micans* in vineyards.

Materials and Methods

We conducted the experiment during 2010, in a greenhouse located at Embrapa Uva e Vinho in Bento Gonçalves, Rio Grande do Sul, Brazil. We used Paulsen 1103 rootstock seedlings (*Vitis berlandieri* Planchon × *Vitis rupestris* Scheele) planted in individual Gallotti Cages (Gallotti 1976) (Fig. 1A).

The Gallotti Cage is a wooden structure (40 cm wide by 50 cm high by 8 cm thick) with a closed bottom and an open top (Figs. 1A and B). The soil is introduced through the top opening and the seedlings are planted. The outer walls are transparent glass (2 mm thick), attached to wooden racks that can be removed to introduce insects and observe their developmental stages. On the outside of the glass is a 1-cm thick wooden mobile cover to exclude light, which when removed allows observation through the glass (Nondillo et al. 2012a).

We also used double Gallotti Cages, two cages connected by a hose covered with red cellophane paper (Fig. 1C) to shade the connection between cages.

Experiment 1. Simple Gallotti Cages. To study the interaction between *E. brasiliensis* and *L. micans*, we established three treatments: 1) soil infestation with *E.*

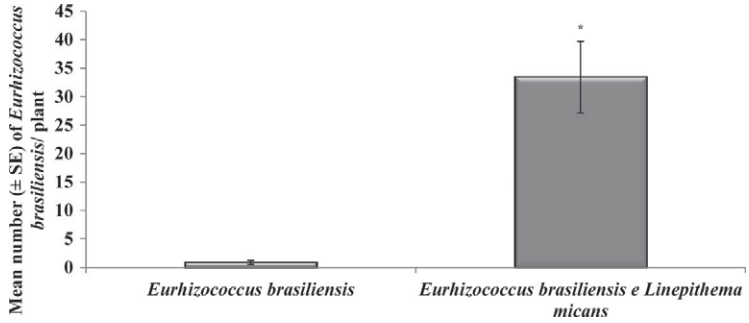

Fig. 2. Mean number (±SE) of *Eurhizococcus brasiliensis* cysts per plant in simple Gallotti Cages without and with the presence of *Linepithema micans* colonies. *Asterisk indicates significant difference between treatments by Mann–Whitney *U* test ($P \leq 0.05$).

brasiliensis, 2) soil infestation with nests of *L. micans*, and 3) soil infestation with *E. brasiliensis* and *L. micans*.

In the *L. micans* treataments, nests of similar size with approximately seven queens and 1,500 workers were transferred to each Gallotti Cage. All the nests contained eggs, larvae, and pupae. The ants were collected from vineyards infested with *E. brasiliensis* and *L. micans*. The ant nests, together with soil, were removed and transported to the laboratory in plastic bags and later 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 sugar solution (25%) was placed between the tiles to stimulate the ants to enter the set of tiles (Nondillo et al. 2012b).

After the colonies established themselves between the tiles, the tiles were placed on the surface of each Gallotti Cage, thus enabling the ants to transfer the colony themselves (Nondillo et al. 2012a).

After 15 d of ant infestation, five mature cysts with eggs and 300 *E. brasiliensis* nymphs were inoculated per cage. The cysts with eggs were collected from infested vineyards and kept in the laboratory (26.5 ± 1.5°C and 80 ± 10% RH) in petri dishes with soil until the nymphs hatched. Daily, the hatched nymphs were removed with a brush, placed in 50-ml plastic cups containing soil, and transferred to the plant roots. The infestation was accomplished by removing the glass cover from the cage and depositing the contents of the plastic cups in three or four holes made next to the roots (Nondillo et al. 2012a).

Replicates of *L. micans* were fed three times per week with larvae of *Tenebrio molitor* L. or adults of *Gryllus* sp. and inverted sugar (25%). Each treatment was repeated 20 times, in a fully randomized design.

Experiment 2. Double Gallotti Cages. In this experiment we measured two treatments: 1) soil infestation with *E. brasiliensis* and *L. micans*, and 2) soil infestation with *E. brasiliensis* only.

The colonies of *L. micans* were established in the cages before an infestation with *E. brasiliensis* was introduced, in only one of the cages of each set. Five mature cysts with eggs and 300 nymphs of *E. brasiliensis* were inoculated per cage; in the treatment with the presence of *L. micans*, nests of similar size with ≈15 queens and 3,000 workers were transferred to each Gallotti Cage. All the nests contained eggs, larvae, and pupae.

Replicates with *L. micans* present were fed three times per week with larvae of *Tenebrio molitor* or adults of *Gryllus* sp. and inverted sugar (25%). Each treatment was repeated 10 times, in a fully randomized design.

Evaluation and Statistical Analysis. The evaluation was carried out by counting the total number of *E. brasiliensis* cysts and ants (eggs, larvas, pupae, workers, queens, and males) in each Gallotti Cage 9 mo after the infestation, because of the time period needed for the *E. brasiliensis* cysts to develop. For evaluation, the cages were opened, the soil was screened and all cysts were removed. Next, the nests together with the soil were transferred to plastic trays with two juxtaposed tiles, to stimulate the ants to enter in the set (Nondillo et al. 2012b). After the colonies established in the tiles, the ants were killed in 70% ethanol and placed in individual vials, and the number of individuals was counted. Differences in the numbers of scales and ants between treatments were compared via Mann–Whitney *U* tests ($P \leq 0.05$) using the Statistica program (StatSoft, Inc. 2011).

Results

In experiment 1, the number of cysts in the treatment with the presence of *E. brasiliensis* and *L. micans* (33.45 ± 6.25 cysts per cage) was significantly ($U = 25.00$; $P < 0.001$) higher than the treatment infested only with *E. brasiliensis* scale (0.9 ± 0.40 cysts/cage) (Fig. 2). In this experiment, we also observed a significant increase in the population size of *L. micans* colonies in the treatment with the presence of *E. brasiliensis* scale ($U = 125.00$; $P < 0.001$) (Fig. 3).

In the experiment with double Gallotti Cages (experiment 2), on the infected sides, the treatment with the presence of *E. brasiliensis* scale and *L. micans* showed a mean of 29.5 ± 5.3 cysts per cage, differing

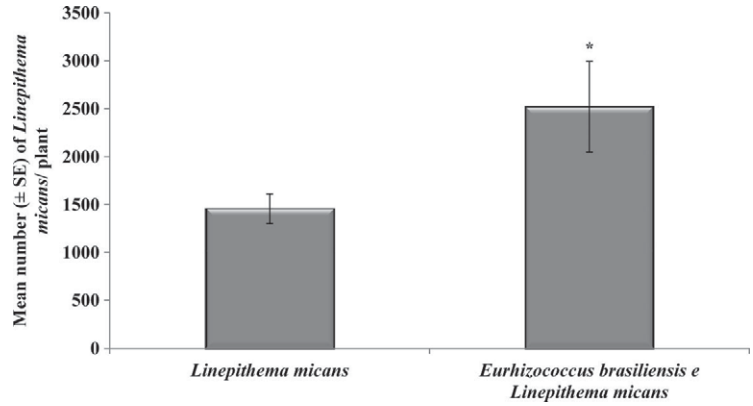

Fig. 3. Mean number (±SE) of ants (egg, larvae, pupae, worker, queen and male) in simple Gallotti Cages without and with the presence of *Eurhizococcus brasiliensis* cysts. *Asterisk indicates significant difference between treatments by Mann–Whitney *U* test ($P \leq 0.05$).

(U = 0.500; $P < 0.001$) from the 1.4 ± 0.95 cysts found in the treatment with ground-pearl only (Fig. 4).

On the noninfested sides, there were no cysts in the treatment with scale only. However, when the plants were infested with *E. brasiliensis* scale and *L. micans* together, a mean of 33.6 ± 5.16 cysts per cage was found (U = 0.000; $P < 0.001$) (Fig. 4).

Discussion

This study is the first to examine the interaction between *L. micans* and *E. brasiliensis*, where the former was the disperser and facilitator for the infestation of the scale in vine plants.

Linepithema humile has been considered the main disperser of *E. brasiliensis* in Brazil (Gallotti 1976, Hickel 1994, Soria and Gallotti 1986). The notorious pest status of *L. humile* made this ant the target of several studies aiming to understand its physiology, ecology, genetics, and social biology, and it is among the best-studied species of ant (Wild 2007). Because of the high visibility of this species and the great similarity between the workers of both species, in Brazil there was a trend to identify all ants of the genus *Linepithema* as *L. humile* (Hickel 1994, Soria and Gallotti 1986, Silva and Loeck 1999, Wild 2007). According to Suarez et al. (2001), some populations of the genus *Linepithema* occurring in Brazil do not belong to the species *L. humile*, although they are commonly referred to as such.

The results showed that *L. micans* is fundamental for the attachment and transport of first-instar *E. brasiliensis* nymphs, as evidenced by the number of cysts found per cage. This number was significantly higher when the scales were tended by ants.

The literature is replete with studies showing that the mutualistic interactions between ants and honeydew-producing insects can increase the population size of both. Cushman and Whitman (1989) found a positive correlation between the population size of *Formica altipetens* Wheeler (Hymenoptera: Formicidae) and the population of *Pubilia modesta* (Uhler)

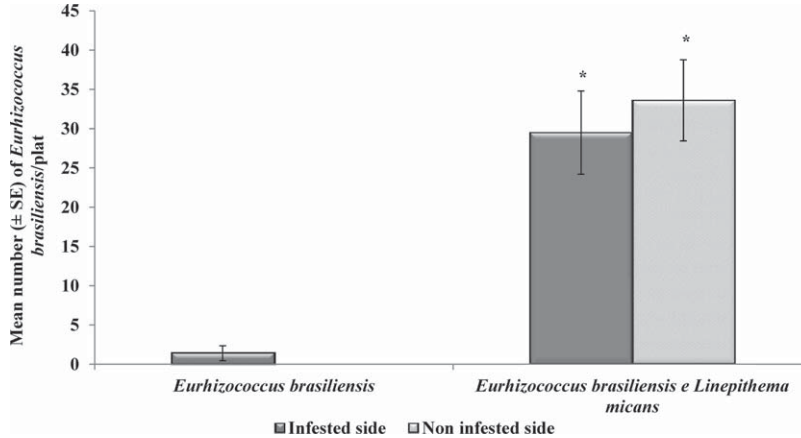

Fig. 4. Mean number (±SE) of *Eurhizococcus brasiliensis* cysts per plant in double Gallotti Cages without and with the presence of *Linepithema micans* colonies in the infested side (gray columns) and noninfested side (hatched columns). *Asterisk indicates significant difference between treatments by Mann–Whitney *U* test ($P \leq 0.05$).

(Hemiptera: Membracidae). Schwartzberg et al. (2010) showed that the biomass of the aphid *Aphis glycines* Matsumura (Hemiptera: Aphididae) increased when the aphids were assisted by ants of the species *Lasius neoniger* Emery (Hymenoptera: Formicidae). The increases in populations of *Toxoptera aurantii* (Boyer de Fonscolombe) (Hemiptera: Aphididae) (Powell et al. 2009), *Aphis gossypii* Glover, and *Myzus persicae* (Sulzer) (Hemiptera:Aphididae) (Powell and Silverman 2010) were greater when these aphids were tended by *L. humile* and *Tapinoma sessile* (Say) (Hymenoptera: Formicidae), compared with nontended populations.

In agricultural systems, *L. humile* has been associated with population increases of phloem-feeding insects such as scales and aphids. These insects exploit honeydew and, in return, they protect scales and aphids against the attack of predators (Buckley and Gullan 1991). In vineyards of California, *L. humile* has been responsible for increases in the populations of *Pseudococcus maritimus*, *Psuedococcus viburni*, and *Planococcus ficus* (Daane et al. 2007, 2008).

In the literature, the situation most commonly described in relation to the ant-Hemiptera mutualism is the ants tending honeydew producers in exchange for protection against natural enemies, thus allowing the populations of both ants and scales to increase. The increased number of *E. brasiliensis* cysts observed in this study probably is not related to defense, as there were no natural enemies in the cages. Even if predation and parasitism are not involved in this situation, a possible benefit provided by the ants may be cleaning services. Removal of the honeydew may improve the scale's habitat, facilitating its establishment on plants (Buckley 1987, Daane et al. 2007, Vanek and Potter 2010). According to Soria and Gallotti (1986), the ants may also open galleries in the soil, allowing the scale to reach other parts of the plant root system. Daane et al. (2007) found that, even in vineyards where there were no natural enemies, the population density of scales was decreased in treatments without ants, suggesting that the ants provided other benefits besides protection.

The growth of the invasive ant colony is often favored by honeydew of hemipterans (Ness and Bronstein 2004). According to Helms and Vinson (2008), *Solenopsis invicta* Buren colonies that had access to scale honeydew grew 50% more than those without access. The importance of carbohydrates for omnivorous ants is also supported by other studies that show a significant increase in the populations of *Solenopsis invicta* and *Tetramorium caespitum* (L.) when fed with sucrose (Porter 1989, Kay et al. 2006). In the first experiment, the population of *L. micans* increased significantly in the presence of *E. brasiliensis*, showing the importance of carbohydrates for the colony. The carbohydrates are fuel for the activities of the workers (Grover et al. 2007).

Carbohydrates from honeydew and plant exudates are important in the ant colony, as workers fed on these liquids play important roles in protecting the colony and caring for offspring (Tobin 1994, Glancey et al. 1981, Helms and Vinson 2008). Ants can obtain carbohydrates from the hemolymph from other predated arthropods (Wyatt and Kalf 1957), but this method would result in a net loss of energy, because of the need to search and compete for food (Helms and Vinson 2008). Carbohydrate sources could also be obtained directly from plants through the nectar; however, nectar availability varies according to season and availability of plants (Rico-Gray and Garcia-Franco 1998). Thus, the ants have found a predictable and renewable source of nutrients in hemipterans (Delabie 2001, Styrsky and Eubanks 2007), providing favorable conditions for colony growth.

In regard to *E. brasiliensis* dispersal, Sacchett et al. (2009) carried out an experiment in two vineyards, exposing cysts and sugar pellets on petri dishes. They observed that workers of *Pheidole* sp. and *L. micans* could transport *E. brasiliensis* cysts. In the experiment carried out here with double Gallotti Cages, in addition to carrying nymphs from one cage to another, the ants helped the nymphs to attach to the roots, and no cysts were observed in the treatment without ants present.

The results of these experiments showed that *L. micans* has an important role in dispersal and infestation by *E. brasiliensis*. The possibility that this can also occur in other species of ants that feed on honeydew should be investigated. However, because of the frequency and abundance of *L. micans* in infested vineyards, an *E. brasiliensis* management program is recommended. Such a program must also involve the control of related disperser ants, which would reduce the survival of scale and also reduce the dispersal of nymphs to new locations in the vineyard.

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