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Foraging Activity and Seasonal Food Preference of *Linepithema micans* (Hymenoptera: Formicidae), a Species Associated With the Spread of *Eurhizococcus brasiliensis* (Hemiptera: Margarodidae)

ALINE NONDILLO,^{1,2} LEONARDO FERRARI,³ SABRINA LERIN,³ ODAIR CORREA BUENO,¹ AND MARCOS BOTTON³

ABSTRACT Linepithema micans (Forel) (Hymenoptera: Formicidae) is the main ant species responsible for the spread of Eurhizococcus brasiliensis (Wille) (Hemiptera: Margarodidae), a soil scale that damages vine plants in southern Brazil. The daily foraging activity of *L. micans* and its seasonal preference for protein- and carbohydrate-based foods were evaluated. The study was carried out in a greenhouse using seedlings of the Paulsen 1103 rootstock (*Vitis berlandieri* × *Vitis rupestris*) planted individually in pots and infested with colonies of *L. micans*. To determine the daily foraging activity and seasonal preference, a cricket (*Gryllus* sp.) and a 70% solution of inverted sugar and water were offered once a month for 12 mo. The ants foraging on each food source were counted hourly for 24 h. *L. micans* foraged from dusk until the end of the next morning, with higher intensity in the spring and summer. Workers of *L. micans* showed changes in food preference during the year, with a predominance of protein-based food during winter and spring and carbohydrate-based food during autumn. The implications of this behavior for control of the species with the use of toxic baits are discussed.

KEY WORDS grapevine, soil scale, daily foraging, seasonal preference

Linepithema micans (Forel) (Hymenoptera: Formicidae) is the main ant species responsible for the spread of Eurhizococcus brasiliensis (Wille) (Hemiptera: Margarodidae), the main pest of vineyards in Brazil (Martins and Bueno 2009, Sacchett et al. 2009, Nondillo et al. 2013). One strategy to reduce infestation by E. brasiliensis in vinevards is to control L. micans through the use of toxic baits (Nondillo 2013), as with *Linepithema humile* (Mayr) (Hymenoptera: Formicidae) in South Africa and California (Addison and Samways 2000; Daane et al. 2006, 2007; Cooper et al. 2008; Nvamukondiwa 2008). A basic requirement for success in the use of toxic baits is knowledge of the foraging period and seasonal food preferences, which will guide the choice of attractive ingredients for bait (Markin 1970, Abril et al. 2007).

L. humile forages only during the coolest hours of the day; during spring and summer, the ants forage mainly at night when temperatures are lower. Workers of *L. humile* change their food preference depending on the stage of the colony reproductive cycle (Abril et al. 2007). Protein foods are preferred during the spring and autumn to feed queens and larvae, and carbohydrate-based foods during the period when the

density of males and workers in the colony is higher (Markin 1970, Rust et al. 2000, Abril et al. 2007).

Because of the lack of information on the foraging behavior and food preference of *L. micans*, this study evaluated the daily and seasonal foraging activity of the species and its seasonal preference for proteinand carbohydrate-based foods.

Materials and Methods

The experiment was carried out from June 2011 through May 2012 in a greenhouse located at Embrapa Uva e Vinho, Bento Gonçalves, Rio Grande do Sul, Brazil. Ten seedlings rooted in Paulsen 1103 vine rootstocks (*Vitis berlandieri* × *Vitis rupestris*) were planted in individual 5-liter pots.

After the planting, the vine seedlings remained in the pots for ≈ 2 mo, after which they were infested with nests of ants. Nests of *L. micans* of similar size, with approximately 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, together with soil, were removed and transported to the laboratory in plastic bags and later transferred to plastic trays. To capture the ants, two tiles (10 by 10 cm) were placed in each tray, with the abrasive faces toward each other and with wooden sticks (2 mm in width) between them. The sticks were placed with a space between their tips, for the ants to enter.

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¹ A. Nondillo, O.C. Bueno, Instituto de Biociências, UNESP – Universidade Estadual Paulista, Campus de Rio Claro, SP, Brazil. Departamento de Biologia, C.E.I.S. - Centro de Estudos de Insetos Sociais. ² Corresponding author, e-mail: alinondillo@vahoo.com.br.

³ L. Ferrari, S. Lerin, M. Botton, Laboratório de Entomologia, Embrapa Uva e Vinho, Bento Gonçalves, RS, Brazil.



Fig. 1. Formic plate of double pick with two food sources: (A) *Gryllus* sp. and (B) an aqueous solution of invert sugar (70%). (C) Detail of ants feeding on food resource during the evaluation period. (Online figure in color.)

Cotton moistened with a 25% sugar solution was placed between the tiles to stimulate the ants to enter the set of tiles (Nondillo et al. 2012, 2013).

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

After the infestation, the ants were fed three times per week with larvae of *Tenebrio molitor* L., adults of *Gryllus* sp., an aqueous solution of inverted sugar (25%), and water ad libitum. The pots were placed in trays filled with talcum powder, with the edges covered with Teflon-30 (DuPont, Wilmington, DE), to prevent the ants from escaping.

To determine the daily and seasonal foraging activity and food preference during the year of workers of *L. micans*, the number of ants foraging on two food sources was counted during each month of the year. The food resources were arranged on a formic plate, allowing the foragers to choose between foods (Fig. 1). The food sources evaluated were crickets (*Gryllus* sp.) and a solution of 70% inverted sugar in water (Fig. 1A and B). The foods selected are preferred by workers of *L. micans*, based on prior food-preference experiments (Nondillo 2013).

After the food was offered, we counted the number of ants around the food source (Fig. 1C). This procedure was repeated each hour for 24 h; in other words, all hours of the day, once a month, for 1 yr. The temperature and air relative humidity were also measured in these intervals.

Each treatment was repeated 10 times in a fully randomized experimental design.

Statistical Analysis. The data were evaluated for normality by the Shapiro–Wilk test, and for variance homogeneity by the Levene test. Non-normal data were square root-transformed.

The numbers of ants foraging at the different times of day (morning, afternoon, night, and dawn) were analyzed using analysis of variance, followed by the comparison of means using the Tukey test at the 5% significance level.

The environmental factors of temperature and humidity, and the daily and seasonal foraging activity were analyzed using Pearson's correlation coefficient.

For analysis of the food preference throughout the year, the data were arcsine-transformed and evaluated

by Student's *t*-test. The software Statistica 10 (StatSoft Inc., Tulsa, OK) was used in all analyses.

Results and Discussion

The ant foraging activity on the vine rootstocks was continuous throughout the year (Fig. 2). The workers foraged most actively at dusk, with peaks in the morning, and were least active during the warmest and least humid hours of the day (Fig. 2).

Although activity was low in the months of lower temperatures (June, July, and August), foraging activity was more intense at night (June: F = 21.064; P < 0.001; July: F = 10.911; P < 0.001; August: F = 35.359; P < 0.001). This pattern continued in September (F = 67.152; P = 0.001) and October (F = 7.580; P < 0.001) with peaks during the first hours of the night (Fig. 2A–E).

In November (F = 76.907; P < 0.001), December (F = 55.242; P < 0.001), February (F = 39.526; P < 0.001), March (F = 49.079; P < 0.001), April (F = 17.251; P < 0.001), and May (F = 2.772; P = 0.042), activity increased in the first hours of dusk and continued until the morning, with a significant decrease in the afternoon (Fig. 2, F–L). In January, a significantly higher peak of foraging was recorded in the morning (F = 17.777; P = <0.001; Fig. 2H).

The daily foraging activity of the colonies of *L. micans* was negatively correlated with temperature and positively correlated with air relative humidity in most months (Fig. 2; Table 1).

The foraging activity pattern is one of the most distinct characteristics of ant species (Hölldobler and Wilson 1990). Interspecific divergences in the foraging activity pattern arise from morphological, physiological, and behavioral characteristics that define the ecological tolerance of a species (Bernstein 1974). This temporal dimension in the foraging behavior depends on abiotic factors that vary seasonally, such as temperature, relative humidity, luminosity, and rainfall, and also on biotic factors such as food and intraspecific competition among others (Carrol and Janzen 1973, Bernstein 1974, Traniello 1989).

The variation in daily foraging activity observed in this study indicates that the temperature and relative humidity strongly affected the foraging pattern, with



Fig. 2. Mean number of workers of *L. micans* foraging per hour (bars), temperature (—), and air relative humidity (.....) in greenhouse. In 2011: (A) June, (B) July, (C) August, (D) September, (E) October, (F) November, and (G) December. In 2012: (H) January, (I) February, (J) March, (K) April, and (L) May. *: Same lowercase letters do not differ by Tukey's test (P < 0.05).

a significant correlation between these variables and the number of ants foraging.

The strategy to reduce the period of exposure to high temperatures was evident, as in all months, the foraging intensity decreased in the first hours of the afternoon when the temperatures are higher and air relative humidity is lower. Similar patterns were observed for *L. humile* by Markin (1970) and Abril et al. (2007) in the warmer seasons of the year, and for other ant species such as *Anoplolepis tenella* Santschi (Formicinae) (Kuate et al. 2008), *Odontomachus chelifer* (Latreille) (Ponerinae) (Raimundo et al. 2009), and





Tapinoma indicum Forel (Dolichoderinae) (Cerdá et al. 1998).

According to Abrams (1991), obtaining food generally involves physiological costs. The highest cost for ants foraging at high temperatures is desiccation, which can be lethal, and represents a high risk for the entire colony (Cerdà et al. 1998). Comparisons with native North American species showed that *L. humile* is sensitive to desiccation, given its small size (Schilman et al. 2005). Because workers of *L. micans* are also small and morphologically similar to *L. humile*, it can be assumed that they use the same behavior pattern to avoid high temperatures.

Table 1. Correlation coefficients (r) between the foraging activity of *L. micans*, with temperature, air relative humidity, and associated probabilities (P) in each month

Month	Temp.		Rel. humidity	
	r	Р	r	Р
June	-0.488	0.013*	0.754	< 0.001*
July	-0.412	0.046*	0.776	0.001*
Aug.	-0.811	< 0.001*	0.807	< 0.001*
Sept.	-0.656	0.001*	0.628	0.001*
Oct.	-0.447	0.029*	0.222	0.298
Nov.	-0.452	0.027*	0.778	< 0.001*
Dec.	-0.709	< 0.001*	0.622	0.001*
Jan.	0.528	0.008*	-0.498	0.013*
Feb.	-0.531	0.008*	0.588	0.033*
Mar.	-0.242	0.255	0.253	0.232
April	-0.490	0.015*	0.550	0.005*
May	-0.405	0.049*	0.514	0.010*

* Significant correlation at the 0.05% level.

The seasonal foraging activity of *L. micans* varied through the year, with a reduction in the activity rhythm from July through September and a higher frequency of workers searching for food from November through February (Fig. 3). A positive correlation was found between the temperature and the monthly foraging patterns (r = 0.646; P = 0.017) with a larger number of ants searching for food in the warmest months. The monthly analysis showed no significant correlation with relative humidity (r = -0.153; P = 0.617).

The increase in foraging activity in the warmest seasons was also reported for *Tetramorium semilaeve* André (Myrmicinae), *Camponotus foreli* Emery (Formicinae) (Cerdà et al. 1998), *A. tenella* (Formicinae) (Kuate et al. 2008), and *O. chelifer* (Ponerinae) (Raimundo et al. 2009) among others. Similar behavior was observed in species of Dolichoderinae, such as *Tapinoma nigerrimum* (Nylander) (Cerdà et al. 1998) and *L. humile* (Markin 1968, Abril et al. 2007).

A possible explanation for the seasonal change in foraging activity may be associated with the reproductive phase of the colony. With the increase in temperature and the beginning of the reproductive cycle, the queen must ingest larger amounts of food to support oviposition. Thus, during the reproductive cycle, the workers increase their own activity to carry out several tasks such as caring for the brood and feeding the queens (Carrol and Janzen 1973, Benois 1973, Keller 1988, Abril et al. 2007), and consequently intensify their search for food. According to Nondillo



Fig. 3. Monthly mean foraging activity of *L. micans* (bars), temperature (continuous line), and air relative humidity (dotted line) in a greenhouse.

(2013), the period of highest oviposition of *L. micans* queens occurs from November to February, coinciding with the months of higher foraging activity.

L. micans workers collected more high-protein food in June (t = -6.114; df = 46; P < 0.001), July (t = 3.259; df = 35.259; P < 0.001), September (t = -6.704; df = 46; P < 0.001), October (t = 0.003; df = 46; P < 0.001), November (t = -4.354; df = 46; P < 0.001), and December (t = -4.354; df = 46; P < 0.001; Fig. 3). In February (t = 12.647; df = 37.302; P < 0.001), March (t = 7.278; df = 46; P < 0.001), and May (t = 4.174; df = 46; P < 0.001) they preferred carbohydrate-based foods (Fig. 4).

This preference for different food sources during the year is probably owing to the nutritional requirements of the colony. According to Fowler et al. (1991) and Parra (1991), the standard food of ants basically consists of proteins, carbohydrates, and lipids. Proteins are acquired from predation on other insects and small invertebrates, carbohydrates from the ingestion of sugars and polysaccharides from the nectar of plants and the excretions of other insects, and lipids from the ingestion of different types of oil.

Carbohydrate-rich foods are an important source of energy for workers (Markin 1970, Abbott 1978, Stradling 1978, Grover et al. 2007), and acquisition of this type of food is indispensable for the maintenance of the entire colony (Glancey et al. 1981, Tobin 1994, Helms and Vinson 2008). A protein-rich diet is essential for the development of the larvae and higher egg production by the queen (Markin 1970, Abbott 1978, Stradling 1978, Rust et al. 2000). Consequently, highprotein food is gathered in higher proportions in the period when the colony needs to increase egg production, thus increasing the population size of the colony (Dussutour and Simpson 2009).

Nondillo (2013) found that larvae in *L. micans* colonies are produced mainly from April through October, corresponding to the period when the collection of protein food increased. These results concord with those found by Rust et al. (2000) and Abril et al. (2007) for *L. humile* colonies.

The development of efficient baits to control ants is a challenging task (Hooper-Bui and Rust et al. 2000, Silverman and Brightwell 2008). In the case of *L. micans*, better results would be obtained by the use of protein matrices during the winter, when the colony needs protein-rich foods to feed the large numbers of larvae (Nondillo 2013). With increasing temperatures, the number of ants in the population increases and oviposition begins (Nondillo 2013), as a consequence



Fig. 4. Ratio of workers of *L. micans* feeding on inverted sugar and *Gryllus* sp. *, significant at 5% probability by the *t*-test.

the, workers require more food, and carbohydratebased foods could be offered in the warmer seasons.

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