Weeds as Alternative Hosts of the Citrus, Coffee, and Plum Strains of *Xylella fastidiosa* in Brazil

S. A. Lopes, S. Marcussi, S. C. Z. Torres, V. Souza, C. Fagan, and **S. C. França,** Unidade de Biotecnologia, Universidade de Ribeirão Preto, Ribeirão Preto, SP 14096-380; **N. G. Fernandes,** Fundecitrus, Araraquara, SP 14801-970; and **J. R. S. Lopes,** Departamento de Entomologia, Fitopatologia e Zoologia Agrícola, ESALQ/Universidade de São Paulo, Piracicaba, SP 13418-900, Brazil

ABSTRACT

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In Brazil, *Xylella fastidiosa* is present in citrus (*Citrus sinensis*), coffee (*Coffea arabica*), and plum (*Prunus* sp.) crops, causing the citrus variegated chlorosis (CVC), coffee leaf scorch (CLS), and plum leaf scald (PLS). Also present in these crops and infesting weeds, which ultimately could serve as sources of inoculum for the cultivated trees, are diverse populations of xylem-feeding leafhopper vectors. In order to assess host range of *X. fastidiosa* among weeds and to better understand their role in epidemics, field surveys, mechanical inoculations, and insect transmission tests were conducted. Polymerase chain reaction (PCR) and culture plating were used to detect the pathogen from plant tissues. *X. fastidiosa* was detected in 10 out of 23 species of the weed plants sampled in two citrus groves affected by CVC. None of the weed plants showed external symptoms. In the greenhouse, the average percentages of infection on plants mechanically inoculated with the CVC, CLS, and PLS strains of *X. fastidiosa* were, respectively, 25, 10, 0 in *Medicago sativa*; 70, 45, 20 in *Echinochloa crus-galli*; 45, 30, 0 in *Brachiaria decumbens*; 72, 70, 40 in *Brachiaria plantaginea*; 13, 10, 0 in *Digitaria horizontalis*; 31, 30, 0 in *Solanum americanum*; and 17, 0, 0 in *Bidens pilosa*. Symptoms were observed only in *S. americanum* and citrus and only when inoculated with the CVC strain. In insect transmission tests, the grass leafhopper *Ferrariana trivittata* was first caged on citrus plants showing CVC symptoms and then on healthy citrus and on the four most common weeds. No plants tested positive by PCR or culture, or showed symptoms for at least 4 months after inoculation. The amount of *X. fastidiosa* cells that may accumulate in weeds inoculated by leafhoppers is probably under insect acquisition thresholds, a factor that would limit their importance to the CVC epidemics, as studies on spatial distribution of diseased citrus trees over time indicate.

In Brazil, the xylem-limited bacterium *Xylella fastidiosa* (37) causes citrus variegated chlorosis (CVC), coffee leaf scorch (CLS), and plum leaf scald (PLS) (4,7,12,17,27). Reduction in plant growth and production of fruits of small size are common symptoms expressed in affected citrus, coffee, and plum trees.

PLS was initially observed in 1978 in Cascata, Rio Grande do Sul State, on Japanese (*Prunus salicina* Lindl.) and European (*Prunus domestica* L.) plum trees (7). The disease, at that time attributed to a Rickettsia-like organism, could have originated from the delta of Paraná River, Argentina, where it was known since 1935. Today, PLS is present in all southern states where plum is grown commercially. CVC was first described in 1987 in Minas Gerais and São Paulo (34). In the following years, CVC was detected in the adjacent regions and in other states. An extensive survey conducted more recently in areas growing the four main citrus cultivars, Pêra Rio, Valência, Natal, and Hamlim, indicated that CVC incidence increased from 22.33% in 1996 to 36.52% in 1999 (3). The adoption of control measures has not been sufficient to reduce the CVC incidence, which has continued at over 30% in 2000, 2001, and 2002. CLS was described in 1995 in São José do Rio Preto, SP (26). Later, the pathogen was detected in samples collected in coffee fields located in northern São Paulo and Minas Gerais states (16,26). Because CLS symptomatology is not well defined under field conditions, detailed surveys have not been conducted and precise data on CLS incidence are not currently available. However, the high frequency of pathogen detection from field samples indicates that CLS is probably more widely distributed than the PLS or CVC.

In citrus, and probably coffee and plum, *X. fastidiosa* is transmitted from plant to plant through adults of leafhoppers that feed on the plant xylem. Under greenhouse conditions, 11 species transmitted the pathogen from diseased citrus to healthy citrus plants (2), with variable levels of efficiency (13,40). Some vector species such as *Plesiommata corniculata* Young, *Ferrariana trivittata* Signoret, and *Sonesimia grossa* Signoret, although detected more frequently on grasses, also have been captured on citrus twigs (9). As the species *X. fastidiosa* may infect a wide range of plant hosts, these results indicate that the bacteria could eventually be transmitted by insects from naturally infected citrus to weed plants. Results of a field survey conducted in Paraná State (36) indicated that the CVC strain was present in several weed species infesting citrus groves. Depending on the susceptibility and level of plant colonization, these alternative hosts could serve as reservoirs of the pathogen to other weed species and, more importantly, to citrus. The importance of weeds as reservoirs of *X. fastidiosa* has been well described for Pierce's disease of grapevine. The pathogen is present in symptomless wild plants in the southern United States and is the major limiting factor for the production of European-type grapes (32). In California, higher incidence of infected vines has been observed in areas adjacent to permanent water sources and weeds known to be hosts of *X. fastidiosa* and feeding hosts of the insect vectors (31). Given the importance that the presence of *X. fastidiosa* in weeds could represent for CVC, as well as for CLS and PLS epidemics, we conducted this work, which included field surveys, mechanical inoculations, and insect transmission experiments.

MATERIALS AND METHODS

Field surveys. The surveys were carried out in February, March, and April 1997 in several rows of citrus (*Citrus sinensis* cv. Hamlin) at Boa Esperança Farm (block 212, 13.24 ha), located in Cajobi, SP, and in citrus (*C. sinensis* cv. Pêra Rio) at São José Farm (blocks 102, 117, 118, and 139 of 45.55, 41.45, 41.85, and 50.86 ha), located in Luis Antonio, SP. The first farm is located in a citrus growing region, and the sampled groves showed over 80% incidence of CVC. The second farm was surrounded by large areas planted with sugarcane, and the incidence of CVC was less than 5% in the surveyed area. The sampled weed plants growing in the groves were identified and selected by chance. A total

Corresponding author: S. A. Lopes
E-mail: slopes@unaerp.br

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of 23 species of 13 distinct families were sampled, including the most common species in citrus groves. Entire plants were collected, transferred to plastic bags, and transported to the laboratory on ice in a foam box. Leaves collected from a few symptomatic citrus trees were used as positive controls. In the laboratory, the stems of the field-collected plants were stripped of leaves and small branches, washed thoroughly with tap water and soap, cut in segments of ca. 2 cm long, and processed for sap extraction. The segments were firmly introduced into 1.5-ml tubes (8) and centrifuged at 10,000 rpm for 2 min. For the citrus samples, which also included leaves collected from plants grown in a greenhouse protected from insects (negative controls), sap was collected from the petioles and midrib by centrifugation in bottom perforated 0.5-ml tubes inserted into 1.5-ml tubes. After removing the plant tissues, 1 ml of extraction buffer (21) was added to the plant sap. The tubes were vortexed 5 s and centrifuged again at 14,000 rpm for 2 min. The buffer was then discarded, and 20 µl of deionized and sterile Milli Q filtered water (Millipore, Bedford MA) was added to each tube. The tubes were transferred to a metal rack, boiled for 10 min, and stored at –20°C for polymerase chain reaction (PCR) analysis. The primer pair CVC-1/272-2int was used for PCR detection of *X. fastidiosa* in the field samples, as described by Pooler and Hartung (28). Each PCR sample consisted of xylem sap extracted from stem segments from 10 to 20 plants of each species.

Plants and inoculation. *Medicago sativa* L. (forage alfalfa), *Echinochloa crus-galli* (L.) Beauv., and five of the most common weeds found in the citrus groves (*Bidens pilosa* L., *Brachiaria decumbens* Stapf., *Brachiaria plantaginea* (Link.) Hitch., *Digitaria horizontalis* Willd., and *Solanum americanum* Mill.) were tested for susceptibility to a CVC isolate of *X. fastidiosa* in three mechanical inoculation experiments. In the first experiment, also considered a screening of potential hosts, seven additional weeds (*Alternanthera tenella* Colla, *Solidago microglossa* DC, *Sorghum halepense* (L.) Pers., *Eleusine indica* (L.) Gaertn., *Ipomoea grandifolia* (Dammer) O'Don., *Eclipta alba* Hassk., and *Commelina virginica* L.) also were inoculated. Only plant species that tested positive for *X. fastidiosa* in the first experiment were included in the second and third experiments. Plants were also inoculated with a CLS isolate in the second and third experiments, and with a PLS isolate in the third experiment.

Weed seeds were purchased from Shokusho (Engenheiro Coelho, SP) and sown on substrate Plantmax for vegetables (Eucatex, Paulínia, SP). Plants circa 5 cm in height were transplanted to 0.5-liter plastic pots containing sandy soil and composted cow manure (3:1). Coffee (*Coffea arabica* cv. Catuaí Amarelo) and citrus (*Citrus sinensis* cv. Caipira) plants were also included in the experiments as positive controls. Due to the fast growth rate, especially of the grasses, test plants were pruned 5 cm above the ground 1 week before inoculation. The experiments were conducted free of potential vectors in a greenhouse equipped with a cooling system that kept the temperature below 32°C, except during a few days of December 1998 and January 1999, when the temperature reached 35°C.

All plants were inoculated twice. Inoculation dates were 10 November and 15 December 1998, 30 April and 15 May 1999, and 2 and 16 July 1999, for the first, second, and third experiments, respectively. The inocula consisted of 20-day-old cultures of *X. fastidiosa* in liquid periwinkle wilt (PW) medium (5) containing 10^8 to 10^9 CFU/ml, as estimated based on dilution plating on solid charcoal yeast extract with ACES buffer (BCYE) medium (38). The isolates used were XfSJ (Farm São José, Taquaritinga, SP), Xf9746, and Xf12288 (Instituto Agronômico do Paraná), recovered from infected citrus, coffee, and plum plants, respectively. Approximately 30 µl of inoculum was injected into 1-month-old weed or 3- to 6-month old coffee and citrus plants. Inocula were injected into the stem at 2 to 3 cm above the soil level, using a 25 × 0.7 mm hypodermic needle. Nine or 10 test plants of each species were inoculated with each strain. Negative controls were injected with liquid PW fresh medium free of bacteria. Forty to 60 days after the second inoculation, stem segments 3 to 4 cm long and midribs of the surviving weed leaves were collected at least 5 cm above the inoculation point. Samples of citrus and coffee plants consisted of midrib and petioles removed from three to five older or symptomatic leaves. Samples were weighed, surface sterilized (21), and macerated in 1 ml of phosphate buffered saline (PBS). A 100-µl aliquot of the macerate was diluted 100-fold in PBS and plated on solid BCYE medium. The remaining macerate was prepared as previously described and stored at –20°C for PCR analysis. The primer pairs CVC-1/272-2 (28) and XF5F/XF5R (21) were used for PCR detection of *X. fastidiosa* in the samples.

Weed colonization by *X. fastidiosa*. Two experiments including *B. decumbens*, *B. plantaginea*, *E. crus-galli*, *Bidens pilosa*, *S. americanum,* and *Nicotiana tabacum* were conducted to assess plant colonization by *X. fastidiosa*. The inoculum consisted of a suspension of the CVC strain of *X. fastidiosa* in PBS, which was pin pricked at the main leaf vein (10 µl per vein) of several plants per species. Just before inoculation, a 100-µl aliquot of inoculum was serially diluted and plated on BCYE agar for quantification. Thirteen, 28, and 41 days (first experiment) and 30 and 50 days (second experiment) after inoculation, samples were collected from the inoculation site and processed for *X. fastidiosa* isolation on BCYE medium as described. The plates were maintained at 28°C for 4 to 6 weeks, when the colonies were counted and the number of CFU per gram of tissue was estimated.

Insect transmission tests. Transmission of the CVC strain (XfSJ isolate) to sweet orange, tobacco, and various weed hosts was tested using the leafhopper vector, *Ferrariana trivittata*. Only weed species that showed susceptibility to XfSJ in the mechanical inoculation experiment were included. Hundreds of *F. trivittata* adults were collected from *B. decumbens* plants in an area at the vicinity of Ribeirão Preto city, located at least 50 km from the main areas grown with citrus or coffee plants. Variable numbers of these insects were caged for acquisition access periods of 48 h on branches of potted sweet oranges (Caipira and Pêra cultivars) showing typical CVC leaf symptoms (source plants). The surviving insects were transferred and caged, in lots of 10 or 20 individuals on each test plant, on 1-month-old potted weeds or on 6-month-old sweet orange potted plants for an inoculation access period (IAP) of 48 h. Citrus test plants were pruned approximately 1 month before inoculation to stimulate the emergence of new leaves. Insect survival rate was evaluated on both the source and the test plants. After the IAP, the insects were eradicated and the test plants maintained in the greenhouse. Two and 3 months later, stem segments (3 to 4 cm long) and midribs of the surviving weed leaves were processed and tested for the presence of *X. fastidiosa* by isolation on BCYE medium and by PCR, as previously described.

RESULTS

Field surveys. A band of approximately 500 bp, indicating the presence of *X. fastidiosa*, was detected in agarose gels from the positive controls (purified DNA or cell lysate of *X. fastidiosa* cells), from leaf samples of Hamlim and Pêra Rio symptomatic sweet oranges, and from stem samples of 10 of 23 species of the collected weeds (Table 1). A higher percentage of species that tested positive by PCR was identified at S. José (53%) than at Boa Esperança (23%). Only *C. benghalensis*, *C. echinatus,* and *D. insularis* were infected in both locations. In S. José, *X. fastidiosa* was detected in five species that were free from the pathogen in Boa Esperança, whereas in Boa Esperança, *X. fastidiosa* was detected in two species that were free from the pathogen in S. José farm.

Mechanical inoculation experiments. In the first experiment, the CVC isolate of *X. fastidiosa* was detected by PCR in most inoculated weeds (Table 2), except (data not shown) *A. tenella*, *S. microglossa*, *S. halepense*, *E. indica*, *I. grandifolia*, *E.*

alba, and *C. virginica*. In the three experiments, all weeds were infected by the CVC strain, except *Bidens pilosa* and *D. horizontalis*, which were negative for this strain in the third experiment. Overall, *B. plantaginea* was the species with the highest rates of infection, with 30, 90, and 90% in the first, second, and third experiments. Considering the average values of infection frequency, the ranking of species with increasing levels of susceptibility is: *M. sativa* (10%), *D. horizontalis* (13%), *Bidens pilosa* (17%), *S. americanum* (31%), *B. decumbens* (45%), *E. crus-galli* (45%), and *B. plantaginea* (70%).

Except for *Bidens pilosa*, all weeds inoculated with CLS strain tested positive by PCR (Table 2). *B. decumbens* and *B. plantaginea* were positive in one of two experiments. *B. plantaginea* was again the species with the highest infection rate. The ranking of species with increasing levels of susceptibility to CLS strain is: *Bidens pilosa* (0%), *M. sativa* and *D. horizontalis* (10%), *B. decumbens* and *S. americanum* (30%), *E. crus-galli* (45%), and *B. plantaginea* (70%). The PLS strain of *X. fastidiosa* was inoculated only in the third experiment; only *E. crus-galli* and *B. plantaginea* were infected by this pathogen, with frequencies of infection of 20 and 40%, respectively.

None of the negative control plants in the three experiments were positive by PCR (data not shown) or showed any foliar symptom. With the exception of *S. americanum*, which showed branch proliferation, short internodes, and small leaves when inoculated with the CVC strain (in a single plant in the first and another in the second experiment), all weeds and coffee plants inoculated with *X. fastidiosa* were symptomless, regardless of the strain used. Symptoms typical of CVC were detected on leaves of citrus inoculated with the

Table 1. Weed species sampled in two geographic locations and analyzed by polymerase chain reaction (PCR) for the presence of *Xylella fastidiosa* in the vascular system

Local common name	Scientific name[a]	Family	Life cycle[b]	Boa Esperança Farm (Cajobi/SP)	São José Farm (Luís Antônio/SP)
Apaga fogo	*Alternanthera tenella*	Amaranthaceae	P	0/4[c]	1/4
Caruru	*Amaranthus* sp.	Amaranthaceae	A	0/2	ns
Trapoeraba	*Commelina benghalensis*	Commelinaceae	P	1/5	1/1
Carrapicho de carneiro	*Acanthospermum hispidum*	Compositae	A	0/2	ns
Picão preto	*Bidens pilosa*	Compositae	A	0/4	1/4
Corda de viola	*Ipomoea* sp.	Convolvulaceae	A	0/1	ns
Erva de S. Luzia	*Euphorbia hirta*	Euphorbiaceae	A	0/2	1/1
Quebra pedra	*Phyllanthus tenellus*	Euphorbiaceae	A	0/1	ns
Capim braquiária	*Brachiaria decumbens*	Gramineae	P	2/6	0/3
Capim marmelada	*Brachiaria plantaginea*	Gramineae	A	ns[d]	0/3
Capim carrapicho	*Cenchrus echinatus*	Gramineae	A	1/5	1/3
Capim colchão	*Digitaria horizontalis*	Gramineae	A	0/3	1/3
Capim amargoso	*Digitaria insularis*	Gramineae	A	1/5	1/3
Colonião	*Panicum maximum*	Gramineae	P	0/4	0/2
Grama seda	*Cynodon dactylon*	Gramineae	P	0/1	0/2
Arranha-gato	*Acacia plumosa*	Leguminosae	P	0/2	ns
Fedegoso	*Senna obtusifolia*	Legumunosae	P	0/2	ns
Guanxuma	*Sida* spp.	Malvaceae	P	0/4	0/1
Beldroega	*Portulaca oleracea*	Portulacaceae	A	0/1	0/2
Erva-quente	*Spermacoce latifolia*	Rubiaceae	A	1/2	0/1
Poaia branca	*Richardia brasiliensis*	Rubiaceae	A	0/1	na
Maria pretinha	*Solanum americanum*	Solanaceae	A	0/1	1/4
Cambará	*Lantana camara*	Verbenaceae	P	0/2	ns
Laranja doce (with CVC symptoms)	*Citrus sinensis* cv. Pêra	Rutaceae	P	ns	3/3
Laranja doce (with CVC symptoms)	*Citrus sinensis* cv. Hamlim	Rutaceae	P	4/4	ns
	Total			10/66	11/40

[a] Weed scientific names based on Lorenzi (22).
[b] Annual (A) or perennial (P).
[c] Number of PCR positive over the total number of analyzed samples. Each sample consisted of xylem sap extracted by centrifugation from stem segments of 10 to 20 plants. The sap was processed and analyzed by PCR using the CVC-1/272-2int primer pair, as described (28).
[d] Not sampled.

Table 2. Frequency of infection of weed, coffee, and citrus plants mechanically inoculated with the citrus variegated chlorosis (CVC), coffee leaf scorch (CLS), and plum leaf scald (PLS) strains of *Xylella fastidiosa*

		CVC strain			CLS strain		PLS strain
Local common name	Scientific name	1st exp.	2nd exp.	3rd exp.	2nd exp.	3rd exp.	3rd exp.
Alfafa-creoula	*Medicago sativa*	…[a]	1/10	5/10	1/10	1/10	0/10
Capim-arroz	*Echinochloa crus-galli*	8/10[b]	6/10	7/10	6/10	3/10	2/10
Capim-braquiária	*Brachiaria decumbens*	2/9	3/10	8/10	0/10	6/10	0/10
Capim-colchão	*Digitaria horizontalis*	3/10	1/10	0/10	2/10	0/10	0/10
Capim-marmelada	*Brachiaria plantaginea*	3/9	9/10	9/10	7/10	7/10	4/10
Maria-pretinha	*Solanum americanum*	2/9	4/10	3/10	4/10	2/10	0/10
Picão-preto	*Bidens pilosa*	4/10	1/10	0/10	0/10	0/10	0/10
Laranja Caipira	*Citrus sinensis* cv. Caipira	10/10	2/6	…	0/9	…	0/10
Café	*Coffea arabica* cv. Catuaí amarelo	…	0/3	…	2/2	…	0/3

[a] Not included in the experiment.
[b] Plants were injected twice with suspensions containing 10^8 to 10^9 CFU of *X. fastidiosa* per ml. Each sample consisted of fibrovascular fluid extracted from 1, 3, or 4 plants present in each pot, 40 and 60 days after the second inoculation date. The samples were processed and evaluated by PCR with the primer pair XF5-F/XF5-R as described (21).

CVC strain. In the first experiment, symptoms were detected in three citrus seedlings at 80 days after the second inoculation date, and with varying degrees of symptoms in all plants 6 months later. In the second experiment, typical CVC symptoms were observed in two of the six inoculated citrus plants 12 months after the second inoculation date.

In the first experiment, attempts were made to isolate the pathogen on solid PW medium from citrus sampled at 3 and 6 months, and from weed species that tested positive by PCR at 2 months after the second inoculation date. *X. fastidiosa* was isolated from all citrus inoculated with the CVC strain. Attempts to isolate the pathogen from weeds were not very successful. The large amount of colonies of contaminant bacteria forced premature elimination of most culture plates. In the second and third experiments, attempts were made to isolate the pathogen from all plants. In order to minimize the number of contaminants, the PW medium was replaced by BCYE, and the samples were diluted 100- and 1,000-fold. In the second experiment, the pathogen was isolated from the two symptomatic citrus and from one *E. crus-galli* plant inoculated with the CVC strain, and from two *B. plantaginea* plants inoculated with the CLS strain. All cultures were confirmed as *X. fastidiosa* by PCR with the primer pair XF5F/XF5R and rep-PCR with the BOX primer (20) (results not shown). The average populations of viable cells in all citrus (including those from the first experiment) and coffee plants were 1.9×10^4 and 2.1×10^5 CFU per gram of leaf midrib. In the third experiment, the pathogen was not isolated from any of the inoculated plants or controls.

Weed colonization by *X. fastidiosa*. The rate of recovery by culturing of the CVC strain of *X. fastidiosa* was variable depending on the species and incubation period (Table 3). *X. fastidiosa* could not be cultured from *E. crus-galli* at 13 days after inoculation. However, at 28 days following inoculation, *X. fastidiosa* colonies were recovered from four of seven plants of this species, in populations ranging from 5.9 to 6.4 $\log_{10}$ CFU per gram of leaf tissue. A similar population estimate was obtained from one plant at 41 days after inoculation, when most of the inoculated leaves were dead. *X. fastidiosa* multiplication was apparently delayed in *B. decumbens*, from which bacterial colonies were recovered only 41 days after inoculation in three of seven plants, in population ranging from 4.5 to 6.3 $\log_{10}$ CFU per gram of tissue.

Among the three grasses included in the study, *B. plantaginea* was the species from which the pathogen was recovered first (Table 3). *X. fastidiosa* was detected in two plants 13 days after inoculation with viable cell population of 4.9 and 5.2 $\log_{10}$ CFU per gram of tissue, and in one plant at 28 days and in another plant at 41 days after inoculation, in populations estimated in 4.6 and 5.6 $\log_{10}$ CFU per gram of tissue. *X. fastidiosa* reached the highest populations in tobacco (5.5 to 7.4 $\log_{10}$ CFU per gram of tissue) at 13 days after inoculation, when most inoculated leaves were showing symptoms consisting of dark orange necrotic spots (21). At the second and third evaluation dates, when all inoculated tobacco leaves and a few lower and upper leaves were symptomatic, all plates had to be discarded due to the presence of contaminants.

On all sampling dates, several plates had to be discarded due to the presence of a large number of contaminants, precluding the isolation of *X. fastidiosa* from many plant samples. Table 3 displays only the results of uncontaminated samples. Leaf midribs seemed to support higher number of *X. fastidiosa* cells since in just one case the CVC strain could be recovered on culture plate from stem. Leaf midrib also had consistently lower numbers of contaminant bacteria than stems.

The second experiment included *B. decumbens*, *B. plantaginea*, *Bidens pilosa*, *S. americanum,* and tobacco. Again the presence of contaminants did not allow *X. fastidiosa* detection in many samples. *X. fastidiosa* was isolated from *Bidens pilosa*, *S. americanum*, and tobacco 30 days after inoculation, with average populations of 5.4, 6.0, and 6.0 $\log_{10}$ CFU per gram of tissue, respectively. Fifty days after inoculation, *X. fastidiosa* was isolated also from *B. decumbens* in an average population of 4.2 $\log_{10}$ CFU per gram of tissue.

Insect transmission trials. During the period of July 1999 to July 2001, four transmission trials were conducted. Between 35 and 80 test plants per plant species of *Bidens pilosa*, *B. plantaginea*, *S. americanum*, *B. decumbens*, *C. sinensis,* and *N. tabacum* were inoculated. The concentration of *X. fastidiosa* in 10 potted plants used as sources of the pathogen for the transmission trials varied from 5.3 to 6.5 $\log_{10}$ CFU per gram of leaf midrib. *X. fastidiosa* was not detected by PCR or culturing 2 and 3 months after inoculation in any of the test plants inoculated in all trials. None of the weed or tobacco test plants developed external symptoms for up to 4 months. Also, no typical CVC symptoms were observed in the citrus test plants, even 12 months after insect inoculations. The survival rate of the leafhopper vector, *F. trivittata*, varied from 36 to 76% on the infected citrus plants used as sources of *X. fastidiosa*. The survival rate of the leafhopper vector on test plants varied from 75 to 96% on *Bidens pilosa*, 71 to 98% on *B. plantaginea*, 81 to 94% in *S. americanum*, 58 to 93% on *B. decumbens*, 46 to 92% on citrus, and 13 to 80% on tobacco plants.

DISCUSSION

X. fastidiosa is known to infect a wide range of plant species (6,10,11,31). We have demonstrated that the strains that cause diseases in citrus, coffee, and plum in Brazil also can infect several herbaceous weed species commonly found in groves of cultivated citrus. The study involving the CVC strain included field surveys, mechanical inoculations, and insect transmission tests. In this study, the CVC strain was detected by PCR in 10 of 23 weed species sampled in two citrus groves affected by the disease. However, only three species, *C. benghalensis*, *C. echinatus,* and *D. insularis* were infected in both locations. The results of this field survey differ from those of a previous survey conducted in Paraná State (36), an area located over 500 km south. *C. benghalensis* and *B. decumbens* were the only species found infected in both regions. In Paraná, *X. fastidiosa* was present in *C. dactylon*, *Sida* sp., and *R. brasiliensis*, which were free from *X. fastidiosa* in São Paulo State. Variations in abundance or number of vector species that are able to transmit the pathogen from infected citrus to weeds could be one reason for the discrepancies observed between the two regions. Because transmission efficiency of the CVC strain by leafhoppers is rather low (13,18), the spread of *X. fastidiosa* to particular weed hosts probably requires the presence of large numbers of vectors that feed both on citrus and on weeds. Vector number also could explain the higher frequency of infected weeds at S. José Farm, a location that had a lower CVC incidence when the surveys were conducted.

Mechanical inoculations were conducted to better assess weed host range for *X. fastidiosa* strains that cause diseases in citrus, coffee, and plum. The detection of *X. fastidiosa* cells at least 5 cm above the inoculation site, 2 months after inoculation, suggests that the bacteria multiplied and were translocated within the weed xylem.

Table 3. Colonization of leaf midribs of weed species and tobacco plants by the citrus variegated chlorosis strain of *Xylella fastidiosa*

	Frequency of infection and concentration of viable *X. fastidiosa* cells ($\log_{10}$ CFU per gram leaf tissue)		
Species	**13 days of incubation**	**28 days of incubation**	**41 days of incubation**
Echinochloa crus-galli	0/5[a]	4/7 (5.9 to 6.4)	1/2 (5.9)
Brachiaria decumbens	0/5	0/8	3/7 (4.5 to 6.3)
Brachiaria plantaginea	2/5 (4.9 and 5.2)	1/5 (4.6)	1/6 (5.6)
Nicotiana tabacum	6/8 (5.5 to 7.4)	Cont.[b]	Cont.

[a] Number of plants positive for *X. fastidiosa* over the total number assayed by culture.
[b] Contamination of all samples prevented culturing.

There was close agreement between CVC and CLS strains concerning weed host species and infection frequencies based on the inoculation experiments. The ranking of species susceptibility was the same for both strains, with *B. plantaginea* as the most susceptible followed by *E. crus-galli*, *B. decumbens*, *S. americanum*, *D. horizontalis,* and *M. sativa*. *Bidens pilosa* was the only host of the CVC strain not colonized by the CLS strain. The PLS strain colonized only *B. plantaginea* and *E. crus-galli*, and at lower frequencies than the CVC and CLS strains. Overall, the mechanical inoculation experiments confirmed the susceptibility of weed species that were positive for *X. fastidiosa* in the field surveys. Close relationship between CVC and CLS strains has been extensively demonstrated based on analysis of DNA fingerprinting (23,24,30,33). Weed inoculations also showed that there is a closer pathological similarity between the CVC and CLS strains than between these strains and the PLS strain of *X. fastidiosa*. The pathological similarity of strains on weeds was not confirmed, however, when inoculated on their original hosts. Although tested in low numbers, citrus and coffee plants were infected by *X. fastidiosa* only when in homologous host/strain combinations. Under greenhouse conditions, only citrus plants developed typical CVC symptoms. The symptomatology described for coffee plants when infected by CLS (16,27) or the CVC strain (15), which includes short internodes, leaf distortion, and necrosis, was not observed in these and in two other cross inoculation experiments (manuscript in preparation).

The higher susceptibility of *B. plantaginea* to the CVC strain was confirmed in the experiment conducted with the objective to assess *X. fastidiosa* multiplication within plant tissues. Differences in incubation periods required for *X. fastidiosa* to be successfully isolated in BCYE suggest differences in multiplication rate of the pathogen inside weeds, as observed for the strain of *X. fastidiosa* responsible for Pierce's disease of grapevine (10). At least 28 days were required for the CVC strain to be isolated from *E. crus-galli*, *B. decumbens*, *S. americanum,* and *Bidens pilosa*, and only 13 days for the pathogen to be isolated from *B. plantaginea*.

Whether weeds play a role in epidemiology of *X. fastidiosa* diseases in citrus, coffee, and plum remains a major question. The natural occurrence, multiplication, and systemic movement of *X. fastidiosa* in weeds commonly present in Brazilian groves indicate that these plants might serve as natural reservoirs for pathogen dissemination in the field. The concentration of viable bacterial cells in mechanically inoculated weed leaves (4 to 6 $\log_{10}$ CFU per gram of leaf midrib) were in the same range observed in symptomatic citrus leaves of plants used as sources of *X. fastidiosa* in the insect transmission experiments, and analogous to population numbers reported for infected citrus in another study (1). This fact suggests that weeds could serve as potential sources of *X. fastidiosa* for vector acquisition and transmission to citrus plants under field conditions. It is possible, however, that those bacterial titers found in weeds resulted from an excessively high number of *X. fastidiosa* cells used as inoculum (6.6 $\log_{10}$ CFU) when compared with the number of cells that would probably accumulate in these plants, after the same time interval, if they had been inoculated by leafhopper vectors. This is supported by the fact that in no case *X. fastidiosa* could be recovered by isolation or PCR from the weed plants inoculated in the insect transmission experiments, even when 30 adults of *F. trivittata* were used per test plant. The amount of cells inoculated by insects during the feeding process is probably much lower than the amount used in the mechanical inoculation. Consequently, the pathogen would require a longer incubation period to reach similar cell titers (4.5 to 6.4 $\log_{10}$ CFU per gram of tissue) within weed leaves. Citrus seedlings inoculated by leafhoppers show CVC symptoms and detectable populations of *X. fastidiosa* only 6 months after inoculation (19).

The short life cycle and the rapid growth rate of the weeds shown to host *X. fastidiosa* also should be considered in the assessment of their importance to CVC epidemics. Most weeds that were PCR positive for *X. fastidiosa* in the field surveys are annuals (22), with life cycles lasting just a few months. They usually grow quickly in the wet summer and die in the dry season (winter and early spring), climatic conditions that prevail in the main citrus growing areas in Brazil. Perennial hosts such as *B. decumbens*, *C. benghalensis*, and *A. tenella* survive through the dry season, but also grow quickly. This can be assessed through analysis of the lifetime of the leaves. Field and experimental observations showed that, during the summer, leaves of *B. decumbens*, the perennial grass most commonly found in citrus groves, do not last longer than 50 days. During this season, mowing is a common practice implemented by the grower to avoid weed infestation in citrus groves. Mowing might contribute to reducing the chances of plant colonization by *X. fastidiosa* by removing eventually infected plant tissues. Conversely, this practice could indeed favor pathogen dissemination among weeds. Although mechanical transmission of *X. fastidiosa* has not been experimentally demonstrated, it constitutes a major means of dissemination of the xylem-inhabiting bacteria *Leifsonia xyli* subsp. *xyli* and *Xanthomonas albilineans* among sugarcane plants during harvesting (35).

Despite the short time frame apparently available for colonization of weeds by *X. fastidiosa*, it should be pointed out that the population of leafhopper species that live on herbaceous weeds is rather high in citrus groves (39). In fact, two sharpshooters known as vectors of *X. fastidiosa* to citrus, *F. trivittata* and *Plesiommata corniculata* (13; J. R. S. Lopes, *unpublished results*), as well as the potential vector *Hortensia similis,* are particularly ubiquitous and abundant on weedy grasses, e.g., *Brachiaria* spp. This high vector population increases the probability of pathogen acquisition and transmission during the transient periods in which the weed hosts would be suitable for bacterial infection and multiplication. The detection of natural infections of *X. fastidiosa* in weeds in the citrus groves is evidence that *X. fastidiosa* is somehow spreading either among weeds or from citrus to weeds.

In this study, we did not evaluate insect transmission of *X. fastidiosa* among weeds, but we found no evidence of transmission of the pathogen from citrus to various weed hosts using *F. trivittata* as a vector, despite the large number of insects confined on the test plants for inoculation. Transmission to citrus and tobacco was not detected either. *F. trivittata* transmitted *X. fastidiosa* from citrus to citrus in another study, but at a very low rate (2.1%) (J. R. S. Lopes, *unpublished data*). Overall, reported transmission rates of the CVC strain to citrus by leafhopper vectors are very low (1 to 12%) (13,19) compared with those of the PD strain to grapevine (>90%) (29). The relatively low population of viable *X. fastidiosa* cells in infected citrus compared with grapevines is thought to be a factor limiting acquisition efficiency by vectors when feeding on citrus source plants (1). Higher populations of *X. fastidiosa* in plants resulted in higher rates of transmission by *Graphocephala atropunctata* (10). We also noticed a significant mortality of *F. trivittata* adults on citrus during the 48-h acquisition access period, suggesting little or no feeding took place on the source plants, which might have affected acquisition efficiency as well. As variation in transmission efficiency of *X. fastidiosa* to citrus has been observed for distinct species of leafhopper vectors (13,18), the results of the present study could be different if vector species more efficient than *F. trivittata* had been used in the insect transmission experiments.

The feeding preference of the vectors is an additional factor that could affect the chances of transmission of *X. fastidiosa* from citrus to weed in the field. Although under experimental conditions caged grass leafhoppers may feed and transmit *X. fastidiosa* from citrus to citrus (13), field studies clearly show a preference of the insect for weedy grasses (25,39). *F. trivittata* adults can eventually be detected in the citrus canopy, but its preferential host is *B. decumbens*, from which all individuals used in our experiments were captured. While feeding preference of *F. trivittata* might reduce the chances of *X. fastidiosa*

transmission between citrus and weeds, it could favor pathogen spread among weeds after successful infection is established in a weed host.

In this study, emphasis was given to assess the potential of weeds as possible reservoirs of *X. fastidiosa* to citrus plants. Evidence was presented indicating that the amounts of *X. fastidiosa* cells that may accumulate in weeds inoculated by leafhoppers are probably under insect acquisition thresholds, a factor that would limit their importance to the CVC epidemics. This is corroborated by spatial and temporal analysis of symptomatic citrus trees in the field, which indicated that infected trees are the main source of inoculum to spread to healthy trees (14). The considerations made on the importance of weeds as a component of the CVC epidemics should not be totally extrapolated to the coffee or plum/*X. fastidiosa* pathosystems. Only field surveys and insect transmission experiments would bring more consistent information for these systems. The apparent lack of specificity of the local CVC and CLS strains toward the weed species tested suggests, however, that these weeds might be colonized by more than one strain of *X. fastidiosa* in the field. Since infected weeds usually do not manifest external and unequivocal symptoms, their identification can be achieved more easily by the use of sensitive techniques such as PCR. The PCR technique will certainly be better explored when primers allowing separation of the distinct *X. fastidiosa* strains are available.

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