Influence of *Xylella fastidiosa* infection of citrus on host selection by leafhopper vectors

R.C. Marucci[1], J.R.S. Lopes[2], J.D. Vendramim[2] & J.E. Corrente[3]

[1]Faculdade de Ciências Agrárias – FACIAGRA/UNIPAM, CP. 485, Patos de Minas, MG 38702-054, Brazil; [2]Departamento de Entomologia, Fitopatologia e Zoologia Agrícola, ESALQ/Universidade de São Paulo, CP. 9, Piracicaba, SP 13418-900, Brazil; [3]Departamento de Ciências Exatas, ESALQ/Universidade de São Paulo, CP. 9, Piracicaba, SP 13418-900, Brazil

Abstract

Infection of plants by pathogens can influence their attractiveness and suitability to insect vectors and other herbivores. Here we examined the effects of *Citrus sinensis* (L.) Osbeck (Rutaceae) infection by the bacterium *Xylella fastidiosa*, which causes citrus variegated chlorosis (CVC), on the feeding preferences of two sharpshooter vectors, *Dilobopterus costalimai* Young and *Oncometopia facialis* (Signoret) (Homoptera: Cicadellidae). Experiments were performed inside observation chambers, in which a healthy plant and an infected one (with or without CVC symptoms) were supplied to a group of 40 sharpshooters. The number of insects that selected each treatment was recorded at several time intervals in 48 h. In another experiment, the ingestion rate on healthy and infected (symptomatic or not) plants was evaluated by measuring the liquid excretion of sharpshooters that were confined on branches of each plant for 72 h. Both sharpshooter species preferred healthy plants to those with CVC symptoms. However, *O. facialis* did not discriminate between healthy citrus and symptomless infected plants. Feeding by *D. costalimai* was markedly reduced when confined on CVC-symptomatic plants, but not on asymptomatic infected ones. The ingestion rate by *O. facialis* was not affected by the presence of CVC symptoms. The results suggest that citrus trees with early (asymptomatic) infections by *X. fastidiosa* may be more effective as inoculum sources for CVC spread by insect vectors than those with advanced symptoms.

Introduction

Xylella fastidiosa is a xylem-limited bacterium that infects and colonizes a large number of plant species, which frequently show no symptoms. It is classified as a single species, although recent genetic studies support the separation of groups of strains in multiple taxa or subspecies (Schaad et al., 2004). The feeding of insect vectors from xylem sap of host plants is a requirement for its transmission (Purcell & Hopkins, 1996). Thus, *X. fastidiosa* can be transmitted by sharpshooter leafhoppers (Homoptera: Cicadellidae) and spittlebugs (Homoptera: Cercopidae), which are xylem-feeders (Purcell, 1989). In the United States, this pathogen is responsible for diseases in important crops, e.g., grapevines, peach, alfafa, almond, plum, and forest species, as well as in plants with no economic relevance (Hopkins, 1989). However, *X. fastidiosa* strains vary in host specificity (Sherald, 1993), in that not all plants are susceptible to the same strain. In Brazil, *X. fastidiosa* is the causal agent of citrus variegated chlorosis (CVC; Chang et al., 1993; Lee et al., 1993), coffee leaf scorch (Paradela Filho et al., 1995), and plum leaf scald (French & Kitajima, 1978), besides infecting a large number of other plants that show no symptoms (Leite et al., 1997; Lopes et al., 2003).

The efficiency of *X. fastidiosa* transmission by sharpshooters in citrus [*Citrus sinensis* (L.) Osbeck (Rutaceae)] is low and variable among vector species (0.3–30%) (Krügner et al., 2000; Yamamoto et al., 2002; Marucci et al., 2003). This could be due to many factors, such as the low concentration of the pathogen in citrus (Almeida et al., 2001) and its irregular distribution in the xylem bundles (Mizubuti et al., 1994), host plant preference (Raju et al., 1982), and behavioral patterns of the sharpshooters related to feeding and movement in orchards (Purcell, 1985). The low rates of sap ingestion and survival by sharpshooters on healthy

*Correspondence: João Roberto Spotti Lopes, Departamento de Entomologia, Fitopatologia e Zoologia Agrícola – ESALQ/USP, Av. Pádua Dias, 11, Piracicaba, SP 13418-900 Brazil. Tel. +55 (19) 3429-4199; E-mail:jlopes@esalq.usp.br

citrus compared with other hosts (Milanez et al., 2003), for example, may reduce the chances of inoculation of *X. fastidiosa* in citrus. Also, the quality of *X. fastidiosa*-infected citrus for vector feeding may affect bacterial acquisition.

The presence of the pathogen in the plant or vector seemed not to influence the sharpshooter feeding habits in prior studies (Houston et al., 1947). However, the obstruction of xylem vessels colonized by *X. fastidiosa* may affect plant physiology and thus sap flow, possibly making sharpshooter feeding more difficult, especially at periods of higher plant transpiration. Vessels can become occluded by dense colonization of *X. fastidiosa*, and high proportions of blocked vessels are associated with disease symptom development (Newman et al., 2003). Infection by *X. fastidiosa* typically induces an internal water stress by clogging xylem vessels and increasing hydraulic resistance in the xylem (Hopkins, 1989; McElrone et al., 2003). In symptomatic leaves of grapevines, Goodwin & Meredith (1988) observed water stress, interruption of photosynthesis, unbalance of some sugars, losses of chlorophyll, and rupture of cell membranes. The mean resistance of the xylem flow at the leaf petioles was 60–200 times higher in diseased grapevines than in healthy plants (Goodwin et al., 1988).

The effects of citrus infection by *X. fastidiosa* on vector behavior and biology are still unknown. Sharpshooters may be more attracted by plants with CVC symptoms, because of the typical leaf chlorosis and yellowish coloration; yellow is a very attractive color to such insects (Purcell, 1975). However, the obstruction of xylem vessels and subsequent changes in physiology of the diseased plants may affect feeding and survival of the sharpshooters. In contrast, plants at an early stage of infection could be more important as sources of inoculum for vector acquisition, because a lower obstruction of xylem vessels may not cause the same level of water and nutritional stress observed in plants with severe symptoms. Therefore, knowing vector feeding preferences in relation to the degree of citrus infection (symptomatic or asymptomatic) by *X. fastidiosa* is relevant for a better understanding of CVC epidemiology.

The goal of this study was to compare the preference of two sharpshooter vectors, *Dilobopterus costalimai* Young and *Oncometopia facialis* (Signoret) (Homoptera: Cicadellidae), for infected citrus plants with or without CVC symptoms, compared with healthy plants. The ingestion of plant sap by individuals when confined on plants of each treatment was also evaluated.

Materials and methods

Insects and infected plants

Sharpshooter adults used in the study were collected using sweep nets on shoots of citrus trees in groves of northern São Paulo State, Brazil. The insects were transported to the laboratory on potted healthy citrus plants covered with fine net (tulle) bags and then kept in the greenhouse for 3–5 days before the experiments. Healthy seedlings of *Citrus sinensis* (L.) Osbeck (sweet orange) (20–30 cm in height) were inoculated by the pin-prick method (Almeida et al., 2001) with a *X. fastidiosa* isolate from citrus (CCT6570), which was deposited at the Tropical Culture Collection, Fundação André Tosello, Campinas, SP, Brazil. The plants were transplanted into 5-l pots containing a mixture of soil (50%), bovine manure (33.3%), and sand (16.7%), and kept in a greenhouse for bacterial colonization and symptom development. Non-inoculated plants of the same variety were produced and kept under the same conditions.

Experiments

The tests were performed inside rectangular wooden observation chambers with dimensions of 63×63 cm (base) and 120 cm (height), which were placed on a greenhouse bench. The chamber top and walls were built with acrylic in order to facilitate visualization, except for the door, which was covered with anti-aphid canvas, with a thin fabric (voile) sleeve for insect transfer and plant irrigation. Forty insects were released at the bottom of each chamber at 17:00 hours and the number of individuals per plant (treatment) was counted after 3 (20:00), 15 (8:00), 21 (14:00), 24 (17:00), 39 (8:00), 45 (14:00), and 48 (17:00 hours) h. The number of dead insects and the number of insects found on the chamber walls were counted as well. The temperature inside the observation chamber was recorded at each observation time.

A first choice experiment was carried out to evaluate the preference of both species of sharpshooters for citrus plants with CVC symptoms compared with healthy plants. Plants of *C. sinensis* ('Westin') with severe symptoms, having mostly chlorotic leaves and viable bacterial population of 10^6 colony forming units (CFU)g^{-1} of leaf tissue were used. Bacterial populations were estimated by culture according to Almeida et al. (2001). A replicate consisted of one healthy plant placed together with one symptomatic plant of the same variety and age in each observation chamber.

A second experiment was performed to compare the preference of *O. facialis* among healthy plants of *C. sinensis* ('Caipira') and plants infected by *X. fastidiosa* but showing no symptoms, with bacterial populations around 10^4–10^5 CFUg^{-1} of tissue. Each observation chamber had one healthy plant and one similarly looking, asymptomatic infected plant of the same age and variety. Only *O. facialis* was tested because of the low availability of *D. costalimai* adults during this second experiment.

For both experiments, the experimental design consisted of random blocks organized in a factorial and split-plot

scheme with 10 blocks (one replicate or observation chamber per block), whereas the plots corresponded to treatments and the subplots corresponded to evaluation times. Data collected were the number of insects per treatment for each experiment. Initially, the occurrence of an over-dispersion was analyzed, using the Oswald routine with S-Plus software (Statistical Science, 1993). Because no over-dispersed data were collected, a Poisson distribution was assumed and a log (x + 1) transformation was used before analysis of variance. Thus, according to the analysis scheme in time-repeated measurements, when there was no significant interaction between treatments and time, only the Tukey test (P ≤ 0.05) was performed, comparing the average number of insects per treatment for the seven observation periods. Otherwise, regression curves for each treatment were obtained for the several time interactions. Additionally, a joint analysis followed by multiple comparison tests was performed for contrasting the two sharpshooter species regarding the average number of individuals that selected plants of both treatments during all assessment times.

Evaluation of sap ingestion in confinement tests

Under laboratory conditions (temperature: 25 ± 2 °C and L14:D10), the rates of sap ingestion by *O. facialis* and *D. costalimai* sharpshooters when confined on healthy citrus plants were compared with those observed on plants infected by *X. fastidiosa*. The experiment was carried out with plants of *C. sinensis* symptomatically infected ('Westin'; 8 months after inoculation) or asymptomatically infected ('Caipira'; 4 months after inoculation), and with healthy (control) plants of respective ages and varieties. In this way, two treatments + controls were prepared. Treatment 1: infected plant without symptom development; Control 1: healthy plant (same age and variety as Treatment 1). Treatment 2: symptomatic infected plant; Control 2: healthy plant (same age and variety as Treatment 2). The ingestion rate was indirectly measured through collecting the liquid excreted by insects per time unit. Sharpshooter adult males were individually confined on branch sections, using a cage similar to that described by Andersen et al. (1992). This cage consisted of a 50-ml transparent plastic centrifuge tube (Cat. no. 430291, Corning Inc., Acton, MA, USA), in which the insect was confined, connected to a 15-ml scaled plastic centrifuge tube (Cat. no. 430791, Corning Inc.). The cage was positioned on the plant branch so that excreta would flow down into the scaled recipient. Before initiating the experiment, one leaf just above and another just below the branch section where the cage was placed (feeding site), were sampled for quantification of *X. fastidiosa* by culturing (Almeida et al., 2001). Leaves of healthy plants were also tested by culture to confirm the absence of the pathogen in the controls. The

sharpshooters were introduced into the cages at 8:00 h and the evaluations were done after 6 (14:00), 12 (20:00), 24 (8:00), 30 (14:00), 36 (20:00), 48 (8:00), 54 (14:00), 60 (20:00), and 72 (8:00 h) h. The death rate and the volume of excreta produced were recorded visually for each observation. In the first evaluation, the dead sharpshooters were replaced, due to the possibility of mortality caused by handling. In the other observations, the survival rate was used as a parameter for comparing treatments.

The experiment consisted of seven replicates (seven plants with one insect per cage) per treatment. Survival curves of both species of sharpshooters were made (SAS Institute, 1996). The survival curves were estimated by the Kaplan-Meier method (1958) and compared by Wilcoxon and log-rank tests (Allison, 1995). Volume of excreta and proportions of insects that excreted after 72 h were compared among treatments with the Tukey test (P = 0.05)

Results

Selection between healthy and symptomatic citrus

For both sharpshooter species, many individuals had not yet chosen a plant in the first 24 h after release. From this time on, most sharpshooters selected one of the plants, showing adaptation to the observation chamber. The number of individuals on the chamber walls was higher for *O. facialis* than for *D. costalimai* during the initial adaptation time. However, by pooling the data across time, no significant difference was found (F = 1.28, d.f. = 1.36, P = 0.265) between the average number of individuals of *O. facialis* (5.76 ± 0.72) and of *D. costalimai* (5.16 ± 0.31) on the plants of both treatments. Mortality was higher for both species during the hottest period (14:00 and 17:00 hours) of the first 24 h, decreasing in the other observation periods.

A clear preference for the healthy plant over the plant with CVC symptoms was observed for both *D. costalimai* (F = 31.74, d.f. = 1.18, P = 0.00009) and *O. facialis* (F = 11.23, d.f. = 1.18, P = 0.0038). By pooling the data across time, the mean numbers of insects per healthy plant for *D. costalimai* (7.9 ± 0.67) and *O. facialis* (7.7 ± 1.14) were significantly higher than those observed on the symptomatic plant for the former (2.4 ± 0.41) and latter (3.8 ± 0.47) species. The interaction treatment*time was significant (F = 5.47, d.f. = 6.11, P = 0.00015) for *D. costalimai.* The regression curves showed a larger number of insects on healthy plants (F = 4.66, R^2 = 0.70, P = 0.03) than on plants with CVC symptoms (F = 6.41, R^2 = 0.69, P = 0.01) since the first evaluation (Figure 1A). As time passed, the difference between the two treatments increased. The interaction treatment × time was also significant for *O. facialis* (F = 3.79, d.f. = 6.11, P = 0.0021). Although a similar number of individuals of this species was observed

on both treatments in the first observation, there was a reduction in the number of insects on the symptomatic plant (F = 11.60, R^2 = 0.49, P = 0.0013) and a proportional increase on the healthy plant (F = 16.68, R^2 = 0.92, P = 0.0002) in the following evaluations (Figure 1B).

The sharpshooter preference for parts (leaves, petioles, and branches) of healthy or symptomatic plants changed through the day (Figure 2), with an inversion in the proportion of insects on leaves or branches during periods of lower (8:00 hours) and higher (14:00 hours) temperatures. On healthy plants, the proportion of individuals of both species on leaves was highest at 8:00 hours, but decreased drastically at 14:00 hours, when a proportional increase of sharpshooters on branches occurred (Figure 2A and 2B). On plants with CVC, the distribution of *O. facialis* was similar to that observed on healthy plants (Figure 2D). For *D. costalimai*, however, a higher proportion of sharpshooters was observed on leaves than on branches most of the time, except for 45 h (14:00 hours) after the experiment began, when the pattern was inverted (Figure 2C). The proportion of individuals from both species on the petiole was low and did not change much among the evaluation periods or between symptomatic and healthy plants (Figure 2).

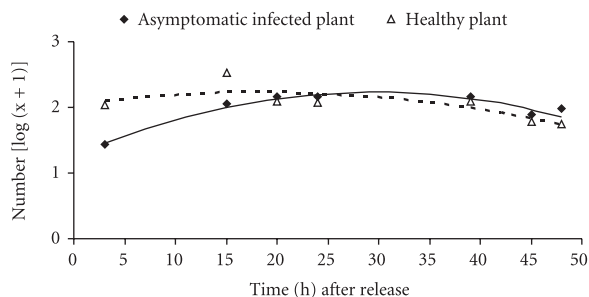**Selection between healthy plants and asymptomatic infected ones**
No significant differences in the number of *O. facialis* (F = 0.23, d.f. = 1.18, P = 0.64) between healthy citrus (7.9 ± 0.83) and symptomless infected citrus (7.0 ± 0.62) were found. The treatment*time interaction was significant (F = 3.47, d.f. = 6.11, P = 0.0038) and the regression curves showed that until the first 3 h (20:00 h), there were more insects on asymptomatic infected plants
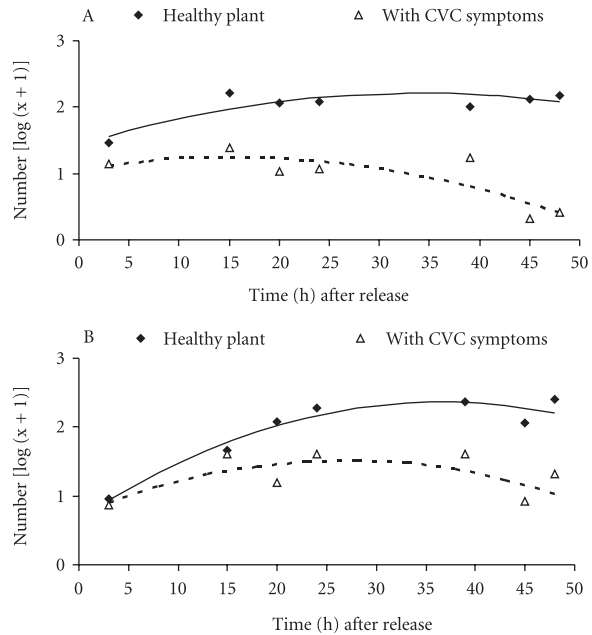
Figure 1 Regression curves of the relationship between observation time (h) and mean number (x) of (A) *Dilobopterus costalimai* and (B) *Oncometopia facialis* per plant, in a choice test between healthy and CVC-symptomatic citrus plants. Regression equations and statistics obtained for healthy and symptomatic plants were, respectively, $y = -0.0007x^2 + 0.046x + 1.43$ (F = 4.66, R^2 = 0.70, P = 0.03) and $y = -0.0008x^2 + 0.025x + 1.05$ (F = 6.41, R^2 = 0.69, P = 0.01) for *D. costalimai* (A); and $y = -0.0013x^2 + 0.093x + 0.67$ (F = 16.68, R^2 = 0.92, P = 0.0002) and $y = -0.0011x^2 + 0.059x + 0.73$ (F = 11.60, R^2 = 0.49, P = 0.0013) for *O. facialis* (B).
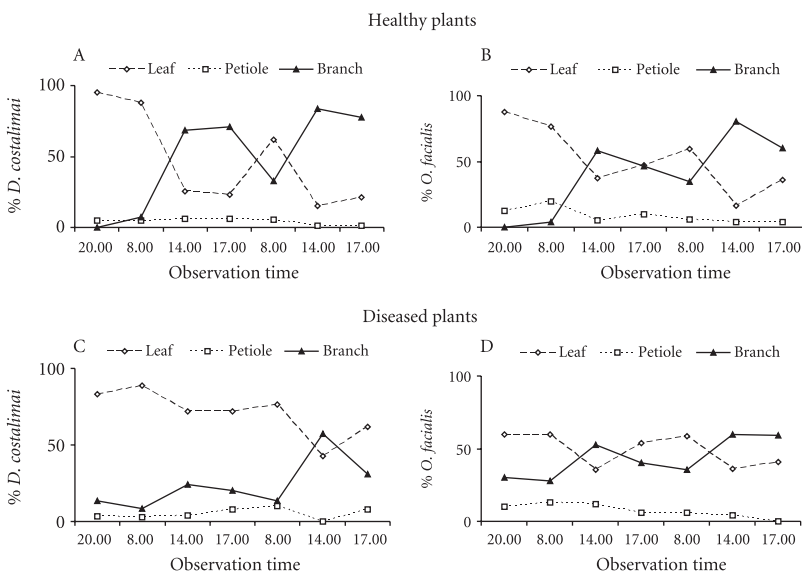
Figure 2 Distribution of *Dilobopterus costalimai* (A and C) and *Oncometopia facialis* (B and D) individuals on leaves, petioles, and branches of the healthy (A and B) and diseased (C and D) plant, during all observations in the choice test between healthy and CVC-symptomatic citrus plants.

Figure 3 Regression curves of the relationship between observation time and mean number (x) of *Oncometopia facialis* per plant in choice test between healthy citrus plants and asymptomatic plants infected by *Xylella fastidiosa*. Regression equations and statistics obtained for healthy and asymptomatic infected plants were, respectively, $y = -0.0005x^2 + 0.020x + 2.06$ ($F = 5.21$, $R^2 = 0.63$, $P = 0.023$) and $y = -0.0011x^2 + 0.065x + 1.27$ ($F = 20.55$, $R^2 = 0.93$, $P = 0.00009$).

($F = 5.21$, $R^2 = 0.63$, $P = 0.023$) than on healthy ones ($F = 20.55$, $R^2 = 0.93$, $P = 0.00009$) (Figure 3). However, similar numbers of sharpshooters were observed on plants of the two treatments in the remaining observations, showing that *O. facialis* apparently does not discriminate between healthy and asymptomatic infected citrus.

The numbers of *O. facialis* individuals on the chamber walls were high only at the first observation (3 h), indicating a faster adaptation to the chamber conditions than in the first choice experiment probably because of the more moderate temperatures (<25 °C) observed in this second experiment. As in the first experiment, the mortality of *O. facialis* was higher during the first 24 h, particularly at 14:00 hours.

Sap ingestion on healthy and infected citrus plants

The survival curves for *D. costalimai* ($P_{Log-Rank} = 0.81$, $P_{Wilcoxon} = 0.68$) and *O. facialis* ($P_{Log-Rank} = 0.99$, $P_{Wilcoxon} = 0.82$) were similar for healthy and infected (either symptomatic or asymptomatic) plants (data not shown). The mean volume of excreta produced and the frequency of *D. costalimai* individuals that excreted on plants with CVC symptoms (Treatment 2) were low compared with the healthy control (Control 2) (Figure 4A). There was almost no excretion after 72 h of confinement on the symptomatic plant. The excretion volume and frequency were similar between healthy and asymptomatic infected plants (Control 1 and Treatment 1). For *O. facialis*, the frequency of excreting individuals was high even on plants with CVC symptoms (Treatment 2) (Figure 4B). For both species, no statistical difference was detected among the treatments regarding excretion, possibly because there were considerable variations in the volumes excreted among individuals subjected to the same treatment.
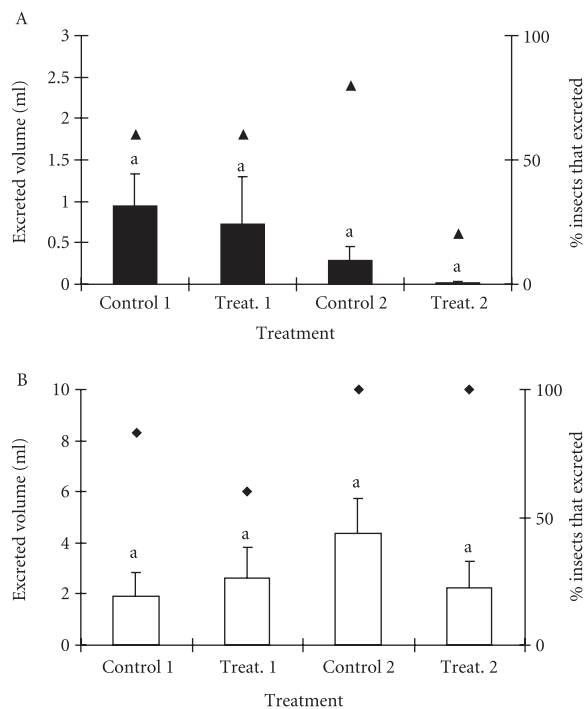
Figure 4 Mean (+ SEM) volume excreted per individual (bars) and frequency of *Dilobopterus costalimai* (A) and *Oncometopia facialis* (B) that excreted (triangles or diamonds) in each treatment in 72 h. Treatment 1: asymptomatic plants infected by *Xylella fastidiosa*. Treatment 2: CVC-symptomatic plant. Controls 1 and 2: healthy citrus plants of same age and variety as Treatment 1 and Treatment 2, respectively.

Discussion

Infection of plants by pathogens may influence host preference and suitability for insect vectors and other herbivores due to changes in plant morphology, physiology, or nutritional quality (Hammond & Hardy, 1988). It has been observed that changes in coloration, e.g., leaf yellowing caused by certain viruses, commonly make a diseased plant more attractive for landing by aphid vectors (Baker, 1960; Macias & Mink, 1969; Ajayi & Dewar, 1983). The yellow coloration is thought to be used as a cue to indicate favorable levels of soluble nitrogen (Kennedy et al., 1961) and might increase the efficiency of an infected plant as source for virus acquisition and spread by the aphids. Plants with CVC also show leaf chlorosis and yellowish coloration, which may be attractive for the sharpshooter vectors. The hypothesis that citrus infection by *X. fastidiosa* influences vector behavior is particularly relevant for the epidemiology of CVC, because infected citrus trees are considered the most important inoculum sources for disease spread in this pathosystem (Laranjeira et al., 1998a).

The first choice experiment showed that symptomatic infection by *X. fastidiosa* in citrus has a negative effect on host attractiveness to the sharpshooter vectors. Plants with CVC symptoms show morphological and biochemical alterations (e.g., increase in levels of lignin and of the enzymes β1,3-glucanase and chitinase), which are associated with plant reaction against pathogens (Martins et al., 1999). Infection by *X. fastidiosa* in citrus caused a 38% reduction on the average daily sap flow, and up to 45% during the hottest hours of the day (Dal Bosco, 2001). In addition, sweet orange plants with CVC show lower water potential in the xylem and lower rates of photosynthesis, transpiration, and stomata conductance compared with healthy plants, especially during periods of higher atmospheric demand, between noon and 15:00 hours (Machado et al., 1994; Medina et al., 1999; Habermann et al., 2003). All these changes in physiology of diseased citrus possibly reduce the nutritional quality of xylem and/or make feeding more difficult; thus, they may explain the preference of the sharpshooters for healthy plants, which show unobstructed vessels, higher rates of photosynthesis, and normal sap flow. Mizell & French (1987) reported a lower incidence and survival of the sharpshooter vector *Homalodisca coagulata* (Say) on peach trees infected by *X. fastidiosa* with symptoms of phony peach disease (PPD) compared with healthy plants. As symptoms develop in peach trees, changes in concentration of elements, xylem water potential, and gas exchange at the leaf level take place.

The observed alternation of citrus leaves and branches as sharpshooter feeding sites along the day may be associated with changes in the physiological status of the plant, which may influence the nutritional quality of xylem sap or the capacity of such insects to suck sap from xylem vessels on leaves or branches during certain periods. For both healthy and CVC-symptomatic sweet orange, a reduction in leaf water potential was observed at periods of higher temperatures (Machado et al., 1994). This could explain the displacement of sharpshooters from leaves to branches at 14:00 hours in the present study. Ribeiro et al. (2004) noted that increases in temperature affects the plant-pathogen system, worsening the disfunction in the photosynthetic metabolism of plants with CVC. In the case of grapevines with Pierce's disease (PD), Goodwin et al. (1988) verified that the symptomatic leaves developed lower water potential and cellular turgescence (near to null values) than healthy plants around noon, which was related to a reduction in the leaf osmotic potential.

Interestingly, the sharpshooter *O. facialis* did not discriminate between healthy and symptomless infected plants in the second choice experiment, showing that early infection of *X. fastidiosa* in citrus does not affect host selection by this hopper species. Because asymptomatic infected plants are as acceptable as healthy ones, they may be more important as inoculum sources for pathogen acquisition by the vector than severely diseased plants. We also observed that viable populations of *X. fastidiosa* in symptomless infected citrus is around 10^4–10^5 $CFUg^{-1}$ of leaf tissue, thus above the minimal threshold (10^4 $CFUg^{-1}$) required for acquisition by vectors in grapevines (Hill & Purcell, 1997).

The excretion experiment allowed an indirect measurement of sharpshooter feeding, which was not possible in the choice experiments. Despite the lack of significant differences in excretion among the treatments, possibly due to high variability and limited number of individuals tested, the data indicate contrasting feeding performances between *D. costalimai* and *O. facialis* under confinement conditions. The ingestion rate of *O. facialis* was not affected by either symptomatic or asymptomatic infection, whereas feeding by *D. costalimai* was markedly reduced when confined on branches of CVC-symptomatic plants. Because nutrients are highly diluted in the xylem's sap, it is necessary that sharpshooters ingest a large amount of sap, about 10–100 times their body weight (Horsfield, 1977). Therefore, the observation of low excretion rates suggests non-preference for certain host species or for parts of a plant. It should be pointed out that the frequency of individuals of *D. costalimai* was higher on leaves than on branches of plants with CVC symptoms in the first choice experiment (Figure 2C). Combined with the results of the excretion experiment, in which the insects were confined on plant branches, these data suggest that symptomatic infection of citrus by *X. fastidiosa* affects the selection of feeding sites by *D. costalimai*, inhibiting its permanence and sap ingestion on the branches of diseased plants.

The observed propensity of *D. costalimai* to remain on the leaves of diseased plants might enhance their acquisition of *X. fastidiosa*. In plants with CVC, the leaves show higher vessel obstruction by *X. fastidiosa* than the stems (Queiroz-Voltan & Paradela Filho, 1999). In grapevines with PD, Hopkins (1981) observed a lower percentage of vessels obstructed by the bacterium in the stem than in the leaves, and higher in leaf veins than in petioles. Therefore, the longer stay of *D. costalimai* on the leaves of infected citrus plants could explain, at least in part, the higher transmission efficiency of *X. fastidiosa* by this vector compared to *O. facialis* (Krügner et al., 2000; Marucci et al., 2003).

Host preference may influence the density of an insect vector on plants and have implications on probability of pathogen infection and disease spread (Purcell, 1985). There is a strong relationship between vector behavior and incidence of phony peach disease caused by *X. fastidiosa* (Gould et al., 1991); the low attractiveness of diseased peach trees to sharpshooters result in low (or no) secondary spread of the pathogen in the peach orchards. In the

present study, the numbers of sharpshooters on healthy citrus plants were similar (*O. facialis*) or higher (*D. costalimai*) compared with those observed on diseased plants in the first 3 h after release in the observation chamber. However, most sharpshooters that initially chose the diseased plants gradually moved to the healthy ones over the 48-h observation period. One may consider this behavior beneficial to *X. fastidiosa*, because it promotes movement of vectors to healthy plants after a few hours on infected plants, favoring rapid inoculation and disease spread in the field. This could be the case when vector transmission is efficient after short acquisition access periods (AAPs). In grapevines, for example, the sharpshooter *Graphocephala atropunctata* (Signoret) can transmit *X. fastidiosa* with high efficiency (>90%) after an AAP of only 3 h on grape with PD (Purcell & Finlay, 1979). In citrus, however, reported transmission efficiencies of *X. fastidiosa* by vectors are much lower (0.3–30%) than in grapes, despite the long AAP (48 h) tested in various studies (Krügner et al., 2000; Yamamoto et al., 2002; Marucci et al., 2003). Therefore, a brief stay of sharpshooters on severely diseased citrus would probably reduce their probability to acquire *X. fastidiosa* from such plants.

Overall, this study shows that selection of diseased citrus plants by sharpshooter vectors is influenced by the level of infection by *X. fastidiosa*. Plants with severe infection and typical CVC symptoms tend to be rejected by both *D. costalimai* and *O. facialis*, whereas asymptomatic ones are equally accepted by the latter species in relation to healthy citrus plants. It should be pointed out that the study was conducted with young potted citrus in a greenhouse; thus, vector responses to adult citrus trees in a commercial grove could be different. Nevertheless, these findings may be helpful for interpretation of epidemiological studies. In sweet orange orchards, asymptomatic infected trees or those with just mild or early CVC symptoms may have a greater impact as sources of inoculum for disease spread than trees with advanced symptoms. Besides, one may speculate that other *Citrus* species tolerant to *X. fastidiosa*, which allow bacterial colonization without expression of CVC symptoms [e.g., some tangerines, tangelos, tangors, and lemons (Laranjeira et al., 1998b)], are important sources of inoculum as well. Further epidemiological research should consider the possible role of asymptomatic systemic hosts on spread of *X. fastidiosa* in citrus and other affected crops.

Acknowledgements

References

Ajayi O & Dewar AM (1983) The effect of barley yellow dwarf virus on field populations of the cereal aphids, *Sitobion avenae* and *Metopolophium dirhodum*. Annals of Applied Biology 103: 1–11.

Allison PD (1995) Survival Analysis Using the SAS System: a Practical Guide. SAS Institute, Cary, NC, 292 pp.

Almeida RPP, Pereira EF, Purcel AH & Lopes JRS (2001) Multiplication and movement of a citrus strain of *Xylella fastidiosa* within sweet orange. Plant Disease 85: 382–386.

Andersen PC, Brodbeck BV & Mizell III RF (1992) Feeding by the leafhopper, *Homalodisca coagulata*, in relation to xylem fluid chemistry and tension. Journal of Insect Physiology 38: 611–622.

Baker PF (1960) Aphid behaviour on healthy and on yellows-virus-infected sugar beet. Annals of Applied Biology 48: 384–391.

Chang CJ, Garnier M, Zreik L, Rossetti V & Bové JM (1993) Culture and serological detection of the xylem-limited bacterium causing citrus variegated chlorosis and its identification as a strain of *Xylella fastidiosa*. Current Microbiology 27: 137–142.

Dal Bosco A (2001) Fluxo de seiva em laranjeira (*Citrus sinensis* L. Osb.) 'Pera' infectada com *Xylella fastidiosa* Wells. Piracicaba, 53p. Dissertação (Mestrado) – Escola Superior de Agricultura 'Luiz de Queiroz', Universidade de São Paulo, Brasil.

French WJ & Kitajima EW (1978) Occurrence of plum leaf scald in Brazil and Paraguay. Plant Disease Reporter 62: 1035–1038.

Goodwin PH, Devay JE & Meredith CP (1988) Roles of water stress and phytotoxins in the development of Pierce's disease of grapevine. Physiological and Molecular Plant Pathology 32: 1–15.

Goodwin PH & Meredith CP (1988) New clues in understanding Pierce's disease. California Agriculture 42: 6–7.

Gould AB, French WJ, Aldrich JH, Brodbeck BV, Mizell III RF & Andersen PC (1991) Rootstock influence on occurrence of *Homalodisca coagulata*, peach xylem fluid amino acids, and concentrations of *Xylella fastidiosa*. Plant Disease 75: 767–770.

Habermann G, Machado EC, Rodrigues JD & Medina CL (2003) CO_2 assimilation, photosynthetic light response curves, and water relations of 'Pêra' sweet orange plants infected with *Xylella fastidiosa*. Brazilian Journal of Plant Physiology 15: 79–87.

Hammond AM & Hardy TN (1988) Quality of diseased plants as hosts for insects. Plant Stress–Insect Interactions (ed. by EA Heinrichs), pp. 381–432. Wiley, NY.

Hill BL & Purcell AH (1997) Populations of *Xylella fastidiosa* in plants required for transmission by efficient vector. Phytopathology 87: 1197–1201.

Hopkins DL (1981) Seasonal concentration of the Pierce's disease bacterium in grapevine stems, petioles and leaf veins. Phytopathology 71: 415–418.

Hopkins DL (1989) *Xylella fastidiosa*: xylem-limited bacterial pathogen of plants. Annual Review of Phytopathology 27: 271–290.

Horsfield D (1977) Relationship between feeding of *Philaenus spumarius* and the amino acid concentration of the xylem sap. Ecological Entomology 2: 259–266.

Houston BR, Esau K & Hewitt WB (1947) The mode of vector feeding and the tissues involved in the transmission of Pierce's disease virus in grape and alfalfa. Phytopathology 37: 247–254.

Kaplan EL & Meier P (1958) Nonparametric estimation from incomplete observations. Journal of American Statistical Association 53: 457–481.

Kennedy JS, Booth CO & Kershaw WJS (1961) Host finding by aphids in the field. III. Visual attraction. Annals of Applied Biology 49: 1–21.

Krügner R, Lopes MTV de C, Santos JS, Beretta MJG & Lopes JRS (2000) Transmission efficiency of *Xylella fastidiosa* to citrus by sharpshooters and identification of two new vector species, p. 423. Conference of International Organization of Citrus Virologists, 14., Campinas. Proceedings. Riverside, IOCV.

Laranjeira FF, Bergamin Filho A, Amorim L & Berger RD (1998a) Aspectos práticos da epidemiologia da clorose variegada dos citros. Laranja 19: 79–90.

Laranjeira FF, Pompeu Jr J, Harakava R, Figueiredo JO, Carvalho SA & Coletta Filho HD (1998b) Cultivares e espécies cítricas hospedeiras de *Xylella fastidiosa* em condição de campo. Fitopatologia Brasileira 23: 147–154.

Lee RF, Beretta MJG, Hartung JH, Hooker ME & Derrick KS (1993) Citrus variegated chlorosis: confirmation of a *Xylella fastidiosa* as the causal agent. Summa Phytopathologica 19: 123–125.

Leite RMVBC, Leite Jr RP & Ceresini PC (1997) Hospedeiros alternativos de *Xylella fastidiosa* entre plantas invasoras de pomares de ameixeira com escaldadura da folha. Fitopatologia Brasileira 22: 54–57.

Lopes S, Marcussi S, Torres SCZ, Souza V, Fagan C, França SC, Fernandes NG & Lopes JRS (2003) Weeds as alternative hosts of the citrus, coffee, and plum strains of *Xylella fastidiosa* in Brazil. Plant Disease 87: 544–549.

Machado EC, Quaggio JA, Lagôa AMMA, Ticelli M & Furlani PR (1994) Trocas gasosas e relações hídricas em laranjeiras com clorose variegada dos citros. Revista Brasileira de Fisiologia Vegetal 6: 53–57.

Macias W & Mink GI (1969) Preference of green peach aphids for virus-infected sugar beet leaves. Journal of Economic Entomology 62: 28–29.

Martins EMF, Guzzo SD, Palazzo DA & Harakava R (1999) Alterações metabólicas constatadas em plantas de laranja Natal infectadas por *Xylella fastidiosa*. Summa Phytopathologica 25: 288–293.

Marucci RC, Lopes JRS & Corrente JE (2003) Análise comparativa da eficiência de transmissão de *Xylella fastidiosa* por cigarrinhas (Hemiptera, Cicadellidae) em plantas de citros e café. Fitopatologia Brasileira 28: 392–392.

McElrone AJ, Sherald JL & Forseth IN (2003) Interactive effects of water stress and xylem-limited bacterial infection on the water relations of a host vine. Journal of Experimental Botany 54: 419–430.

Medina CL, Machado EC & Gomes MMA (1999) Condutância estomática, transpiração e fotossíntese em laranjeira 'Valência' sob deficiência hídrica. Revista Brasileira de Fisiologia Vegetal 11: 29–34.

Milanez JM, Parra JRP, Custódio IA, Magri DC, Cera C & Lopes JRS (2003) Feeding and survival of citrus sharpshooters (Hemiptera: Cicadellidae) on host plants. Florida Entomologist 86: 154–157.

Mizell RF III & French WJ (1987) Leafhopper vectors phony peach disease: feeding site preference and survival on infected and uninfected peach, and seasonal response to selected host plants. Journal of Entomology Science 22: 11–22.

Mizubuti ESG, Matsuoka K & Parizzi P (1994) Associação de bactéria do tipo *Xylella* em laranjeiras com sintomas de clorose variegada na região da Zona da Mata de Minas Gerais. Fitopatologia Brasileira 19: 241–244.

Newman KL, Almeida RPP, Purcell AH & Lindow SE (2003) Use of a green fluorescent strain for analysis of *Xylella fastidiosa* colonization of *Vitis vinifera*. Applied and Environmental Microbiology 69: 7319–7327.

Paradela Filho O, Sugimori MH, Ribeiro IJA, Machado MA, Laranjeira FF, Garcia Jr A, Beretta MJG, Harakawa R, Rodrigues Neto J & Beriam LOS (1995) Primeira constatação em cafeeiro no Brasil da *Xylella fastidiosa* causadora da clorose variegada dos citros. Laranja 16: 135–136.

Purcell AH (1975) Role of blue-green sharpshooter, *Hordnia circellata*, in the epidemiology of Pierce's disease of grapevines. Environmental Entomology 4: 745–752.

Purcell AH (1985) The ecology of bacterial and mycoplasma plant diseases spread by leafhoppers and planthoppers. The Leafhoppers and Planthoppers (ed. by LR Nault & JG Rodriguez), pp. 351–380. Wiley, NY.

Purcell AH (1989) Homopteran transmission of xylem-inhabiting bacteria. Advances in Disease Vector Research, Vol. 6 (ed. by KF Harris), pp. 243–266. Springer-Verlag, NY.

Purcell AH & Finlay AH (1979) Evidence for noncirculative transmission of Pierce's disease bacterium by sharpshooter leafhoppers. Phytopathology 69: 393–395.

Purcell AH & Hopkins DL (1996) Fastidious xylem-limited bacterial plant pathogens. Annual Review of Phytopathology 34: 131–151.

Queiroz-Voltan RB & Paradela Filho O (1999) Caracterização de estruturas anatômicas de citros infectados com *Xylella fastidiosa*. Laranja 20: 55–76.

Raju BC, Wells JM, Nyland G, Brlansky RH & Lowe SK (1982) Plum leaf scald: isolation, culture, and pathogenicity of the causal agent. Phytopathology 72: 1460–1466.

Ribeiro RV, Machado EC & Oliveira RF (2004) Growth-and leaf-temperature effects on photosynthesis of sweet orange seedlings infected with *Xylella fastidiosa*. Plant Pathology 53: 334–340.

SAS Institute (1996) Guia do Usuário SAS/STAT, versão 6. SAS/STAT Inc., Cary, NC.

Schaad NW, Postnikova E, Lacy G, Fatmi M & Chang CJ (2004) *Xylella fastidiosa* subspecies: *X. fastidiosa* subsp. *piercei*, subsp. nov., *X. fastidiosa* subsp. *multiplex* subsp. nov., and *X. fastidiosa* subsp. *pauca* subsp. nov. Systematic and Applied Microbiology 27: 290–300.

Sherald JL (1993) Pathogenicity of *Xylella fastidiosa* in American elm and failure of reciprocal transmission between strains from elm and sycamore. Plant Disease 77: 190–193.

Statistical Science (1993) S-Plus for Windows: user's manual. Insightful Corporation, Seattle.

Yamamoto PT, Roberto SR, Pria Júnior WD, Felippe MR, Miranda VS, Teixeira D do C & Lopes JRS (2002) Transmissão de *Xylella fastidiosa* por cigarrinhas *Acrogonia virescens* e *Homalodisca ignorata* (Hemiptera: Cicadellidae) em plantas cítricas. Summa Phytopathologica 28: 178–181.