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Detection of *Xylella fastidiosa* in Weeds and Sharpshooters in Orange Groves Affected with Citrus Variegated Chlorosis in Misiones, Argentina

O. R. de Coll, A. M. M. Remes Lenicov, J. P. Agostini, and S. Paradell

ABSTRACT. Pecosita was first found in Valencia sweet orange groves around Montecarlo, Misiones in 1984. Pecosita has similar symptoms to citrus variegated chlorosis (CVC) of Brazil, and it is positive for CVC by serological tests using antiserum UF-26 for *Xylella fastidiosa*. The objective of this work was to detect the potential sharpshooter vectors of *X. fastidiosa* and weed hosts of the bacterium in sweet orange groves with CVC symptoms using dot immunobinding assays (DIBA). The weed and sharpshooter surveys were carried out in two sweet orange groves four times: February, July, October, and November 1995. The insects were caught from citrus trees and natural vegetation using yellow sticky traps and nets, respectively. The following adult life stage sharpshooters were positive for *X. fastidiosa*: *Bucephalogonia xanthophis*; *Scopogonalia subolivacea; Hortensia similis*; *Rotigonalia limbatula*; *Sonesimia grossa; Chlorotettix* latocintus; and *Curtara samera* from the Cicadellidae family and two species of the Membracidae family: *Ceresa* ustulata; and *Entylia carinata*. Furthermore, *S. grossa* was positive in the nymph stage. At least once during the year the following weeds were positive for *X. fastidiosa*: *Conium maculatum*; *Chloris halophila; Digitaria sp.*; *Paspalum regnelli*; *Axonopus compresus*; *Blainvillea biaristata; Commelina erecta*; *Medicago polymorpha; Phalaris angusta; Lepidium aetes*; *Stachys arvensis; Senecio cfr. geisebachii*; *Talinum paniculatum*; and *Sida rhombifolia*.

In 1984 a new disease, locally called pecosita, which is characterized by small fruit and chlorosis in the leaves with the presence of the gum spots on the lower side of the leaves (2), was detected in Valencia sweet orange groves in Misiones, Argentina. The symptoms of pecosita are very similar to those described for citrus variegated chlorosis (CVC) detected in Brazil in 1987, which is caused by the bacterium *Xylella fastidiosa* Wells & Raju and transmitted by sharpshooters (17, 26).

In Argentina, plum leaf scald has been present in the Paraná Delta since 1935 (8), however, 40 yr later, the causal agent of this disease was identified as *X. fastidiosa* (14). This bacterium has been associated with a disease of almond in other areas (22).

Xylella has been found in other symptomatic and non-symptomatic plant hosts, which represent several plant species from different families. At least 28 families of monocotyledonous or dicotyledonous plants are natural hosts of the bacterium (11, 21), and they could be a natural source of the pathogen, and, of course, they may play an important part in the epidemiology of different diseases (9).

Sharpshooters (Homoptera: Auchenorrhyncha), are insects which feed on plants by sucking plant sap from xylem, causing serious problems of varying severity to certain plant hosts by their ability to transmit viruses or procaryotes (23). Two surveys carried out in Misiones citrus groves (6, 7, 25) detected a large number of sharpshooters which are considered vectors of viruses and procaryotes causing several diseases around the world on many plants (19, 20).

Different serological techniques can be used as diagnostic tools in epidemiological studies, in the detection of healthy plants, for detection of natural hosts or potential vectors of different diseases (3, 9, 10). In Brazil, the serological dot immunobinding assay (DIBA) has been used to select potential vectors of CVC from citrus groves of São Paulo, Brazil (4, 16). DIBA is practi-

cal and economical, which allows use of a large number of samples to detect the bacteria from different hosts such as citrus trees, weeds or sharpshooters from the field or in experimental transmission trials.

The objective of this work was to use DIBA to determine the ability of sharpshooters detected in sweet orange groves to be potential vectors; and to find possible hosts of *X. fastidiosa* in the natural weeds surrounding citrus trees affected by CVC.

MATERIALS AND METHODS

Samples were collected from two citrus groves with a high incidence of CVC located near Montecarlo, Misiones, Argentina. The first grove was an experimental plot of 3 ha of 16-yr-old Valencia sweet orange on several rootstocks planted at a density of 7m × 6m between rows and trees, respectively. In this grove, CVC symptoms were detected in 1984 and the disease incidence was about 71% in 1995 (1). The second grove was a commercial grove and the samples were collected from a 2 ha plot of 7-yr-old Valencia sweet orange on Volkamer lemon planted on a spacing of 4m × 6m with an incidence of CVC of 13% in 1994 and an increase to 55% in the following year (1). The CVC incidence in both groves was recorded during the winter when the symptoms in leaves and fruit size were more evident.

The weeds and sharpshooter samples were collected at the same time from both groves on four dates: February; July; October; and November, 1995. The Cicadellidae present on the natural vegetation of both groves were caught with the classical entomological net and hand captured from the citrus trees. Five samples were taken from each grove at each time, and the sample unit was 50 successive strokes of the net. The total number of sweeps taken as unit was previously determined by the accumulated frequency method.

Insects captured were placed in glass tubes with cotton caps and transferred to the laboratory, where they were classified to species. In less than 24 hr after collection the cibaria were separated under stereoscopic microscopy and placed in 2 ml microfuge tubes. Samples had from 1 to 5 cibaria depending on the availability of the species.

Weeds were collected at each sample time depending on their presence in the groves. The weeds were collected in a diagonal transect of the plot, taking mainly those with different degrees of chlorosis caused by unknown factors. The plants were placed in plastic bags for transport to laboratory. Four to 10 samples of each weed species and five subsamples from each were prepared.

The DIBA serology test was applied to both types of samples according to the technique of Lee et al. (15). Valencia sweet orange leaves with CVC symptoms were used as positive controls; whereas leaves of sweet orange trees and *Catharanthus roseus* (L.) G. Don growing in a greenhouse from seeds were used for negative controls.

RESULTS

Using the DIBA technique, 13 species of weeds were positive for *X. fastidiosa*, of which nine were from the experimental plot and four were from the commercial plot (Table 1). The species *Chloris halophila*, *Digitaria sp.*, *Sida rhombifolia*, were present at all survey times, but only were positive during the survey at the end of summer. *Commelina erecta* was present at each sample date, but they were positive only during the winter survey in samples collected from the experimental plot.

During the spring and summer surveys, a large number of species were observed, but no samples were positive in October (spring) and only 10% were positive in November. Positives were obtained in the samples

TABLE 1
WEEDS POSITIVE FOR THE PRESENCE OF *XYLELLA FASTIDIOSA* BY DOT IMMUNOBINDING ASSAY IN TWO VALENCIA SWEET ORANGE GROVES WITH SYMPTOMS OF CITRUS VARIEGATED CHLOROSIS DURING 1995

	February		July		October		November	
Location and weed species	No. samples	% Positive[z]	No. samples	% Positive[z]	No. samples	% Positive[z]	No. samples	% Positive[z]
Experimental Plot								
Commelina erecta L.	0	0	6	33	0	0	0	0
Blainvillea biaristata DC.	0	0	0	0	0	0	10	10
Senecio cfr. Geisebachii Baker	0	0	0	0	0	0	10	10
Axonopus compresus (Sev.) P. Beaur	4	50	0	0	0	0	0	0
Chloris halophila Parodi	4	50	0	0	0	0	0	0
Digitaria sp.	4	50	0	0	0	0	0	0
Stachys arvensis K (L.)	0	0	6	66	0	0	0	0
Medicago polymorpha L.	4	100	0	0	0	0	0	0
Sida rhombifolia L.	4	50	0	0	0	0	10	10
Commercial Grove								
Senecio cfr. Geisebachii Baker	0	0	0	0	0	0	10	20
Paspalum regnellii Schrad. Ex Schult.	0	0	20	70	0	0	0	0
Phalaris angusta Nees ex Trin.	0	0	6	33	0	0	0	0
Talinum paniculatum (Jacq.) Gaeiln	0	0	0	0	10	20	0	0
cfr. Conium maculatum L.	0	0	0	0	0	0	10	20
Controls								
Positive (sweet orange leaves w/CVC)	4	100	2	100	4	100	2	100
Negative (sweet orange leaves greenhouse)	4	0	2	100	4	0	2	0
Catharanthus roseus (greenhouse)	1	0	1	0	1	0	1	0

[z]Dot immunobinding assay test using the UF-26 antiserum.

of *Blainvillea biaristata*, *Senecio cfr.* geisebachii, and *Sida rhombifolia*. In the commercial grove in the October survey, only 20% of the samples from one species, *Talinum paniculatum*, were positive and during November 20% of the samples of *Conium maculatum* and *S. cfr. geisebachii* were positive.

Of the sharpshooters associated with citrus, 10 species and 3 families of Cicadellidae were found to be positive by DIBA for *X. fastidiosa*. Of these samples six species and two families were collected from the experimental plot at different times; and six species and one family from the commercial grove were also positive (Table 2). Two species, *Ceresa ustulata* and *Bucephalogonia xanthophis,* were positive for *X. fastidiosa* at both sample sites.

More species that were positive were collected during the July survey from the experimental plot and in November for both locations. In the July survey, all the samples collected of the subfamily Gyponinae and those of the species *Hortensia similis* were 100% positive. Also, 91% of the samples of *Sonesimia grossa* were positive for *X. fastidiosa*. For the latter sample, adult insects were analyzed independently of the nymph state. All samples from fifth nymphal stage of this species were positive (data not shown).

In the October survey, only samples from the experimental plot were positive. The species *B. xanthophis* had 33% of the positive samples. For the same location 100% of the samples of *C. ustulata* and *Rotigonalia limbatula* in the November survey were positive, whereas the adults of *S. grossa* were 80% positive and those *Curtara samera* were 50% positive. The summer survey was the only time of all sample dates where sharpshooter were be positive by DIBA at the commercial grove. However, a larger number of positive samples were detected at this time in this location. Also in this survey, 50% of the fifth nymphal instars of the Cicadellidae collected were positive.

DISCUSSION

With DIBA, the presence of *X. fastidiosa* was detected in some weeds surrounding citrus trees with CVC symptoms in the region of Montecarlo, Misiones, Argentina and in the sharpshooters associated with this pathosystem. Among the sharpshooters that were identified as positive for *Xylella* are the following species: *Bucephalogonia xanthophis*; *Scopogonalia subolivacea; Hortensia similis; Rotigonalia limbatula; Sonesimia grossa; Chlorotettix latocintus;* and *Curtara samera* in the Cicadellidae family; and two species in the Membracidae: *Ceresa ustulata* and *Entylia carinata*. Different life stages of the different species were analyzed, but only the adult was positive with exception of the nymphal stage of *Sonesimia grossa*. This species was found in both citrus trees and in the weeds.

Some cicadellids such as *Acrogonia sp.*, *Dilobopterus costalimai*, and *Oncometopia* sp. are recognized as positive for *Xylella* in Brazil (18). Also *Oncometopia undulata*, *Homalodisca coagulata*, and *Cuerna costalis* were found to be vectors of Pierce's disease of grapes and for *X. fastidiosa* to the weed *Ambrosia artemisifolia* (5).

In California, *X. fastidiosa* was detected by ELISA in *Conium maculatum*, *Paspalum dilatatum*, and *Vinca minor* among different weeds associated with grape vineyards, in studies related to Pierce's disease (11). In Brazil, where CVC is present; *X. fastidiosa* has been detected in weeds such as *Digitaria sp.* and *Lolium sp.* in the São Paulo State; or in the Paraná State where in addition to CVC in citrus, *Xylella* has been detected also in plum causing the plum leaf scald and in other weeds such as the *Chloris sp.*

TABLE 2
CICADELLIDAE POSITIVE FOR *XYLELLA FASTIDIOSA* BY DOT IMMUNOBINDING ASSAY IN TWO VALENCIA SWEET ORANGE GROVES WITH SYMPTOMS OF CITRUS VARIEGATED CHLOROSIS DURING 1995

Location and Cicadellidae species	February		July		October		November	
	No. samples	% Positive[z]	No. samples	% Positive[z]	No. samples	% Positive[z]	No. samples	% Positive[z]
Experimental Plot	0	0	0	0	0	0	0	0
Bucephalogonia xanthophis	0	0	0	0	6	33	0	0
Hortensia similis	0	0	2	100	0	0	0	0
Rotigonalia limbatula	0	0	0	0	0	0	2	100
Sonesimia grossa	0	0	14	91	0	0	0	0
Curtara (C.) samera	0	0	0	0	0	0	2	50
Ceresa ustulata	0	0	0	0	0	0	4	100
Cicadellinae	4	100	0	0	0	0	0	0
Gyponinae	0	0	2	100	0	0	0	0
Commercial Grove								
Bucephalogonia xanthophis	0	0	0	0	0	0	2	100
Macugonalia leucomelas	0	0	0	0	0	0	2	100
Scopogonalia subolivacea	0	0	0	0	0	0	4	100
Chlorotettix latocinctus	0	0	0	0	0	0	2	100
Ceresa ustulata	0	0	0	0	0	0	2	100
Entylia carinata	0	0	0	0	0	0	2	100
Cicadellinae 5th instars	0	0	0	0	0	0	2	50
Controls								
Positive (sweet orange leaves w/CVC	4	100	2	100	4	100	2	100
Negative (sweet orange leaves greenhouse	4	0	2	0	4	0	2	0
Catharanthus roseus (greenhouse)	1	0	1	0	1	0	1	0

[z]Dot immunobinding assay test using the UF-26 antiserum.

The different CVC incidence through time, recorded in citrus groves of Misiones since 1984 when the disease appeared in the region, show that this disease has alternative cycles of incidence with high peaks followed by years where the incidence decreases (1). This seasonal variation occurs not only in the CVC symptoms in Brazil (24) but also in the *Xylella* concentration of blighted trees and grapes from Florida (12, 13), where a larger number of positive samples were detected by serology at the end of the summer and fall. In the Misiones environment, the presence of symptoms in trees affected with CVC are very difficult to find after the spring flush, but this could be due to the long incubation time of the disease for symptom development in the newer leaves.

Considering the number of sharpshooters and weeds that are positive serologically, a large number of host-vector combinations must be tested in the near future to transmit CVC by vectors.

The weed species *C. erecta*, *Chloris sp*, *Digitaria sp.* and *Sida rhombifolia* are present year round in citrus groves with CVC in our region, thus these weeds together with *S. cfr. geisebachii* (also positive at both groves) could be a primary inoculum source of *X. fastidiosa*. As potential vectors, transmission tests for CVC could be conducted with *Ceresa ustulata* and *Bucephalogonia xanthophis* because they were found at both groves, and also with *Sonesimia grossa* because of the higher population level on citrus groves (7).

Previous to these trials, more specific techniques such as PCR or Western blot should be used to determine whether the *X. fastidiosa* involved in these hosts and vectors are the same as that present in the citrus trees affected by CVC, or other strains of *X. fastidiosa* related to plum leaf scald or phony peach, that also could be present in the region. This objective should be pursued when weeds and possible vectors of CVC were positive at the end of the summer or fall months, because the probability of positive samples is higher.

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