CROP PROTECTION

Feeding Site Preference of *Dilobopterus costalimai* Young and *Oncometopia facialis* (Signoret) (Hemiptera: Cicadellidae) on Citrus Plants

Rosangela C. Marucci[1], João R.S. Lopes[2], José D. Vendramim[1] e José E. Corrente[3]

[1]*Centro Universitário de Patos de Minas – UNIPAM, Faculdade de Ciências Agrárias – FACIAGRA, C. postal 485 38702-054, Patos de Minas, MG*
[2]*Depto. Entomologia, Fitopatologia e Zoologia Agrícola;* [3]*Depto. Ciências Exatas. ESALQ/USP, C. postal 9, 13.418-900, Piracicaba, SP*

Neotropical Entomology 33(6):759-768 (2004)

Preferência de *Dilobopterus costalimai* Young e *Oncometopia facialis* (Signoret) (Hemiptera: Cicadellidae) por Locais de Alimentação em Plantas Cítricas

RESUMO - A eficiência de transmissão de *Xylella fastidiosa* por cigarrinhas em citros é baixa e variável com a espécie vetora. O comportamento alimentar do vetor relacionado à aquisição e inoculação de *X. fastidiosa* é um dos prováveis fatores condicionantes da eficiência de transmissão. Assim, os objetivos desse trabalho foram avaliar o comportamento de duas espécies de cigarrinhas, *Dilobopterus costalimai* Young e *Oncometopia facialis* (Signoret), na seleção de mudas cítricas e de locais de alimentação e verificar a influência das brotações na atratividade aos vetores. Realizaram-se testes de livre escolha em câmaras de observação (63 x 63 x 120 cm) com as duas espécies, liberando-se 40 indivíduos por câmara. Avaliou-se o número de insetos mortos e que escolheram ou não um dos tratamentos, após 3, 15, 21, 24, 39, 45 e 48h da liberação. Para *D. costalimai*, as folhas foram importantes no início da seleção hospedeira, sendo substituídas com o passar do tempo pelo ramo secundário. *D. costalimai* preferiu pousar na nervura secundária da folha, seguida pelo ramo secundário. Para *O. facialis* não houve diferença na preferência para pouso entre folhas (nervura central e secundária) e ramos secundários das mudas cítricas, sendo esta variável com o horário do dia. Comprovou-se que a presença de brotação nas mudas aumenta a atratividade das plantas para as duas espécies de cigarrinhas, elevando as chances de aquisição ou inoculação de *X. fastidiosa* pelos vetores. Assim, sugere-se adotar medidas de controle desses insetos em períodos de maior vegetação dos pomares de laranja.

PALAVRAS-CHAVE: *Citrus sinensis*, clorose variegada dos citros, cigarrinha vetora, seleção hospedeira

ABSTRACT - The efficiency of *Xylella fastidiosa* transmission by sharpshooters on citrus plants is low and varies with the vector species. The feeding behavior of the sharpshooter vector related to *X. fastidiosa* acquisition and inoculation is one of the probable conditioning factors in transmission efficiency. The objective of this study was to assess the behavior of two sharpshooter species, *Dilobopterus costalimai* Young and *Oncometopia facialis* (Signoret), in selection of citrus nursery trees and feeding places and to ascertain the influence of sprouting on plant attractiveness to the vectors. Free choice tests were performed with the two species in observation chambers (63 x 63 x 120 cm), releasing 40 individuals per chamber. The number of dead insects were assessed as well as those that chose, or not, one of the treatments, at 3, 15, 21, 24, 39, 45 and 48h after the release. For *D. costalimai* the leaves were important at the start of host selection, and were substituted over time by the secondary branch. *D. costalimai* preferred to stay on the secondary leaf nervures followed by the secondary branch. *O. facialis* showed no difference in preference for staying on the leaves (central and secondary nervures) and secondary branch on citrus plants, and this varied with the time of day. The presence of sprouting in the nursery trees increased the attractiveness of the plant to the two sharpshooter species raising the chances of *X. fastidiosa* acquisition or inoculation. So, control measures should be adopted against these insects in periods of greater vegetation in orange orchards.

KEY WORDS: *Citrus sinensis*, citrus variegated chlorosis, sharpshooter vector, host selection

Hemiptera feeding is intriguing regarding plant pathogen transmission. Unfortunately, feeding studies involving sharpshooters are particularly difficult because the penetration of the mouth apparatus and subsequent salivation and ingestion occur inside the plant tissue (Triplehorn *et al.* 1984). The stylets are certainly important in host and feeding site selection because they receive feeding stimuli and are the means of pathogen acquisition and inoculation by the leafhopper vector species (Forbes & Raine 1973).

In addition to specialization in certain host species, the Hemiptera have specialized in certain parts of the plant and plant tissues for feeding (Backus 1988). They exploit the phloem, xylem, mesophyll, and all the three tissues depending on the species (Backus 1985). Sharpshooters probably vary their preference in function of the physiological and phenological stage of the plants and/or the microclimatic characteristics of the habitat (Lopes 1999).

Xylella fastidiosa is a bacteria limited to the xylem vessels in the plants. It causes diseases in various fruit plants, and can infect plants without symptom manifestation. In Brazil it is the pathogen of citrus variegated chlorosis (CVC) (Chang *et al.* 1993) and causes coffee tree atrophy (Paradela Filho *et al.* 1995) and plum leaf scald (French & Kitajima 1978). It is transmitted by grafting or sharpshooters, which are xylem-feeding leafhoppers (Hemiptera: Cicadellidae) of the Cicadellinae sub-family.

Sharpshooters feed on a wide diversity of host plants in attempts to meet the deficiencies of their extremely poor diet (Novotny & Wilson 1997). Furthermore, they do not destroy the cells inoculated with pathogen and promote inoculation success (Purcell 1985).

Although a requirement for *X. fastidiosa* transmission is feeding on the xylem, variable efficiency has been observed in pathogen transmission between the sharpshooters in citrus plants.

Several factors may determine the variations in *X. fastidiosa* transmission efficiency, such as low bacteria concentration in citrus (Almeida *et al.* 2001) and/or irregular distribution in the xylem bundles of the plants (Mizubuti *et al.* 1994), sharpshooter behavior patterns related to feeding and movement in orchards (Purcell 1985) and, perhaps, the low ingestion rates observed in citrus plants compared to other host plants of these insects (Milanez *et al.* 2003).

X. fastidiosa transmission efficiency depends on a set of variables, including acquisition and inoculation efficiency, the time spent by the sharpshooter vector in feeding on infective tissue, the rates of natural infectivity of the sharpshooter population and the survival of initial bacterial infections (Purcell 1981, Almeida *et al.* 2001).

The feeding behavior seems to be the first step in pathogen acquisition and the last in transmission to the plants (Purcell 1985). Thus, sharpshooter vector behavioral characteristics and feeding preferences are probably related to the variations in the transmission efficiency among species in the same plant host.

The objectives of this study were to determine the preferences of two sharpshooter vector species of *X. fastidiosa*, *Dilobopterus costalimai* Young and *Oncometopia facialis* (Signoret), in selection of the plant parts and permanency sites on citrus nursery trees, and ascertain the effect of sprouting on citrus attractiveness to these insects.

Material and Methods

D. costalimai and *O. facialis* sharpshooter behavior was studied in three free choice experiments in a greenhouse, using healthy 'Pêra' sweet orange [*Citrus sinensis* (L.) Osbeck] nursery trees budded on 'Rangpur' lime (*Citrus limonia* Osbeck), about 70 cm high and kept in plastic bags with *Pinus* substrates (Plantmax®). About 45 to 50 days before the experiments, the plant stem was pruned 10 cm above the graft region for secondary branch and sprout formation.

The experiments were carried out inside rectangular observation chambers measuring 63 x 63 cm at the base and 120 cm in height, with a wooden structure and transparent acrylic walls. The door was covered with small mesh screening, with a voile type cloth sleeve to transfer the insects and irrigate the plants. The insects used in the experiments were collected in citrus orchards in the Bebedouro (SP) region and kept in a greenhouse on sweet orange (*C. sinensis*), *Vernonia condensata* Baker and *Aloysia virgata* (Ruiz & Pavan) Juss.] plants until the tests were carried out. Forty non-sexed insects were released in the central portion of the chamber at the end of the afternoon (5 p.m.). The experiments were assessed for 48h at fixed time intervals: 3, 15, 21, 24, 39,45 and 48h after installation, which corresponded to the following times: 8 p.m., 8 a.m., 2 p.m., 5 p.m., 8 a.m., 2 p.m., and 5 p.m., respectively. At each assessment, the number of dead insects and those that had chosen, or not, one of the citrus nursery trees or one of the parts of the citrus plant was scored; the temperature inside the observation chamber was also recorded.

Selection on the Citrus Plants. The first experiment was carried out to identify the part of the citrus nursery trees that attracted the insects during host selection. A complete plant, a plant with leaves covered with transparent adherent plastic PVC type film and another with the secondary branches covered were placed in the same observation chamber. The secondary branch was considered to be that derived from the main stem above the graft region.

The leaves and branches were covered on the same day that the experiments were set up, 1h before releasing the insects.

Plants of uniform size and coloring were placed in the chambers without touching each other. The leaves and branches were covered with transparent plastic film because their removal might alter the plant physiology or permit volatile emissions, altering the insect behavior.

Selection of the Permanency Sites on the Citrus Plant. In the second experiment, a single citrus plant was placed in an observation chamber to determine the preferred permanence site of the sharpshooters among the petiole (P), central nervure (CN) or secondary leaf nervure (SN) and primary branch (PB) or secondary branch (SB) of the plants. The primary branch was considered the main stem formed by the

'Rangpur' lime rootstock and the secondary branch was that derived from the main stem, above the graft point.

Selection Between Sprouting and Non-Sprouting Citrus Plants. The third experiment was carried out to assess the role of sprouting in citrus attractiveness to sharpshooters by placing two orange tree nursery trees in the same chamber, one with and one without sprouting at the tip.

The plant shoots in the non-sprouting treatment were removed at the start of emission, about 15 days before in the experiments.

Experimental Design and Analysis of Preference Studies. A randomized block design was used, with 10 plots (one replication or observation chamber per plot) in a split plot design where the plots corresponded to the treatments and the split plots to the times. The collected data represented the count of the number of insects per treatment for each experiment. First, occurrence of over dispersion was analyzed using the Oswald routine with the S-Plus software (Statistical Science 1993). As there were no over-dispersed data, a Poisson distribution was assumed and a log (x + 1) transformation was used to proceed to the analysis of variance using the SAS Institute program (1996). Thus according to the analysis design in measurements repeated in time, when there was no significant treatment with time interaction, only the Tukey test was performed to ($P < 0.05$), comparing the mean number of insects per treatment in the seven assessment periods. If to the contrary, regression curves were also obtained for each treatment for the various time interactions.

Joint analysis was also carried out, followed by multiple comparison tests between the two sharpshooter species in each experiment.

Results

Shortly after release the insects remained on the sides of the observation chamber. Landing on the citric plant occurred gradually during the first 24h (Figs. 1A, C and E). In the first two experiments, the number of *D. costalimai* adults that remained on the chamber sides was higher compared to *O. facialis* (Figs. 1A and C). However, in the case of the sprouting and non-sprouting plants the number of insects of the *O. facialis* species that remained on the sides of the chamber was more accentuated (Fig. 1E).

Death was observed mainly starting 15h after insect release, but declining after 24h (Figs. 1B, D and F). In experiment 2, *D. costalimai* continued with accentuated mortality up to 39h after insect release.

The mean temperature inside the observation chamber oscillated over time in the three experiments (Figs. 2A and B), and may have influenced insect death, movement and localization in the chamber.

Selection on the Citrus Plant. There was no significant difference between the complete plant, plants with covered leaves or plants with the secondary branches covered for the mean number of *D. costalimai* ($F = 3.26$; $P = 0.052$) and *O. facialis* ($F = 0.30$; $P = 0.75$) adults (Table 1), considering the seven assessment times. For *D. costalimai* there was treatment x time interaction ($F = 2.28$; $P = 0.01$) and significant regression for the three treatments. In the first assessments the complete plant ($F = 10.71$; $P = 0.0017$; $R^2 = 0.65$) and the plant with covered branches ($F = 10.97$; $P = 0.0015$; $R^2 = 0.71$) presented a greater number of insects (Fig. 3), indicating that the leaves guide the start of host selection. With time, especially after 39h, there was an increase in the number of insects on the plant with covered leaves ($F = 6.98$; $P = 0.0089$; $R^2 = 0.82$), showing preference for the branch compared to the leaves. There was no treatment x time interference for *O. facialis* ($F = 1.55$; $P = 0.111$) so the number of insects in the three treatments could not be compared by the assessment times.

When the two sharpshooter species were compared ($F = 52.76$; $P = 0.00001$), a greater mean number of *O. facialis* (7.5 ± 0.38) than *D. costalimai* (2.0 ± 0.24) were detected in the treatments. This difference was expected because the latter presented a higher mean number of insects on the chamber sides and more accentuated mortality than *O. facialis* (Figs. 1A and B).

Selection of the Permanency Sites on the Citrus Plant. *D. costalimai* ($F = 51.56$; $P = 0.0001$) presented a greater preference for the secondary leaf nervures (SN) followed by the secondary branch (SB). The other parts of the plant, petiole (P), central leaf nervure (CN) and the primary branch (PB) did not differ (Table 2). Significant interaction was observed among treatments and time ($F = 6.82$; $P = 0.00001$) and the analyses of regression showed that the preference for NS prevailed in all the assessments ($F = 22.70$; $P = 0.00004$); $R^2 = 0.58$), decreasing as the preference for SB increased ($F = 49.83$; $P = 0.00001$; $R^2 = 0.33$) (Fig. 4A). The preference for the petiole ($F = 4.82$; $P = 0.027$; $R^2 = 0.95$) and for leaf CN ($F = 3.97$; $P = 0.044$; $R^2 = 0.39$) remained low during the assessments, and the regression curves for P and CN were, respectively, $Y = -0{,}0005x^2 + 0{,}027x – 0{,}076$; $R^2 = 0{,}95$ e $Y = -0{,}0054x + 0{,}43$; $R^2 = 0{,}38$.

For *O. facialis* ($F = 19.77$; $P = 0.00001$) there was no difference regarding the preference for SN, CN and SB, but P and PB were less preferred than the other sites and did not differ (Table 2). The treatment x time interaction was significant ($F = 6.6$; $P = 0.00001$) and the number of insects decreased with time on SN ($F = 48.31$; $P = 0.0001$; $R^2 = 0.43$) and increased on RS ($F = 15.91$; $P = 0.00025$; $R^2 = 0.78$), and became equal in the last assessments (Fig. 4B). Nothing can be stated regarding the number of insects on P and CN because the regression curves were not significant for the two parameters.

When the percentages of *D. costalimai* and *O. facialis* on the leaves, petioles and branches were analyzed, its presence on the leaf petiole was small and stable during the assessment times (Figs. 5A and B). In contrast, the percentages of insects on the leaves and branches alternated according to the time of day. There was a tendency for greater sharpshooter concentration on the leaves in the periods with lower temperatures (5 p.m., 8 p.m., and 8 a.m.) (Figs. 2A and B), and for the individuals to move to the branches with increased temperature at the 2 p.m. assessment.

Comparative analysis between the two species again

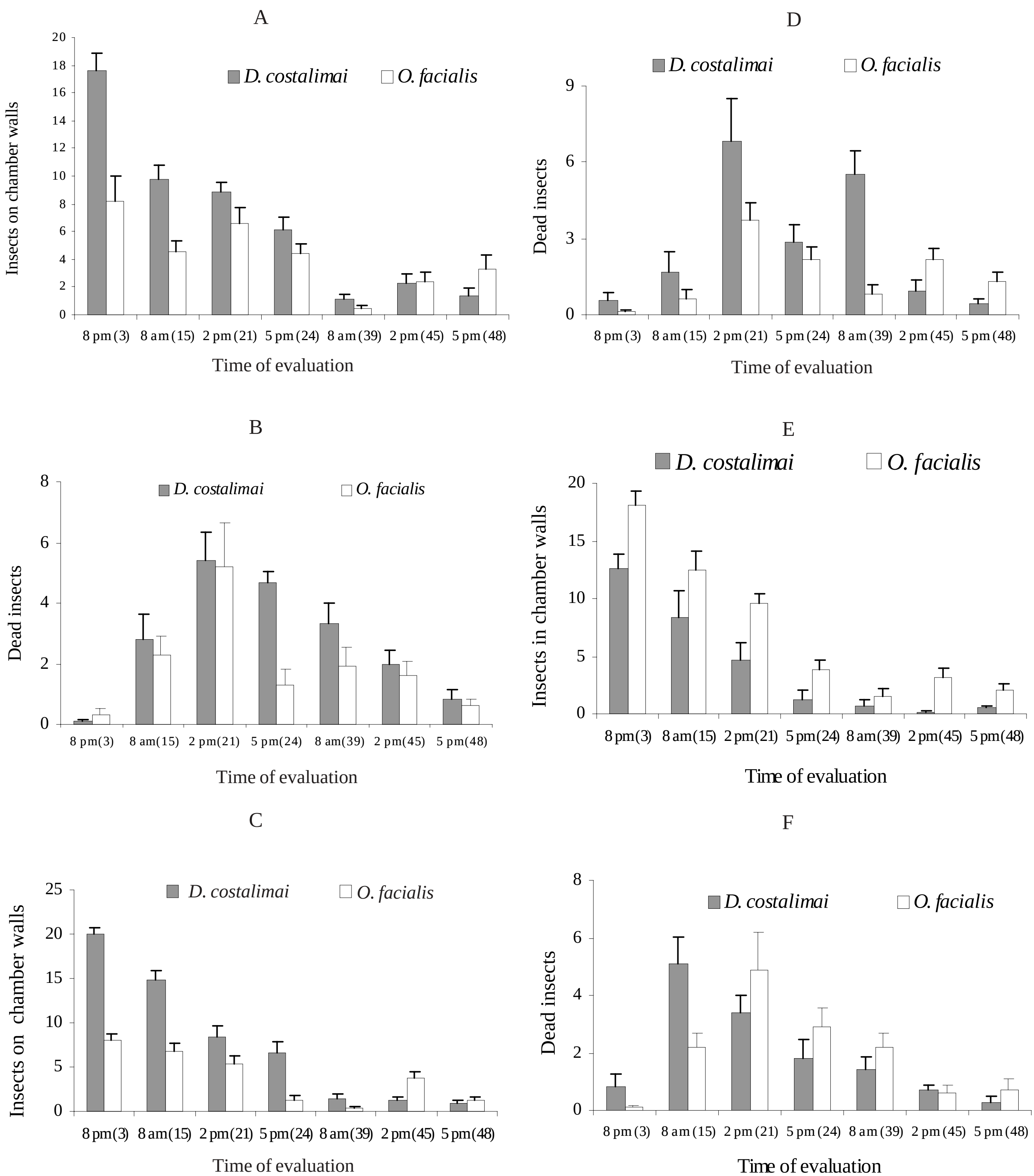
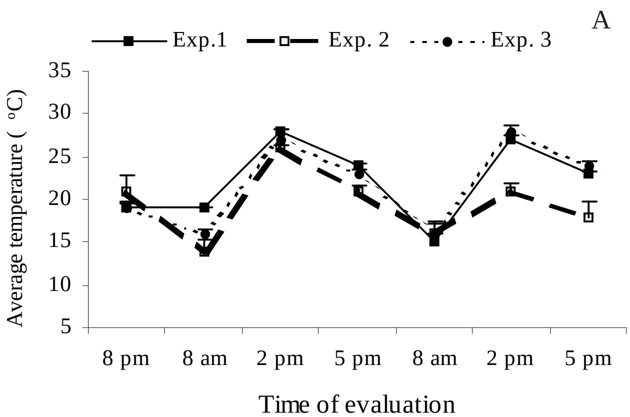


Figure 1. Mean number (± SEM) of *D. costalimai* and *O. facialis* adults that remained on the sides of the observation chamber (A, C, E) and dead (B, D, F) in three free choice experiments. Experiment 1 (A and B): selection of whole plant, plant with covered leaves and plant with covered secondary branches; experiment 2 (C and D): selection of petiole, central and secondary leaf nervures, primary and secondary branches; experiment 3 (E and F): selection between sprouting and non-sprouting plants, during the seven assessment periods. Numbers between parentheses corresponds to time (h) after insect release.



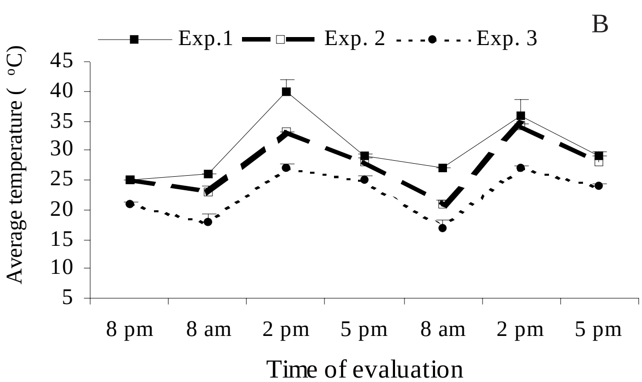


Figure 2. Mean temperature (± SEM) in the observation chamber at each assessment time of the three experiments with *D. costalimai* (A) and *O. facialis* (B). Experiment 1: selection of whole plant, plant with covered leaves and plant with covered secondary branch; experiment 2: selection of petiole, central and secondary leaf nervures, primary and secondary branches; experiment 3: selection between sprouting and non-sprouting plants.

showed a greater mean number of *O. facialis* (1.63 ± 0.16) compared to *D. costalimai* (1.3 ± 0.13) in the different plant sites (F = 6.56; P = 0.011), probably resulting from the lower mortality and smaller number of insects in the first species that stayed on the observation chamber sides (Figs. 1C and D).

Selection Between Sprouting and Non-Sprouting Citrus Plants. Both *D. costalimai* (F = 22.34; P = 0.00033) and *O. facialis* (F = 37.41; P = 0.00005) showed clear preference for the plants with sprouting (Fig. 6). The treatment x time interaction was not significant (F = 2.17; P = 0.051) for *D. costalimai* and did not permit comparison of the number of insects on the sprouting and non-sprouting plants in relation to the assessment times. The treatment x time interaction was significant for *O. facialis* (F = 9.16; P = 0.00001); 3h after insect release there was no difference in attractiveness between the two plants, but after 15h reference increased for the sprouting plant (F = 5.22; P = 0.023; R^2 = 0.95) with corresponding reduction for the non-sprouting plant (F = 17.86; P = 0.00017; R^2 = 0.55) (Fig. 7).

Table 1. Mean number (±SEM) of *D. costalimai* and *O. facialis* adults in a free choice test among citrus plants with total or partial exposure of their structures.

Treatment	*D. costalimai*	*O. facialis*
Complet plant	2.6 ± 0.34	8.7 ± 0.62
Plant with covered leaves	2.3 ± 0.37	6.4 ± 0.47
Plant with secondary branches covered	1.3 ± 0.18	7.3 ± 0.79

The means were not significantly different according to ANOVA.

The comparative analysis of these experiments showed a greater number of *D. costalimai* (6.4 ± 0.39) than *O. facialis* (5.7 ± 0.34) on the plants (F = 4.23; P = 0.044) as a higher number of insects in the latter species remained on the sides of the chamber at practically all the assessment times (Figs. 1E and F). This finding indicates the influence that sprouting plays on host localization for *D. costalimai*, because in the other two experiments few individuals of this species landed on the plant compared to *O. facialis*, in addition to having recorded greater mortality.

Discussion

This research showed that two of the main *X. fastidiosa* sharpshooter vectors in citrus plants, *D. costalimai* and *O. facialis*, present preferential permanency sites on citrus plants which can vary during the day or over time of plant exposure to the insects. Even when there was a highly significant treatment x time interaction, the coefficient of determination R^2 was low (0.3-0.5) for some regression curves, which was expected because of the larger movements of the insects in the observation chamber at certain times of day. Even so, these data are useful because they show oscillations regarding the feeding preference of these insects over time. Variation in the feeding or permanence sites of the insects may be related to possible diurnal alterations in the sap nutrient concentration in certain plant structures (Brodbeck *et al.* 1993) or with microclimatic variations in the citrus tree canopy (Leite & Nakano 2000).

The feeding preferences of the sharpshooters that feed on xylem sap are largely determined by differences in the nutrient content among the plant species (Brodbeck *et al.* 1990, Thompson 1994) and plant parts (Horsfield 1977), and

Table 2. Mean number (± SEM) of *D. costalimai* and *O. facialis* adults in a free choice test among citrus plant parts.

Treatment	*D. costalimai*	*O. facialis*
Petiole	0.3 ± 0.06 c	0.4 ± 0.11 b
Central leaf nervure	0.5 ± 0.11 c	2.2 ± 0.23 a
Secondary leaf nervure	4.1 ± 0.52 a	3.1 ± 0.71 a
Primary branch	0.01 ± 0.01 c	0.09 ± 0.04 b
Secondary branch	1.6 ± 0.052 b	2.3 ± 0.45 a

Means followed by the same letter in the column do not differ by the Tukey test (P < 0.05).

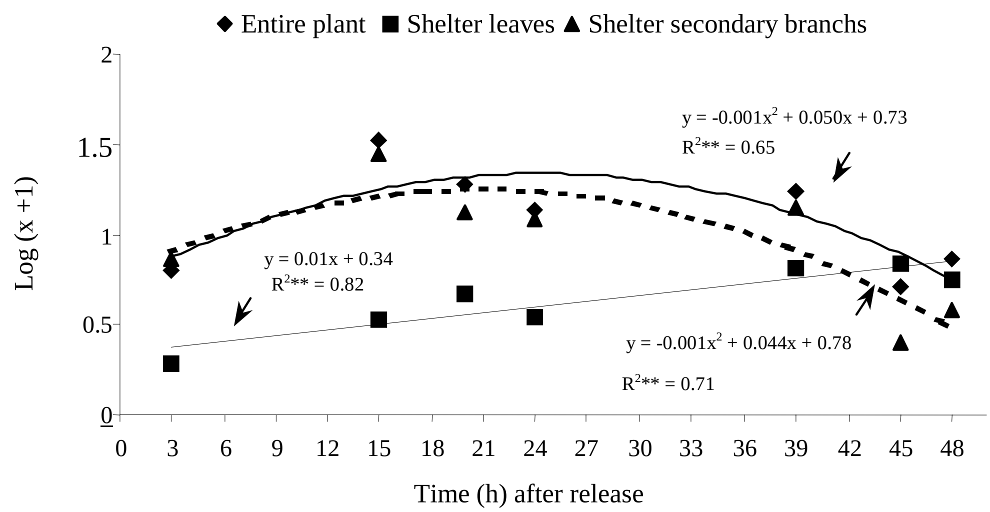
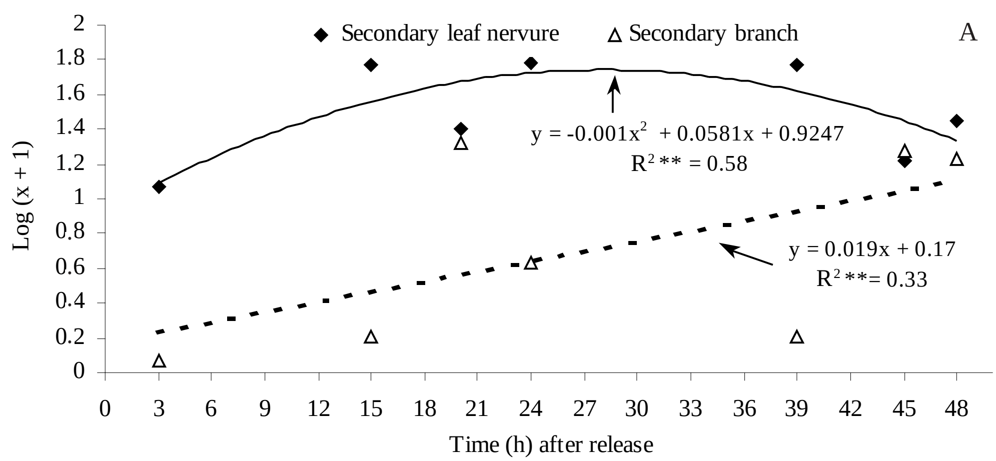


Figure 3. Relation between mean number (x) of *D. costalimai* adults per plant and time after release, in a free choice test involving three categories of citrus plants: whole plant; plant with covered leaves and plant with covered secondary branches.



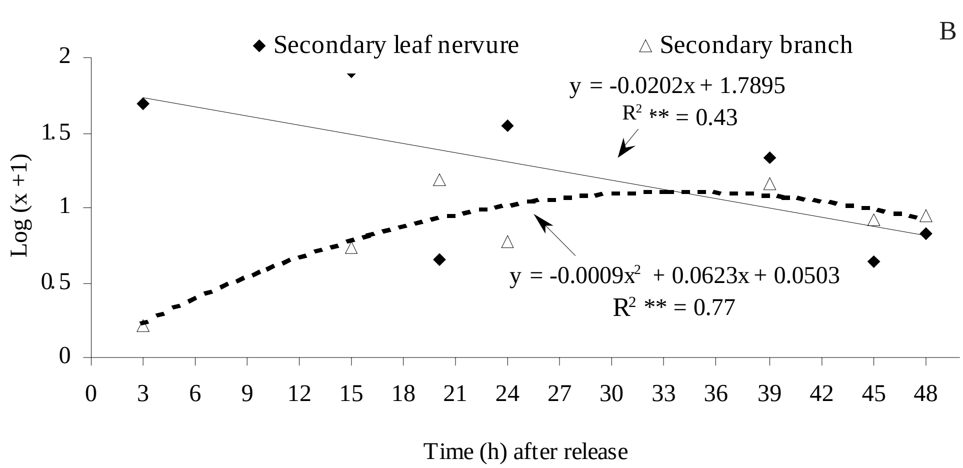


Figure 4. Relation between the mean number (x) of *D. costalimai* (A) and *O. facialis* (B) adults on different parts of the citrus nursery trees and time after release, in a free choice test.

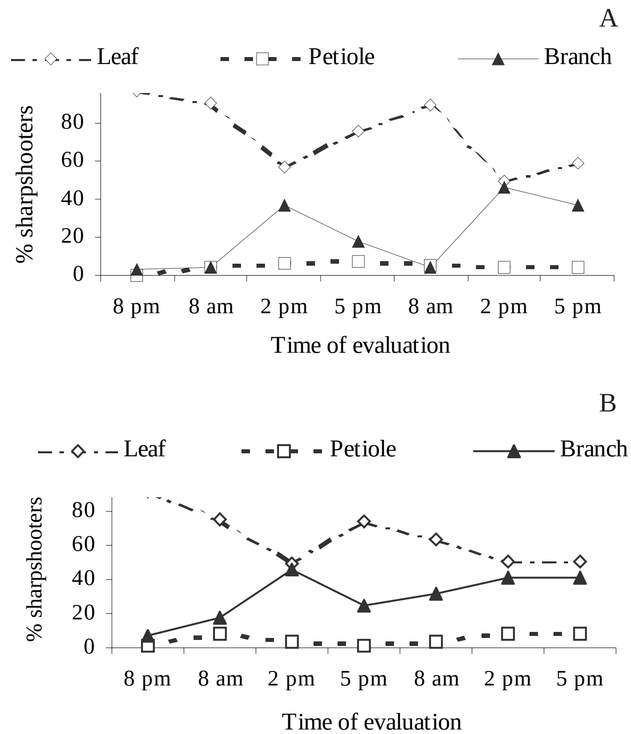


Figure 5. *D. costalimai* (A) and *O. facialis* (B) adult distribution on citrus plant leaves, petioles and branches at the seven assessment times in a free choice test.

also adjust to the daily changes in the xylem chemistry (Brodbeck *et al.* 1993). It should be taken into consideration therefore that the quantity and composition of the xylem sap nutrients vary with age, season, time of day, feeding site and health of the plant (Andersen & Brodbeck 1989, Moreno & Garcia Martínez 1993, Hopkins 1995).

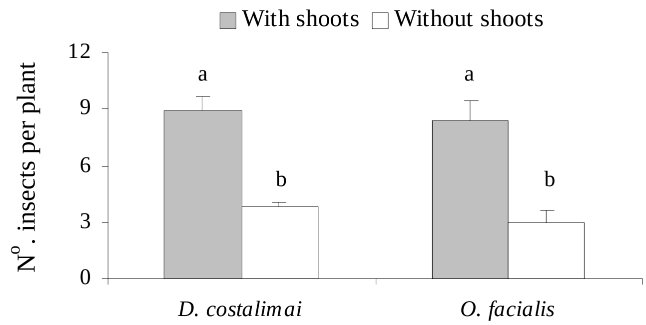


Figure 6. Mean number (± SEM) of *D. costalimai* and *O. facialis* adults per plant in a free choice test between sprouting and non-sprouting citrus plants, in seven assessment periods. For the same species means with the same letter do not differ by the Tukey test ($P < 0.05$).

The choice of the preferred feeding site among the leaves, branches and petioles, varies among the sharpshooter species (Gravena *et al.* 1997). According to Leite & Nakano (2000), *D. costalimai* prefers new branches and young leaves while *O. facialis* prefers a wider variety of plant organs. However, the results obtained in the present study, under the more restricted environmental conditions of a greenhouse, showed similar food preferences between the two sharpshooters species, and *D. costalimai* showed an initial preference for leaves compared to the other parts of the citrus plant.

The analysis of the feeding site of the sharpshooter vectors on the plant (branches, twigs or leaves) is relevant for *X. fastidiosa* transmission studies, because it can affect the efficiency with which the different sharpshooter species acquire or inoculate the bacteria (Lopes 1996). In grapevines with Pierce's disease, the bacterial concentration expressed in percentage of colonized vessels is lower in the branches

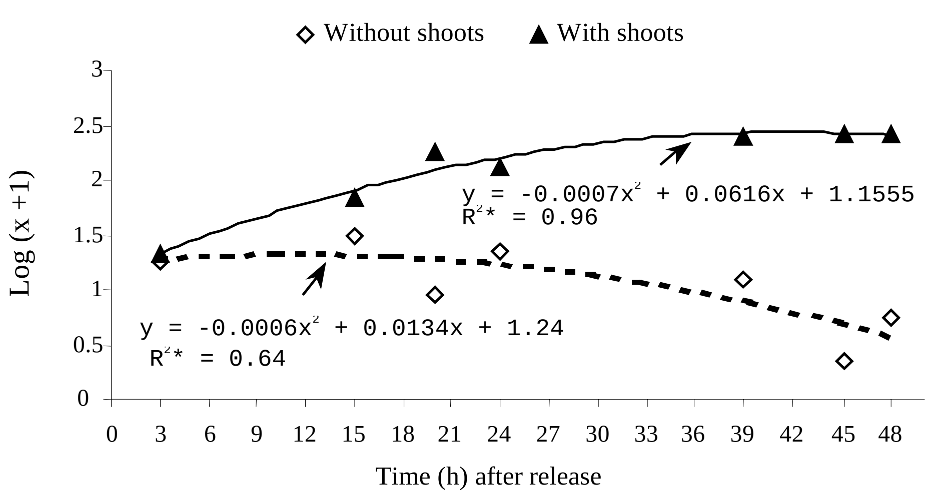


Figure 7. Relation between mean number (x) of *O. facialis* adults per plant and time after release, in a free choice test between sprouting and non-sprouting citrus plants.

than in the leaves (Hopkins 1981). Furthermore, in the leaves with symptoms of edge necrosis, there is a greater percentage of vessels containing bacteria in the leaf nervure than in the petioles. The bacteria are confined to the xylem vessel lumen, more frequently in the small vessels, where they form clusters that apparently restrict water passage (Mollenhauer & Hopkins 1974). Thus it can be considered that in vines there are greater chances for *X. fastid* acquisition during feeding on leaf nervures.

In citrus plants with CVC, vessel obstruction decreases from the leaves to the stem (Queiroz-Voltan & Paradela Filho 1999). Thus it is postulated that *X. fastidiosa* acquisition efficiency may be determined by the preferential feeding site of the sharpshooter vector on the infected plant. Although the two sharpshooter species assessed in this study are similar regarding preference for citrus plant parts, a tendency was observed in the *D. costalimai* individuals to remain longer on the leaves (Figs. 4A and 5A), which showed higher rates of vessels colonized by *X. fastidiosa*. This difference in feeding behavior may increase the chance of pathogen acquisition by *D. costalimai*, which might explain, at least partially, the greater efficiency of this sharpshooter vector compared to *O. facialis*, reported by Marucci (2003). It would be interesting to investigate the rate of vessels colonized by *X. fastidiosa* in the secondary nervure of citrus leaves, which is the preferential permanency (and probably feeding) site of *D. costalimai* but, little assessed in previous microscopy studies.

Preference for sprouting may also influence the transmission eficiency by the sharpshooter vectors. In vines, the older leaves of many cultivars present lower indices of *X. fastidiosa* infection after inoculation by sharpshooters than the younger leaves (Hopkins 1983). The higher infection rate may be related to the amino acid concentration that is greater in the xylem of young leaves than in the older leaves (Purcell 1989). In vines, sharpshooters have a strong preference for succulent tissues (Hewitt *et al.* 1949, Purcell 1975), and this is more pronounced in the recently emaciated leaves and branches at the end of spring and beginning of summer (Purcell & Feil 2001).

The present study showed sharpshooter preference for citrus plants that present sprouting. Roberto & Yamamoto (1998) reported that the sharpshooter population fluctuation in orchards is influenced by climate, and in years with more prolonged drought after winter, late sharpshooter infestation is observed, probably because of the interference of the drought on the host, delaying the start of sprouting in the citrus plants. Thus water deficiency in non-irrigated areas results in slow sprout formation by citrus trees that may affect the population and the sharpshooter preference (Garcia Junior *et al.* 1997).

Management of the diseases caused by vetor-borne pathogens can be facilitated by knowledge of the period in which control tactics should be adopted for a greater effect on the vector-pathogen-host plant interaction. These tactics can include the use of insecticides that affect the sharpshooter vectors before they can inoculate a healthy plant or acquire the bacteria from an infected plant (Blua *et al.* 2001). Thus, the observed sharpshooter preference for citrus sprouting has important implications for CVC management, and control measures against these insects should be adopted in periods of greater vegetation in orange orchards, which are probably critical for *X. fastidiosa* acquisition and transmission.

Acknowledgments

The authors thank researcher Ivani Pozar Otsuk, of the Instituto de Zootecnia de Nova Odessa, for help in the statistical analyses; CAPES for the scholarship to the first author and to FAPESP and FUNDECITRUS, for funding the experiment execution. This study was taken from the doctorate theses of the first author, and developed at the Departamento de Entomologia , Fitopatologia e Zoologia Agrícola - ESALQ/ USP, Piracicaba, SP.

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Received 12/11/03. Accepted 12/06/04.