Citrus Stubborn Disease in Iraq and Syria: Correlation Between Symptom Expression and Detection of Spiroplasma citri by Culture and ELISA

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ABSTRACT. Detection of *Spiroplasma citri* Saglio *et al.* by enzyme-linked immunosorbent assay (ELISA) and by culturing has been used to diagnose citrus stubborn disease in Syria and Iraq. There was an excellent correlation between symptom expression and detection of *S. citri* by these techniques. Stubborn symptoms in Syria are similar to those in California and Mediterranean countries. The disease is widespread within the Lattaquia-Tartous area. Young, 3-4 year-old trees propagated in this area from budwood found to be free of stubborn by repeated indexing in Corsica, showed stubborn symptoms and were found to be infected with *S. citri*. Natural spread of the spiroplasma is probably involved.

In Iraq, three types of symptoms were used to detect stubborn trees in January: small, lopsided fruit with aborted seeds, sucker-like shoots with blunt, ovoid or palmate leaves, and leaf mottle. All the trees showing these symptoms were infected by *S. citri*. These symptoms can be used to reliably diagnose stubborn in the winter in Iraq. Stubborn seems to be widespread in Iraq since the disease was detected in all the areas surveyed: Fahama, Rachidiya, Abou-dali, Houwesh, Kerbala, Tarmia and Beled.
Index words. aborted seeds, fruit axis.

Natural transmission of *Spiroplasma citri*, the causal agent of citrus stubborn disease, has been demonstrated in California (4) and Morocco (1, 7). Recently, young citrus trees propagated in Syria from budwood free of stubborn (as determined by indexing on Mme Vinous sweet orange seedlings in Corsica) developed typical stubborn symptoms. Infection of these trees by the stubborn agent implies natural transmission by insect vectors. It was therefore important to assess that *S. citri* was indeed present in these trees as well as in trees of commercial orchards throughout the area by criteria other than symptomatology. ELISA and culturing have been used to detect *S. citri* in 56 trees and compared to detection based on symptoms.

Citrus in Iraq consists mainly of the local Mahali sweet orange on sour orange rootstock. Trees are grown under the shade of date palms and this practice is supposed to protect them from the heat of the summer when the temperatures go well above 40-45°C. Surveys carried out in the winter of 1980 and 1981 by J. M. Bove resulted in the isolation and culture of *S. citri* from trees with unusual symptoms. These trees did not have, or showed only poorly, the bushiness and the bunchy type of growth so characteristic of stubborn trees in open (unshaded) orchards. Instead, the trees had rather sparse foliage with relatively large leaves, but little or none of the small cupped leaves or shoots with erect, upright leaves so typical of stubborn elsewhere. The following three symptoms were always well pronounced: 1) fruit of small to very small size, often lopsided with many or all seeds aborted; 2) presence of a few sucker-like shoots, sticking out of the top or the sides of the tree, with pale-green leaves of various types (ovoid with short midrib, palmate, pinched-in or yellow at the tip) and often abnormally short internodes. 3) Leaf mottle, affecting relatively well-developed, mature leaves.

During a third survey, in January 1982, trees were selected in commercial orchards on the basis

of the above three symptoms. Fruit was collected from these trees and analyzed by ELISA and culture for the presence of *S. citri*. In this paper we report an excellent correlation between symptom expression of stubborn as it occurs in Syria or Iraq, and the detection of *S. citri* by ELISA and culture. The presence of stubborn disease in many orchards of Syria and Iraq is therefore established.

MATERIALS AND METHODS

Detection of *S. citri* by ELISA (8) and by culturing (2, 9) was as described previously.

In Syria, 54 trees were selected from eleven different locations in the Lattaquia-Tartous area in October 1982. Most had stubborn symptoms. Twenty-one of these trees, representing 19 different species or varieties, were 3 to 4 years-old and had been propagated at El Annadeh from stubborn-free budwood of Corsican origin. All had stubborn symptoms. The 33 other trees from 10 different orchards were mostly old-line Washington navel or Valencia sweet orange trees, 10 to 20 years old. Most of these trees had stubborn symptoms. A few were selected as apparently healthy controls for *S. citri* detection. The following plant material was used, according to availability: very young leaves, mottled leaves, flowers, aborted seeds and peduncular ends of fruit axes.

In Iraq, in January 1982, 26 trees showing the three symptoms described above and seven trees showing questionable symptoms were selected in 15 commercial orchards, North of Baghdad, along the left bank of the river Tigris. All trees were of the local Mahali sweet orange variety except in one orchard where Washington navel sweet orange trees were planted. All trees were on sour orange rootstocks. Five to ten fruits with stubborn symptoms (small size, lopsided, aborted seeds) were collected on each tree and taken to Bordeaux. Each fruit sample was subdivided into seeds and peduncular ends of fruit axes (3). The ELISA and the culture assay were carried out on both the seeds and the fruit axes.

RESULTS AND DISCUSSION

Stubborn in Syria. Table 1 shows that 72% of the 47 trees with stubborn symptoms gave a positive ELISA, 81% yielded a *S. citri* culture and 89% were found positive on the basis of both assays. The 3 trees with questionable symptoms and the 4 apparently healthy trees gave negative results.

The results of ELISA and culture are shown in Table 2 for the three different plant materials used for the assays, and compared with symptom expression of stubborn. The fruit axes from 28 trees were analyzed by both techniques. Table 2 shows that 20 trees tested positive by either one or both assays. These 20 trees showed stubborn symptoms. Both assays were positive for 17 (85%) of the 20 trees. Seeds from seven trees with stubborn

TABLE 1
DETECTION OF *SPIROPLASMA CITRI* IN SYRIA BY ELISA AND BY CULTURING

Symptoms	No. of trees analyzed	Number of trees with positive detection of *S. citri* by:		
		ELISA (E)	Culture (C)	(E) + (C)
Stubborn symptoms	47	34	38	42
Dubious symptoms	3	0	0	0
No symptoms	4	0	0	0

TABLE 2
CITRUS STUBBORN DISEASE IN SYRIA: CORRELATION BETWEEN SYMPTOM EXPRESSION AND DETECTION OF *SPIROPLASMA CITRI* BY ELISA AND BY CULTURING

Plant material	*S. citri* detection by ELISA (E)	*S. citri* detection by Culture (C)	Number of trees with indicated (E) or (C) results		Number of trees with: Stubborn symptoms	Dubious symptoms	No symptoms
Fruit axis	+	+	17	20	17	0	0
	+	—	1		1	0	0
	—	+	2		2	0	0
	—	—	8		2	2	4
Seeds	+	+	7	7	7	0	0
	+	—	0		0	0	0
	—	+	0		0	0	0
	—	—	2		0	0	2
Leaves	+	+	14	20	14	0	0
	+	—	3		3	0	0
	—	+	3		3	0	0
	—	—	6		4	1	1

symptoms tested positive by both assays. Leaves from 14 of 20 trees (70%) tested positive by both assays; all 20 trees with stubborn symptoms tested positive by at least one assay.

Table 2 also shows that in the case of fruit axes, eight trees gave negative results for both ELISA and culture. Of these eight trees, four did not show stubborn symptoms, two had dubious symptoms and two expressed definite symptoms of the disease. With leaves, four trees of six showed stubborn symptoms even though both ELISA and culture were negative.

In summary, 91% (20 of 22 stubborn trees) of fruit axes, 100% (seven of seven) of seeds and 83% (20 of 24) of leaves gave a positive ELISA and/or a positive culture assay. Combining the results of tables 1 and 2, it appears that in Syria, when trees are examined for symptoms in October and when at the same time fruit and leaves are collected for *S. citri* detection, about 90% of the trees with stubborn symptoms gave a positive culture. The two assays failed to confirm the presence of *S. citri* in the remaining 10% of stubborn trees.

The relatively high percentages (10%) of stubborn trees failing to give a positive ELISA and/or culture assay is probably due to the use of leaves (in the absence of fruit on young trees) as the plant material for some assays. Leaves give less consistent results than aborted seeds and/or fruit axes. In Iraq (see below) where only fruit was used, *S. citri* could be detected in all trees with stubborn symptoms.

The presence of stubborn, suspected for many years on the basis of symptomatology (5) was confirmed by ELISA and/or culture of *S. citri* in all ten commercial orchards surveyed between Lattaquia and Tartous. In certain orchards, the percentage of affected trees was very high. Thus, stubborn disease seems to be widespread in the surveyed area. Within this area, at El Annadeh, 3-4-year-old trees, propagated in 1978-1979 from budwood found free of stubborn by repeated indexing in Corsica, show stubborn symptoms (Table 3). ELISA and/or culture have confirmed the presence of *S. citri* in many of these trees (Table 3). According to these results a high incidence of natural spread of *C. citri* from 1978 to 1982 must have occurred.

Stubborn in Iraq. During the January 1982 survey, 26 trees were selected on the basis of their fruit symptoms, their abnormal shoots and their leaf mottle, as indicated above. Seven trees with dubious symptoms were also chosen. *S. citri* detection was carried out by ELISA and culture, on both seeds and peduncular ends of fruit axes. Four assay results were obtained for each fruit sample. Table 4 indicates that among the 26 affected trees, 18 (70%) were indexed positive by all four assays, six (23%) had three positive assays, and two had one or two positive assays. None of the affected trees indexed negative. These results show that the great majority of trees (96%) had either three or four positive assays. In the case of the trees with dubious symptoms, three yielded *S. citri* cultures, while four were negative by all assays. Table 5 presents (for all 33 trees) the number of positive and negative trees for a given assay or combination of assays. Seeds were somewhat superior to fruit axes when culture assay was used, since 27 *S. citri* cultures were obtained from seeds and only 21 from fruit axes. The two tissues seemed equally good for ELISA, and ELISA and culture seemed to have the same sensitivity. However, the highest number of positive trees

TABLE 3
CITRUS STUBBORN DISEASE IN SYRIA: DETECTION OF *SPIROPLASMA CITRI* IN YOUNG TREES PROPAGATED FROM STUBBORN-FREE BUDWOOD*

Species or Varieties†	Origin and year of release or introduction in Corsica	Result of *S. citri* detection in plant tissue‡ by:				
		ELISA (E)		Culture (C)		(E) and (C)
Atwood navel 157	California 1966	L, F§	+	L, F	+	+
Atwood navel 157	California 1966	Fl	+	Fl	+	+
Newhall navel 182	California 1972	L	—	L	—	—
Washington navel 39	Tunisia	L	+	L	—	+
Washington navel 141	California	L	+	L	—	+
Washington navel 141	California	L, Fl	—	Fl	+	+
Washington navel 204	Corsica (Nucellar) 1977	L	+	L	+	+
Washington navel 215	Corsica (Nucellar) 1974	L	+	L	+	+
Washington navel 216	Corsica (Nucellar) 1974	L	—	L	+	+
Cadenera 232	Corsica (Nucellar) 1977	L	+	L	+	+
Hamlin 250	Corsica (Nucellar) 1980	L	+	L	+	+
Madam Vinous	Corsica (Nucellar)	L	+	L	+	+
Valencia late 35	Tunisia	F	+	L, F	+	+
Valencia late 139	California 1966	L	+	L	+	+
Valencia late 246	Corsica (Nucellar)	L	+	L	+	+
Little River grapefruit 187	California 1972	L	+	L	+	+
Clementine 15	California 1961	L	+	L	—	+
Encore mandarin 190	California 1972	L, Fl	—	L, Fl	—	—
Malvasio mandarin 115	Morocco 1960	L	+	L	+	+
Owari satsuma 221	Corsica (Nucellar)	L	—	L	—	—
Nova tangelo 158	California 1967	L	+	L	+	+

*Based on repeated indexing in Corsica.
†All trees showed stubborn symptoms.
‡L = leaf; F = stem end of fruit axis; Fl = flower.
§Assay was carried out on both plant tissues.

TABLE 4
CITRUS STUBBORN DISEASE IN IRAQ: CORRELATION BETWEEN SYMPTOM EXPRESSION AND DETECTION OF *SPIROPLASMA CITRI* IN SEEDS AND FRUIT AXES BY ELISA AND BY CULTURING

S. citri detection				Stubborn symptoms	Doubtful symptoms
in Seeds		in Fruit Axis		No. of trees with indicated (E) & (C) results	No. of trees with indicated (E) & (C) results
ELISA (E)	Culture (C)	ELISA (E)	Culture (C)		
+	+	+	+	18	1
+	+	+	—	5	0
—	+	+	+	1	0
+	—	+	—	1	0
—	+	—	—	1	1
—	—	—	+	0	1
—	—	—	—	0	4
				Totals 26	7

(29) and the smallest number of negative ones (4) were obtained when all four assays (Table 5, column on right) were used.

All 26 trees selected on the basis of positive symptoms yielded *S. citri* cultures (except tree number 27) and positive ELISAs (except tree number 16). Tree 27 (negative in the culture assay) gave a positive ELISA with both seeds and fruit axes. A *S. citri* culture was obtained from the seeds of tree 16 (negative in ELISA).

These results indicate that there is an excellent correlation between the symptoms used for stubborn diagnosis in Iraq at the time of the survey (January) and the presence of *S. citri* in these trees.

Trees infected by *S. citri* in Iraq do not show or show only poorly the bushy type of growth, the many small, cupped leaves with pale or yellow tips, the shoots with upright leaves, so typical of stubborn trees in California and Mediterranean countries. They had abnormal, sucker-like shoots with ovoid or palmate leaves. Others have indicated that high temperatures in the summer (44-50°C) rather than stubborn disease, could be responsible for the development of atypical shoots with abnormal ovoid or palmate leaves in Iran (6). In Iraq it seems that only the trees infected with *S. citri* had shoots with abnormal leaves. The hot, shaded Iraq environment might favor the development of the atypical shoots on the infected trees.

Every one of the 15 orchards

TABLE 5
DETECTION OF *SPIROPLASMA CITRI* BY ELISA (E) AND CULTURE (C) IN SEEDS AND FRUIT AXES OF 26 STUBBORN AND 7 QUESTIONABLE TREES IN IRAQ: COMPARISON OF ASSAYS

S. citri detection assay	Number of positive and negative trees per assay or combination of assays						
	Seed (S)		Fruit Axis (FA)		(S) or (FA)		(S) or (FA)
	E	C	E	C	E	C	E or C
+	25	27	27	21	26	28	29
—	8	6	7	12	7	5	4