

Seasonal Occurrence and Distribution on Grapevine Roots of *Eurhizococcus brasiliensis* (Wille) (Hemiptera: Margarodidae) in Brazil

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The ground pearl, *Eurhizococcus brasiliensis* (Wille) (Hemiptera: Margarodidae), is the most important grapevine pest in Brazil. Its seasonal occurrence and distribution on the roots of the different development stages were determined to allow better monitoring of this insect and better targeting of its vulnerable life stages. Yellow cysts (after the first nymphal moult) showed the lowest density in October, followed by a gradual increase towards August. White cysts (cysts with enclosed pre-pupal males or females) occurred from August to December, with a peak in November. Mobile females (adult females emerging from the white cysts) were found from August to December, with a peak in August. Parthenogenetic females that remain in the ruptured white cysts for egg laying were present from August to April, with a peak in November. Mobile nymphs (first instar) were also found from August to April, with a peak in December. Yellow cysts were most abundant at depths of 0 to 25 cm. The horizontal survey showed that cysts occurred mostly on the trunk below the ground (trunk of the rootstock), and that almost all occurred in an area of 20 cm width around the trunk. These results provide important information for better monitoring of this pest and to develop better methods for and timing of chemical control.

INTRODUCTION

Margarodes (Hemiptera: Margarodidae) are scale insects whose immature stages remain enclosed in a waxy cyst with a shining pearl-like appearance of different colours in different species. These cysts remain underground and therefore are commonly also known as 'ground pearls'.

Margarodes, as is typical for scale insects, show extreme sexual dimorphism, with apterous, neotenic females and small, winged, ephemeral males. The mobile first-instar nymphs, characterised by the presence of antennae and fossorial prothoracic legs, are responsible for the dispersal of the species. Adults lack mouthparts, but the encysted nymphal stages have long sucking mouthparts and their feeding on roots has a marked deleterious effect on plant growth and vigour. Furthermore, encysted nymphs have an extraordinary ability to resist adverse conditions, providing many species with an added advantage in becoming agricultural pests (Hoffman & Smith, 1991).

Eurhizococcus brasiliensis (Wille) is associated with various plant species and often develops large infestations, seen as dense aggregates on the roots of host plants. This species is the main insect pest in most Brazilian vineyards, with one generation per year. Feeding by these insects results in serious damage to grapevines that is characterised by a progressive decline in overall plant health, with a concomitant decrease in grape production (Botton *et al.*, 2004).

E. brasiliensis is predominantly thelytokous, facultatively parthenogenetic, with two wingless mobile sexual stages (adult females and pre-pupal males) that crawl to the soil surface, where the male pre-pupae become pupae and, later, winged, ephemeral males. The mobile adult females mate with the winged males and then return underground to lay their eggs in a bundle covered with white wax filaments in the vicinity of the trunk and roots (below the ground).

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The parthenogenetic females remain below ground, protected within the white cyst in which they lay their eggs. After the eggs hatch, the white cysts break up and the first instar nymphs emerge (Botton *et al.*, 2004; Soria & Dal Conte, 2005). These nymphs settle on roots and begin to feed, while simultaneously secreting layers of wax to form new cysts. During the first moult, the nymphs lose their legs and antennae and continue to feed and become enclosed within the new yellow cysts (Foldi, 2005). The cysts change to white when the adult female or male pre-pupae develop.

Besides *E. brasiliensis*, *Margarodes vitis* (Phillipi) from Chile is also an economic pest in vineyards in South America (Foldi & Soria, 1989). One margarodes species (*M. meridionalis*) attacks grapevines in California, USA (Barnes *et al.*, 1954). Ten indigenous species of *Margarodes* are known to occur in South Africa, five of which infest vine roots in most of the vine-growing areas. All of the vine-infesting species are economically important, and severe infestations can kill vines within four years after planting (De Klerk, 1985).

Little research has been done on the biology and seasonal occurrence of *E. brasiliensis*, primarily because of difficulties in sampling and rearing the insect in the laboratory. Past attempts to control margarodes with insecticides have failed, largely because of the unusually long time that immature stages spend underground in the resistant encysted stage. The success of future attempts at chemical control of *E. brasiliensis* depends on a better understanding of the nymph and adult bionomics (Hoffman & Smith, 1991).

The aim of this study was to examine the seasonal occurrence of *E. brasiliensis* and its distribution on vine roots. This information will be useful in monitoring margarodes and choosing the appropriate time and place to target the most vulnerable life stages.

MATERIALS AND METHODS

Seasonal occurrence

This study was done in a 1.2 ha commercial vineyard of Niagara Rosada grafted onto IAC 766, in the region of Louveira, São Paulo state, south-eastern Brazil. The vines were seven years old, planted in a sandy loam soil (clay: 12.2%, silt: 9.9%, total sand: 77.9%) and spaced 1.0 m x 1.7 m apart.

Observations were made at different intervals from August 2008 to August 2009, on six randomly selected grapevines. A circular trench 30 cm in diameter and 30 cm deep was dug around each vine and the number of individuals of each life stage were counted visually on the roots, as well as in the soil around the roots. The soil around the roots was removed carefully to count the insects on the vine roots as well as in the soil around the roots, after loosening and spreading the soil by hand.

Distribution on roots

The study on their distribution on the roots was done during February and March 2010, in two vineyards with six-year-old Niagara Rosada grafted onto IAC 766 in 0.2 ha of clay loam soil (clay: 36.1%, silt: 36.8%, total sand: 27.1%) (field 1), and onto Riparia do Traviú in 0.1 ha of sandy loam soil (clay: 16.3%, silt: 17.1%, total sand: 66.6%) (field 2). Both

vineyards were located in the region of Louveira, about 2 km from each other.

In each vineyard, 25 vines infested with *E. brasiliensis* were randomly selected and a circular trench 60 cm in diameter and 60 cm deep was dug around each vine. The soil in this 60 cm-wide circle was carefully loosened in layers of about 10 cm deep, removed from the roots and taken out of the trench with the roots still intact. As the excavation of the soil progressed, all roots were cut loose at a distance of 60 cm from the trunk of the rootstock. At the depth of 60 cm, all the roots present were also cut and each vine was lifted out of the trench with all the remaining roots attached. Each vine was placed separately in a single bag and taken to the laboratory of the Instituto Biológico, Campinas, SP. Almost all the cysts were still attached to the trunk below the ground and to the roots after lifting the vine from the soil, with very few lying loose in the bags on arrival at the laboratory. The cysts were still small and in the early nymphal stage (February to March), resulting in lower resistance to the soil during the removal of the plants and less vibration during transport.

In the laboratory, the yellow cysts found on the trunk below the ground and on the roots, at a certain depth and width around the trunk (hereafter referred to as the counting intervals) of 0 to 5, 5 to 10, 10 to 15, 15 to 20, 20 to 25, 25 to 30, 30 to 35, 35 to 40, 40 to 45, 45 to 50 and > 50 cm, were counted visually. The number of roots was also recorded for each trunk interval.

Statistical analysis

The seasonal and distribution data were Log (x+1) transformed and statistically compared by one-way analysis of variance (ANOVA), followed by Tukey's studentised range test. Pearson's coefficient of correlation (r) was used to examine the correlation between the number of yellow cysts on the trunk below ground and on the roots, and the number of roots arising from the trunk at each soil depth. In this analysis, $P < 0.05$ indicated significance.

RESULTS AND DISCUSSION

Seasonal occurrence

Five developmental stages were observed in *E. brasiliensis*: mobile nymphs (first instar nymphs or "crawlers"), yellow cysts (after the first moult), white cysts (pre-pupal males or females), immobile adult females (parthenogenetic females within ruptured white cysts), and mobile females (adult females).

Yellow cysts

This stage (after first moult) was the most ubiquitous throughout the sampling period, with the lowest densities (< four/vine) in October, followed by a gradual increase to 20.2/vine in August (Fig. 1), but with no significant differences between evaluations ($F = 1.775$; $df = 11, 60$; $P = 0.79$). The yellow cyst develops after the first ecdysis, 60 days after nymph eclosion (Monteiro & Soria, 1996). Surveys done by Foldi (1990) of vines in Veranópolis, Rio Grande do Sul, Brazil, showed that the first moult occurred in February and March. Second instar nymphs lose their legs, but continue to feed while remaining inside the exuvia, which

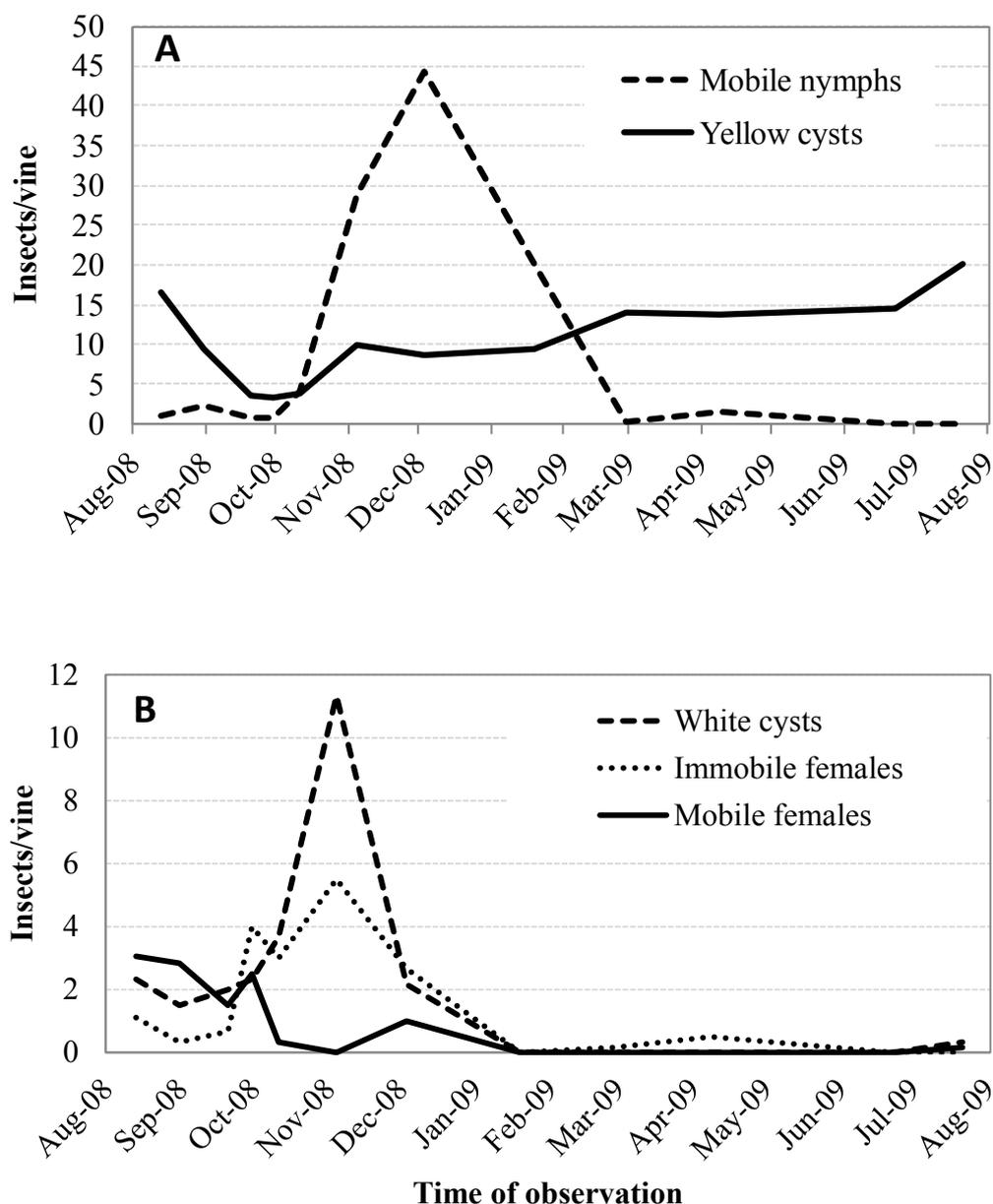


FIGURE 1

Seasonal occurrence of the life stages of *Eurhizococcus brasiliensis* in a commercial vineyard in the municipality of Louveira, SP, Brazil, in 2008 and 2009. A = nymph stages; B = older stages. Yellow cysts = after the first nymphal moult. White cysts = containing a pre-adult or adult. Mobile females = adult females. Immobile adult females = parthenogenetic. Mobile nymphs = first instar.

becomes a protective, spherical capsule approximately 4 mm in diameter (Botton *et al.*, 2004; Haji *et al.*, 2004, Soria & Dal Conte, 2005).

Monteiro and Soria (1996) studied the development of *E. brasiliensis* on beet and found that yellow cysts are fully developed, with all their peculiar characteristics, at 140 to 160 days after nymph eclosion. The formation of the cyst wall in hypogean species, including *E. brasiliensis*, was described by Foldi (1997). The yellow cyst, as well as the white cyst, makes the insect resistant to water loss, environmental factors (high temperature, low humidity), unfavourable nutritional conditions and natural enemies (Soria & Gallotti, 1986; Foldi, 1997; Gullan & Kosztarab, 1997).

White cysts

White cysts were found from August to December, with a significant peak (11.3/vine) in November (Fig. 1) ($F = 5.501$; $df = 11, 60$; $P < 0.001$).

The white cyst population occurred during the highest rainfall period (from October to March), suggesting that adult formation and emergency may be favoured by moisture and warmth. According to Hoffman and Smith (1991), the emergence of adult *Margarodes meridionalis* is triggered by an increase in soil moisture. Cysts of *E. brasiliensis* increase considerably in size and reach approximately 7 mm in length 270 days after nymph eclosion, giving rise to females (Monteiro & Soria, 1996).

Mobile females

The mobile females emerged from white cysts and were present from August to December. Mobile females reached a significant population peak (3.1/vine) in August ($F = 4.006$; $df = 11, 60$; $P < 0.001$), followed by a gradual decrease in density, with small oscillations, until they finally disappear from December onwards (Fig. 1). Their gradual decrease in density from August onwards may partly reflect the fact that their life stages are highly susceptible to entomopathogens, which may increase in density during the highest rainfall period (October to March). Mobile adult females of *E. brasiliensis* are susceptible to the nematode *Steinernema carpocapsae* (Hickel & Schmitt, 1997) and to the fungus *Isaria fumosoroseus* (Carneiro *et al.*, 1994), with up to 100% mortality. In contrast, yellow cysts show low susceptibility or are resistant to these natural enemies (Soria & Gallotti, 1986; Foldi, 1997; Gullan & Kosztarab, 1997).

In South African vineyards, pre-pupal males of *Margarodes prieskaensis* develop from cysts during April and burrow up through the soil, where they moult and pupate 20 to 30 mm beneath the soil surface (De Klerk, 2010; De Klerk *et al.*, 2011). The pupae moult into winged male adults and appear above ground from June to August. From the beginning of June until August, adult females emerge from the mature cysts and burrow directly to the soil surface. After mating, the males die and the females burrow down into the soil to deposit their eggs in the vicinity of the roots.

As shown here, the emergence of mobile females of *E. brasiliensis* starts two to three months after the emergence of mobile females of *M. prieskaensis* in South Africa, but lasts for five months (August to December), the same length of time required for the total time of emerging of mobile females of the South African ground pearl (May to September). Surveys of *M. prieskaensis* in 2008 showed an earlier and higher population of pre-pupal males compared to adult females (*i.e.* $> 108/m^2$ versus $> 20/m^2$ in June) (De Klerk, 2011). However, Foldi (1990) noted that in Veranópolis, Rio Grande do Sul, Brazil, pre-pupal males of *E. brasiliensis* emerged in September/October, whereas pupae occurred in October/November and adult winged males in November/December. In the present study, no male pupae or winged adults were found on the soil surface, leading to the assumption that male pre-pupae did not occur or occurred in very low populations in the experimental site in the year of surveying. Hickel *et al.* (2009) and Botton *et al.* (2000) also mentioned that adult males have not been found in vineyards of Southern Brazil. Future studies on *E. brasiliensis* are necessary to investigate the conditions required for the development of males.

The sexual stages may, however, be less important for reproduction because of their low population density compared to immobile parthenogenetic females; in addition, no mobile adult females were observed depositing their eggs in the survey area, whereas almost all immobile adult females were found depositing eggs. These findings are in agreement with those obtained by Hickel *et al.* (2009), who surveyed *E. brasiliensis* populations in vineyard areas and found only 16% of the mobile female stage among all other stages.

Immobile females

Immobile or encysted females within the ruptured white cysts (parthenogenetic females) were found between August and April (Fig. 1), with a significant population peak (5.5/vine) in November ($F = 3.431$; $df = 11, 60$; $P = 0.001$), when the incidence of mobile females was already low.

Parthenogenesis allows *E. brasiliensis* to reproduce within the cyst and to complete its entire development in the soil, with no need for males (Foldi, 2005). The predominance of immobile females during the greater part of the adult period makes parthenogenetic females more important for reproduction than mobile adult females, with an oviposition rate varying from 278 to 319 (Soria & Gallotti, 1986, Haji *et al.*, 2004). Furthermore, the protection of immobile females within the cyst excludes this adult stage as a potential target of chemical control.

Mobile nymphs

Mobile nymphs (first instar nymphs) were found between August and April, with a significant population peak (44.2/vine) in December (Fig. 1) ($F = 6.665$; $df = 11, 60$; $P < 0.001$). The significant increase in the nymphal population resulted from a previous significant increase in the population of parthenogenetic (immobile) females in October and November; these females had laid a large number of eggs inside the cysts. Similar results were obtained in the Brazilian states of Santa Catarina (Haji *et al.*, 2004) and Rio Grande do Sul (Botton *et al.*, 2004), where nymphs prevailed from November to February and from November to March respectively. Nymph eclosion also peaked in the highest rainfall period (October to March), suggesting that mobile nymphs may also be favoured by moisture, which allows individuals to overcome desiccation and ensures that the soil is soft enough for underground migration.

First instar nymphs showed the highest population peak, *i.e.* 44/vine, twice the density observed for the yellow cyst peak (20.2/vine). This difference suggests that some insects had died or dispersed during development from nymphs to yellow cysts. High mortality of first instar nymphs was also pointed out by Hickel *et al.* (2009) as the main reason for the population decrease of *E. brasiliensis* during the development of the nymphal stage observed in the field.

The underground stages of *E. brasiliensis* most vulnerable to chemical control are the first instar nymphs and mobile sexual stages (adult females and pre-pupal or winged adult males), primarily because they are not protected by a cyst wall. Mobile adult females and pre-pupal males are also a vulnerable target for chemical insecticides, because they migrate towards and remain close to the soil surface. Good chemical control of these stages of *M. prieskaensis* was obtained on vines in South Africa (De Klerk, 2010). Since the mobile sexual stages of *E. brasiliensis* occur in lower numbers than that of immobile females, and therefore are a less important target, it is reasonable to conclude that first instar nymphs are the best target for effective pest control.

In general, the population densities of the different stages fluctuated during the observation period (August 2008 to August 2009), in a manner similar to that described by Soria and Dal Conte (2005) for southern Brazil. In our data, however, mobile females, eggs (in the white cysts) and

first instar nymphs appeared earlier in the season (August), in contrast to the findings of Soria and Dal Conte (2005), who recorded the appearance of these stages in October, December and February. These differences indicate that the time of occurrence of peaks varies depending on the region and on environmental aspects, which may fluctuate annually.

Furthermore, our observations of the life cycle of the margarodes agree with those of Foldi (2005), *i.e.* reproduction is primarily parthenogenetic and the yellow cyst stage lasts longer than all other stages and is less vulnerable to chemical control because of the protective cyst.

Distribution on roots

Only yellow cysts (after the first moult) were found on

grapevine roots in the two fields surveyed. The number of cysts varied significantly along the cross-sections of the horizontal and vertical counting intervals (Tables 1 and 2).

The vertical survey in field 1 (Table 1) showed two peaks of cyst densities, one at 10 to 15 cm (mean: 17.24 cysts) and another at 40 to 45 cm (mean: 22.71 cysts), with no significant difference between them ($F = 6,108$; $df = 10, 220$; $P < 0.430$). These two peaks of yellow cyst densities were explained by the finding that these two depths had the highest concentrations of roots, as confirmed by the positive correlation between the number of cysts and the number of roots ($r = 0.603$; $P = 0.04$). Thus, margarodes colonise the vine according to the distribution of the roots, with a tendency to cluster at sites with a higher number of roots because these

TABLE 1

Vertical distribution of *Eurhizococcus brasiliensis* (yellow cysts) in two vineyards with different rootstocks in the region of Louveira, SP, Brazil.

Soil depth (cm)	Field 1 = Rootstock IAC 766			Field 2 = Rootstock Riparia do Traviú		
	Average number of roots per vine	Average number of cysts per vine \pm SE		Average number of roots per vine	Average number of cysts per vine \pm SE	
0-5	0.81	0.24 \pm 0.17	cd	1.64	2.64 \pm 1.69	cd
5-10	1.77	5.38 \pm 1.92	abcd	2.07	10.86 \pm 2.72	ab
10-15	1.90	17.24 \pm 4.04	a	2.43	15.43 \pm 4.35	a
15-20	1.57	15.43 \pm 5.72	ab	1.07	5.43 \pm 1.99	abc
20-25	1.55	11.33 \pm 5.01	abc	0.64	4.14 \pm 1.85	bcd
25-30	1.23	6.00 \pm 2.06	abcd	1.14	4.71 \pm 2.84	cd
30-35	0.95	3.76 \pm 2.89	bcd	2.71	1.43 \pm 0.54	cd
35-40	3.43	3.19 \pm 1.25	bcd	2.79	1.36 \pm 0.63	cd
40-45	4.57	22.71 \pm 10.48	abcd	1.21	0	d
45-50	1.76	0.67 \pm 0.62	cd	0.00	0	d
> 50	0	0	d	0.07	0	d
Total	19.54	85.95		15.79	46.00	

Means followed by the same letter within a row did not differ significantly (ANOVA followed by the Tukey test, $P < 0.05$).

The data were $\text{Log} \sqrt{x+1}$ transformed prior to analysis. SE = standard error.

TABLE 2

Horizontal distribution of *Eurhizococcus brasiliensis* (yellow cysts) from the trunk below ground of two vineyards with different rootstocks in the region of Louveira, SP, Brazil.

Distances from the trunk (cm)	Average number of cysts per vine \pm SE			
	Field 1 = Rootstock IAC 766		Field 2 = Rootstock Riparia do Traviú	
Cysts on the trunk	46.76 \pm 14.75	a	28.00 \pm 5.90	a
0-5	26.14 \pm 5.21	a	12.50 \pm 2.79	b
5-10	11.62 \pm 3.21	b	2.36 \pm 0.78	c
10-15	0.90 \pm 0.45	c	2.00 \pm 0.78	c
15-20	0.43 \pm 0.38	c	1.00 \pm 0.54	c
20-25	0.10 \pm 0.07	c	0	c
25-30	0	c	0	c
30-35	0	c	0	c
35-40	0	c	0.14 \pm 0.11	c
> 40	0	c	0	c
Total	85.95		46.00	

Means followed by the same letter in the rows did not differ significantly (ANOVA followed by the Tukey test, $P < 0.05$).

The data were $\text{Log} \sqrt{x+1}$ transformed prior to analysis. SE = standard error.

roots provide more lodging and food. Furthermore, clusters of cysts were commonly found at forks between the trunk below ground level and the roots, or in bark crevices, which provided greater protection for the ground pearl, as also observed by Haji *et al.* (2004).

In field 2 (Table 1), the vertical survey showed only one peak at 10 to 15 cm (15.4 cysts), which differed significantly from the lowest densities ($F = 6.348$; $df = 8, 117$; $P < 0.001$). However, a slightly higher number of roots were found below this depth, suggesting that other factors, such as root condition, may also influence *E. brasiliensis* clustering on the trunk or roots.

Cysts were found mostly at a depth of 0 to 25 cm (57%) in field 1, and at 0 to 15 cm (63%) in field 2 (Table 1). No cyst were found at depths > 50 cm in field 1 and > 40 cm in field 2. This absence of cysts corresponds with the average length of the trunk below ground, *i.e.* 42 cm in field 1 (range: 28 to 49 cm) and 40 cm in field 2 (range: 25 to 71 cm), and with the absence of roots below these depths (Table 1). The rootstocks in many vine-growing areas of Louveira region, Brazil, are very long and planted very deep in the soil.

Hickel *et al.* (2009) surveyed *E. brasiliensis* in "Meio-Oeste Catarinense" and found 65% to 80% of the population concentrated in the zero to 20 cm depth. Figueiredo (1970) stated that *E. brasiliensis* occurred at depths of up to 30 cm, whereas Soria and Dal Conte (2005) reported that this species was found mainly at depths of 5 to 30 cm. According to Haji *et al.* (2004), some cysts were also found at a depth of 1 m. Barnes *et al.* (1969) found an increasing concentration of *Margarodes vitis* cysts up to 1,14 m deep in the soil of a 36-year-old vineyard and concluded that cyst dispersal paralleled root distribution.

Horizontal surveys conducted laterally from the trunks below ground (Table 2) showed the highest cyst densities on the trunks of plants in fields 1 and 2 (46.8 and 28.0 cysts respectively), followed by the 0 to 5 cm interval on the roots (26.1 and 12.5 cysts respectively), with a significant difference between these two sites for field 2 ($F = 33.826$; $df = 9, 240$; $P < 0.001$), but not for field 1 ($F = 34.845$; $df = 9, 200$; $P < 0.001$). These two sites differed significantly from the other horizontal intervals that all showed very low cyst densities.

Thus, cysts occurred mostly on the trunks below ground of vines in both fields (54% in field 1 and 61% in field 2). More than 80% of the population was included in the 5 cm width around the stem, whereas almost all cysts occurred in the 20 cm width around the stem.

Other studies have reported different horizontal distributions for *E. brasiliensis* on roots. Haji *et al.* (2004) reported that, in a vineyard just a few years old, *E. brasiliensis* mostly colonised the trunk below ground and its proximities. On the other hand, in a 10-year-old vineyard, the ground pearl were found mostly on the roots, 80 cm from the trunk at a depth of 20 cm. This implies that margarodes can colonise the roots further away from the trunk and to deeper levels in the soil as plants get older. Mobile females, male pre-pupae and particularly nymphs have specially adapted legs to easily crawl in the soil. According to Hickel (1994) and Botton *et al.* (2000), ants may also play a role by carrying the nymphs and small cysts underground to other areas on

the root system or to other vines.

An average infestation of 82 and 46 cysts/vine was observed for the 25 vines examined in fields 1 (IAC 766) and 2 (Riparia do Traviú) respectively. Despite the higher insect infestation observed for IAC 766, this rootstock may provide the vine with better survival and vigour compared to Riparia do Traviú when grown in soil infested with the ground pearl, as shown by Lourenção *et al.* (2002). Meanwhile, before concluding that IAC766 is more tolerant to the ground pearl, other studies should be done to investigate the susceptibility of these two rootstocks to other factors or agents, such as trunk diseases, which may also be in the field affecting the plants, alone or together with margarodes. According to Hickel *et al.* (2009), *E. brasiliensis* predisposes the plant to a complexity of disease, especially trunk diseases.

CONCLUSION

The study over one season gave a good indication of what development stages in the lifecycle of *E. brasiliensis* occur in the Louveira area, São Paulo, and at what time of the year. This information, as well as the results on the vertical and horizontal distribution of each development stage, are vital for introducing and managing effective chemical control. Although further observations during another one or two seasons are needed to verify these results, it is reasonable to conclude that the first instar nymphs would be the best target for effective control.

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