New Findings on the Epidemiology of *Spiroplasma citri* in the Eastern Mediterranean Region of Turkey

U. Kersting, H. Başpinar, A. Çinar, Ç. Şengonca and N. Uygun

ABSTRACT. Noncitrus plant hosts and leafhopper vectors of *Spiroplasma citri* found in the citrus-growing area of the Eastern Mediterranean region of Turkey were determined by ELISA, culture and transmission tests. Four plant hosts of *S. citri* were found, the most important being sesame, a common crop in the region. *S. citri* was cultured from seven leafhopper species, including two closely related, but distinct species of the genus *Circulifer* Zakhvatkin belonging to two subgeneric species complexes, namely *C. haematoceps* (M. & R.) complex and *C. opacipennis* (Lethierry) complex. They differed in both their range of host plants and in the male genitalia shapes. The apex of the style of *C. haematoceps* was slightly bent, while *C. opacipennis* possessed strong laterally curved apical extensions of the style. The only host plant of the latter complex was *Salsola kali*, while *C. haematoceps* was shown to be natural vector of *S. citri*, transmitting it from sesame to periwinkle. It is assumed that sesame and the associated *C. haematoceps* complex play an important role in the epidemiology of stubborn, while that of the *C. opacipennis* complex remains unclear.

Noncitrus hosts play an important role in the epidemiology of *Spiroplasma citri* because citrus is not an adequate host of most leafhopper vectors (1, 2, 7), and it is questionable whether or not leafhoppers can acquire and transmit *S. citri* from stubborn-diseased trees several years after the original infection (3). Besides periwinkle, *Hirschfeldia incana* and *Sisymbrium irio* (Brassicaceae) were found to be host plants of the stubborn pathogen in the Eastern Mediterranean (5). The only known vector in this area is *Circulifer haematoceps*, and prickly saltwort (*Salsola kali*, Chenopodiaceae) is supposed to be its most important host plant there (2, 3, 6). Recently Klein and Raccah (9) reported that species of the *C. haematoceps* complex differ strongly in distribution and host range in Israel. Members of neither of the two populations they studied were capable of transmitting *S. citri*.

The purpose of this study was to identify the hosts and natural vectors of *S. citri* in the Eastern Mediterranean region of Turkey.

MATERIAL AND METHODS

Hosts of *S. citri*. During the two-year period from 1988 to 1990, we collected weeds and crop plants which were growing in the Eastern Mediterranean region of Turkey, and which appeared either healthy or stunted and chlorotic. ELISA tests were performed on midribs of young leaves processed as described by Saillard and Bové (13), using a polyclonal antibody prepared against SPA-strain Israel. Midribs with a narrow band of accompanying leaf blade were cut from plants suspected of harboring the pathogen, and chopped in 5 ml SMC-medium (12). Resulting preparations were incubated at 32 C, and the presence of spiroplasma in positive cultures was verified by dark-field microscopy. ELISA was used to determine the serological relationship of cultured spiroplasmas to *S. citri*.

Leafhoppers and association with *S. citri*. Leafhoppers were collected from various wild and cultivated plants using a D-VAC aspirator throughout

the year from 1988 to 1990 and again in 1992. Field identification of the leafhoppers was confirmed by microscopic examination of dissected male genitalia in the laboratory. ELISA of leafhoppers was performed as described previously (8), and culture of the stubborn pathogen from leafhoppers was carried out according to Markham, *et al.* (10) and confirmed by ELISA.

To determine if various field-collected leafhoppers were inoculative for *S. citri*, they were caged on young healthy periwinkle held at 32 ± 1 C, 75 ± 5% RH and 16 hr of artificial light. After 72 hr the plants were sprayed with Supracide and kept in a greenhouse at 30 ± 2 C for at least 12 weeks. Periwinkle plants that developed symptoms were tested for the stubborn pathogen using ELISA and the direct culture method.

RESULTS

Hosts of *S. citri*. About 250 plant samples, representing 27 species in 15 families were collected and assayed for *S. citri*. Only six species showed positive ELISA readings (Table 1). *Xanthium* sp. (Asteraceae) and maize reacted nonspecifically using healthy plant sap. Interestingly, ELISA results suggested that *Sorghum halepense* (Poaceae) was infected with *S. citri*, but the organism could not be cultured from any of 21 samples of this species. *S. citri* was cultured from periwinkle, *Crepis echiodes* (Asteraceae), *Echium* sp. (Boraginaceae) and sesame (*Sesamum indicum*, Pedaliaceae) and showed a close antigenic relationship between all isolates and *S. citri* (SPA strain Israel) was shown by ELISA. A low percentage of tested plants of *Digitaria sanguinalis* (Poaceae) and *S. kali* tested positive by ELISA, but *S. citri* was not culturable from either species.

Leafhopper and association with *S. citri*. In all, members of 41 different identifiable taxa of Cicadellidae were collected during the study. Adult forms belonging to two distinct sub-generic groups of the genus *Circulifer* were recognized, which differed strongly in distribution, host plants and shape of the male genitalia. These complexes were tentatively assigned to the *C. haematoceps* complex and the *C. opacipennis* complex. Those of the latter exhibited a style with a truncate or sometimes rounded, and abruptly laterally directed apex, while in those assigned to the *C. haematoceps* complex, the apical extension of the style was slightly bent (Fig. 1). We were only able to collect members of the *C. opacipennis* complex from *S. kali*, the geographical occurrence of these being remarkably similar. Leafhoppers of the *C. haematoceps* complex were found widely and evenly distributed throughout the region on at least 27 host plants.

Members of 31 distinguishable taxa were tested by ELISA or culture for the presence of *S. citri*. It was cultured from seven species, including both complexes of *Circulifer* (Table 2). In tests of natural inoculativity, only leaf-

TABLE 1
NATURAL PLANT HOSTS OF *SPIROPLASMA CITRI* AS DETERMINED BY ELISA AND CULTURE IN THE EASTERN MEDITERRANEAN REGION OF TURKEY IN 1988-1990

Plant species	No. of positive ELISA samples/ no. of ELISA samples	No. of positive cultures/ no. of cultures
Catharanthus roseus L.	3/3	3/3
Crepis echiodes (L.) All.	2/2	2/2
Digitaria sanguinalis (L.) Scop.	1/18	0/7
Echium sp.	na[a]	2/3
Salsola kali L.	3/14	0/9
Sesamun indicum L.	8/26	3/6
Sorghum halepense (L.) Pers.	52/79	0/21
Xanthium sp.	1/2	na[a]
Zea mays L.	1/8	na[a]

[a]na: not attempted.

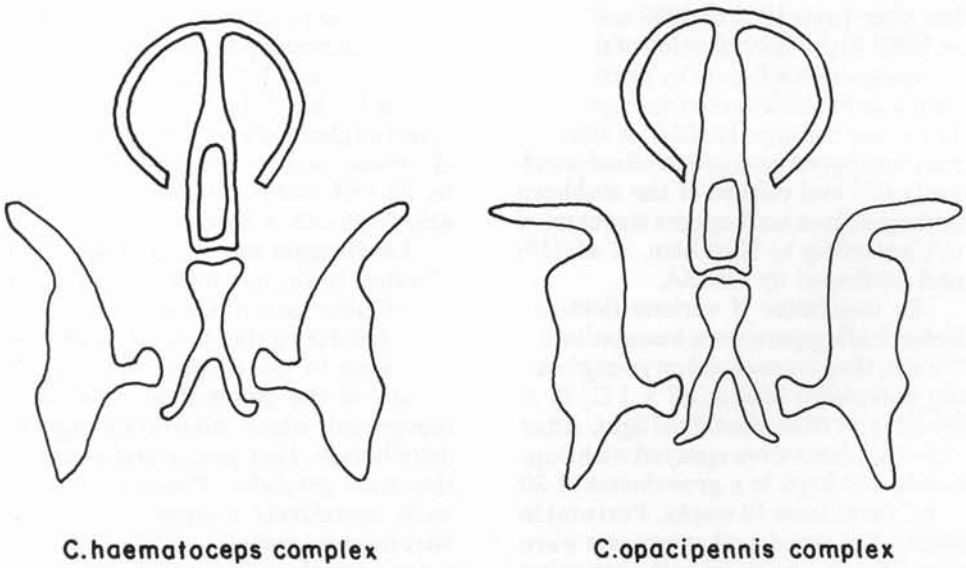

Fig. 1. Male genitalia of *Circulifer*. Styles, connective and aedeagus (dorsal view) of *C. opacipennis* complex on *Salsola kali* and *C. haematoceps* complex on *Sesamum indicum*.

hoppers collected from sesame and assigned to the *C. haematoceps* complex transmitted *S. citri* to periwinkle. It is noteworthy that those of the *C. opacipennis* complex did not transmit *S. citri* to periwinkle (Table 2), even though we cultured *S. citri* from their bodies. Leafhoppers of the *C. haematoceps* complex collected from sesame at different locations in August 1990 and 1992 were assayed by ELISA for *S. citri*. An average of 408 specimens was collected by a standardized D-VAC sampling at the six locations in 1990. We were able to demonstrate the highest infection rate in leafhoppers collected at Incirlik 1 (89%). On average, 62% of all leafhopper batches of the *C. haematoceps* complex tested positive for *S. citri* by ELISA in 1990. Numbers of these leafhoppers on sesame were lower 1992; on average only 268 individuals were collected by a standardized D-VAC sampling in August. Similarly the rate of infection of leafhoppers by *S. citri* did not exceed 12 5% (Adana 3) in 1992 and the average infection rate of leafhoppers from all locations during the same period was only 4.5% (Table 3).

DISCUSSION

The successful culture of *S. citri* from field-collected samples of *C. echoides*, *Echium* sp. and sesame adds these

TABLE 2
ISOLATION OF *SPIROPLASMA CITRI* FROM, AND RATE OF TRANSMISSION TO PERIWINKLE BY, LEAFHOPPERS COLLECTED FROM FIELD IN THE EASTERN MEDITERRANEAN REGION OF TURKEY IN 1988-1990 AND 1992

Leafhopper species	Culture of *S. citri*	Transmission tests
	No. of pos. cultures/ no. of cultures	No. of infected plants/ no. of exposed plants
Balclutha hebe (Kirkaldy)	2/13	0/9
Cicadulina bipunctella (Matsumura)	2/14	0/18
Circulifer haematoceps complex (M.&R.)	10/21	12/57
Circulifer opacipennis complex (Leth.)	4/6	0/18
Exitianus capicola (Stål)	5/18	0/9
Orosius orientalis (Matsumara)	4/7	0/10
Psammotettix sp.	3/5	0/8

TABLE 3
RATE AT WHICH LEAFHOPPERS OF THE *CIRCULIFER HAEMATOCEPS* COMPLEX COLLECTED FROM SESAME AT SIX LOCATIONS IN AUGUST 1990 AND SEVEN LOCATIONS IN AUGUST 1992 IN THE EASTERN MEDITERRANEAN REGION OF TURKEY HARBORED *SPIROPLASMA CITRI* (AS INDICATED BY ELISA READINGS)

August 1990			August 1992		
Location	No. of collected leafhoppers	No. of positive ELISA samples/ no. of ELISA samples[a]	Location	No. of collected leafhoppers	No. of positive ELISA samples/ no. of ELISA samples[b]
Adana 1	116	5/10	Adana 1	311	0/27
Adana 2	265	7/10	Adana 2	699	3/32
Çatalan	576	5/10	Adana 3	122	1/8
Incirlik 1	1270	20/25	Erzin	89	0/6
Incirlik 2	134	4/10	Imamoğlu	157	1/11
Misis	86	4/6	Iskenderun	286	0/10
			Silifke	214	0/17
Mean	408	62.0%	Mean	268	4.5%

[a]Each sample with five individuals.
[b]Each sample with ten individuals.

plants to the list of natural hosts of this agent in the Eastern Mediterranean and expands the known host range of *S. citri*. Sesame is an important cultivated plant in this region. The infection rate of up to 20% in 1990 indicates that this plant may constitute an extensive natural reservoir from which the leafhopper can acquire the pathogen. *S. kali*, reported earlier as an important plant in the epidemiology (2, 3), was found only in small numbers restricted to the seashore. Several attempts to culture *S. citri* from these species failed (11). Another interesting plant seems to be *S. halepense*. Yellow or stunted specimens were positive in ELISA tests, but it was not possible to culture the pathogen.

Two distinct species complexes of the genus *Circulifer*, which differed in the shape of the male genitalia, were found in the Eastern Mediterranean. This study and that of Başpinar, *et al.* (1) on distribution and host plants showed clearly that those of the *C. haematoceps* complex and of the *C. opacipennis* complex shared no common host plant. This is further supported by the findings of Klein and Raccah (9), who indicated that leafhoppers of the *C. opacipennis* complex are only able to reproduce on *S. kali*.

Of the seven leafhopper taxa from which *S. citri* was isolated, only those of the *C. haematoceps* complex were demonstrated to be natural vectors of the pathogen. Sesame, the main host plant of this taxon, also proved to be an excellent host for the pathogen. Thus, in 1990 up to 80% of the groups of *C. haematoceps* that were tested proved to be inoculative. However, in 1992 the infection rate of *C. haematoceps* collected on sesame averaged only 4.5% for unknown reasons. Leafhoppers of the *C. opacipennis* complex collected from *S. kali* were incapable of transmitting *S. citri* to periwinkle, although many harbored the pathogen as determined by ELISA and culture. Klein, *et al.* (cited in 4) reported similar failure to transmit *S. citri* by *Circulifer* collected from *S. kali*.

Our results indicate that the *S. citri*-vector relationship should be further evaluated, in light of evidence that *C. haematoceps* was found to consist of two distinct species complexes, most likely with different potential in spreading the pathogen. We found no evidence that leafhoppers belonging to the *C. opacipennis* complex, that are commonly found on *S. kali*, transmit the pathogen, and there is no evidence that this plant is a host of *S. citri*. By contrast, members of the *C. haematoceps* complex associated with at least 27 host plants showed frequent ability to inoculate plants with naturally harbored *S. citri*, and one imported plant, sesame, is also a source of the pathogen. Our data and that of others point to an important epidemiological role for the *C. haematoceps*-like leafhopper is spreading *S. citri* in the Eastern Mediterranean region of Turkey.

ACKNOWLEDGEMENTS

The authors want to express their thanks to the Volkswagenwerk Foundation, FRG, for partly supporting this study. Ulrich Kersting gratefully acknowledges his scholarship from the Alexander von Humboldt Foundation, Feodor-Lynen-programme, FRG.

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