Inoculativity of Leafhopper Vectors of Stubborn Disease in California*

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ABSTRACT. Leafhoppers collected in citrus growing districts and elsewhere in California were tested for the presence of the citrus stubborn pathogen, *Spiroplasma citri* Saglio *et al.*, either by feeding them on Madagascar periwinkle plants or by culturing. Although field-collected *Scaphytopius nitridus* (DeLong) and *Scaphytopius acutus delongi* (Young) (both of which reproduce on citrus and transmit *S. citri* in the laboratory) were often encountered, only *Circulifer tenellus* (Baker) frequently harbored and transmitted *S. citri*. *S. citri* was not found in association with field-collected specimens of several other commonly collected leafhoppers. Laboratory-reared specimens of these species acquired *S. citri* from infected plants and retained it internally for at least 2 weeks, but none was able to transmit the pathogen.

The isolation of *Spiroplasma citri* Saglio *et al.* from the bodies of field-collected *Circulifer tenellus* (Baker) (4) and the subsequent demonstration of plant to plant transmission of *S. citri* by *Scaphytopius nitridus* (DeLong) (3, 9), *C. tenellus* (8), and *Scaphytopius acutus delongi* (Young) (2) allowed the initiation of studies of natural inoculativity of leafhoppers during the middle 1970s in California. The reports by Markham and Townsend (5) and Kaloostian *et al.* (3) of leafhopper transmission of *S. citri* to Madagascar periwinkle, led to the utilization of periwinkle as an indicator plant in studies of natural inoculativity as well as in studies of the relationship between *S. citri* and its vectors. Starting in 1974, leafhoppers were collected from several locations in California and assayed for the presence of *S. citri* by feeding them on indicator plants or by attempting to culture *S. citri* from their bodies. Species collected in the field were reared in the laboratory and their ability to acquire, retain and transmit *S. citri* under experimental conditions was studied. This paper reports the results of these studies.

MATERIALS AND METHODS

Insects were collected from citrus and various wild and cultivated plants in citrus growing districts and elsewhere in California using a De-vac® insect collecting machine. Leafhopper species were sorted in the laboratory and fed on young greenhouse grown Madagascar periwinkle plants, 8-12 cm tall, for one week, then the insects were killed by methyl bromide fumigation. Test plants were maintained at least two months after exposure to leafhoppers and infection of apparently diseased plants was verified by culturing *S. citri*. Other field-collected leafhoppers were assayed for the presence of spiroplasmas in groups of 10-25 adults using the technique of Lee *et al.* (4). The serological deformation test (12) was used to determine the apparent relationship and identities of selected cultures of spiroplasmas from field-collected *C. tenellus*. Spiroplasmas cultured from each new location were tested by this method. Laboratory colonies of leafhoppers were maintained in a greenhouse under a 16:8-hour light:dark regimen at 30 ± 3 C,

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and leafhoppers were transferred to new plants every one to two months.

A colony of *C. tenellus*, started by leafhoppers collected at Riverside, California in 1973, was maintained on sugarbeet. Colonies of *Aceratagallia curvata* Oman and *Texananus spatulatus* (Van Duzee), both maintained on sugarbeet, were started by leafhoppers collected in Riverside County in 1976. Colonies of *Graminella sonora* (Ball) and *Ollarianus strictus* Ball, maintained on barley and asparagus, respectively, were started by leafhoppers collected near Riverside in 1976. *S. acutus delongi* and *S. nitridus*, both reared on celery, were from colonies started by leafhoppers collected respectively near Exeter, Tulare County, California in 1974, and near Riverside in 1971. Three species, *Colladonus montanus* Van Duzee, *Euscelidius variegatus* (Kirschbaum) and *Macrosteles fascifrons* (Stål), were obtained from laboratory colonies from the University of California at Berkeley in 1976 and were maintained on celery, barley, and barley, respectively.

Leafhoppers from each laboratory colony were tested periodically for contamination by *S. citri*. Adult leafhoppers were allowed to feed for two days on a newly infected Madagascar periwinkle plant, and then returned for predetermined periods to feed on their respective rearing hosts. At the end of the feeding period, attempts were made to culture *S. citri* from the insects' bodies or the insects were fed for one week on healthy Madagascar periwinkle plants. The —215 isolate of *S. citri*, originally obtained from a naturally infected sweet orange tree at Moreno, California in 1973 was used in all laboratory acquisition and transmission tests. It was perpetuated by periodic transmission to new Madagascar periwinkle plants, using *S. nitridus* as the vector.

RESULTS

Transmission of naturally harbored S. citri **by field-collected leafhoppers.** Among eight leafhopper taxa collected and fed on Madagascar periwinkle, only *C. tenellus* was frequently inoculative (table 1). One hundred nineteen of 1464 plants developed infection by *S. citri*, i.e., 8% of exposed plants, with a mean 10 leafhoppers/plant. Field-collected *S. nitridus*, a recognized vector of *S. citri*, transmitted to one of 136 plants, a rate of 0.7%, with a mean 25 leafhoppers/plant. Field-collected *S. acutus delongi*, in fewer tests, failed to transmit to any of 25 plants with a mean

TABLE 1
TRANSMISSION OF NATURALLY HARBORED SPIROPLASMA CITRI TO MADAGASCAR PERIWINKLE PLANTS BY LEAFHOPPERS COLLECTED FROM THE FIELD IN CALIFORNIA DURING 1974-82

Leafhoppers			No. infected plants/no. exposed plants	Infected (%)
Name	No. tested	Mean no./plant		
Aceratagallia spp.	2492	17	0/149	0
Circulifer tenellus	15367	10	119/1464	8
Colladonus montanus	13	7	0/2	0
Graminella sonora	206	21	0/10	0
Empoasca spp.	7444	90	0/83	0
Ollarianus strictus	8778	70	0/126	0
Scaphytopius acutus delongi	572	20	0/25	0
Scaphytopius nitridus	3431	25	1/136	<1

20 leafhoppers/plant. Field-collected *Aceratagallia* spp., *C. montanus, G. sonora, Empoasca* spp., and *O. strictus* failed to transmit.

Isolation of spiroplasmas from field-collected leafhoppers. As in the case of natural inoculativity, only *C. tenellus* frequently harbored spiroplasma (table 2). Isolates of *S. citri* cultured from the bodies of *C. tenellus* from each location in California reacted as *S. citri* to high dilutions of antisera of the type strain (Maroc) and/or the California strain (C-189) of *S. citri*. Spiroplasmas were cultured from 88 of 358 groups of *C. tenellus* (25%), from only one of 76 groups of *S. nitridus*, and from none of 21 groups of *S. acutus delongi*. Spiroplasmas were cultured from six of 162 groups (4%) of *O. strictus*. No spiroplasma was cultured from groups of *Aceratagallia* spp., *G. sonora, Empoasca* spp., or *Erythroneura* spp.

Isolation of S. citri **from bodies of laboratory-reared leafhoppers.** *S. citri* was cultured from the bodies of each of several tested species except *T. spatulatus* (table 3). *S. citri* was cultured from 50% or more of the examined groups in five species: *A. curvata*, 75%; *C. tenellus*, 50%; *O. strictus*, 83%; *S. acutus delongi*, 100%; and *S. nitridus*, 50%.

Transmission of —215 isolate of S. citri **by leafhoppers.** As shown in table 4, three weeks or more after initiation of acquisition feeding, *C. tenellus, S. acutus delongi*, and *S. nitridus*, all recognized vectors of *S. citri*, transmitted the —215 isolate to 27 of 35 plants (77%), 23 of 36 plants (64%), and two of seven plants (29%), respectively. *A. curvata, G. sonora, E. variegatus, M. fascifrons* and *O. strictus*, all of which were able to acquire and retain *S. citri* by feeding on infected Madagascar periwinkle (see table 3) failed to transmit *S. citri*. *C. montanus*, not tested for ability to acquire and retain *S. citri* in its body, did not transmit *S. citri*. *T. spatulatus* neither acquired (see table 3) nor transmitted *S. citri* (table 4) in these tests.

Several species that failed to transmit *S. citri* after a three-week incubation feeding period on the rearing host also failed to transmit after a five-, six-, or eight-week incubation feeding period. Thus, *C. montanus* and *E. variegatus* failed to transmit after three weeks (table 4) and after five weeks (table 5). *M. fascifrons*, which failed to transmit after three weeks (table 4) also failed to transmit after five and six weeks (table 5). *O. strictus*, a species from which

TABLE 2
ISOLATION OF NATURALLY HARBORED SPIROPLASMAS FROM BODIES OF LEAFHOPPERS COLLECTED FROM THE FIELD IN CALIFORNIA DURING 1974-82

Name	Leafhoppers		No. positive cultures/no. cultures	Positive cultures (%)
	No. tested	Mean no./ culture		
Aceratagallia spp.	1104	20	0/58	0
Circulifer tenellus	4538	13	88/358	25
Graminella sonora	195	15	0/13	0
Empoasca spp.	2018	22	0/93	0
Erythroneura spp.	150	22	0/7	0
Ollarianus strictus	2110	13	6/162	4
Scaphytopius acutus delongi	240	11	0/21	0
Scaphytopius nitridus	1054	14	1/76	1

TABLE 3
ISOLATION OF SPIROPLASMA CITRI FROM BODIES OF LABORATORY-REARED LEAFHOPPERS GIVEN TWO-DAY ACQUISITION ACCESS ON INFECTED MADAGASCAR PERIWINKLE AND 12-DAY FEEDING PERIOD ON REARING PLANT

	Leafhoppers		No. cultures with spiroplasmas	Positive cultures (%)
	No. tested	No. of cultures		
Aceratagallia curvata	180	12	9	75
Circulifer tenellus	60	4	2	50
Graminella sonora	607	43	8	19
Euscelidius variegatus	627	48	1	2
Macrosteles fascifrons	1080	85	1	1
Ollarianus strictus	90	6	5	83
Scaphytopius acutus delongi	15	1	1	100
Scaphytopius nitridus	420	28	14	50
Texananus spatulatus	165	33	0	0

TABLE 4
TRANSMISSION OF —215 ISOLATE OF SPIROPLASMA CITRI TO MADAGASCAR PERIWINKLE BY LEAFHOPPERS*

Leafhoppers		No. infected plants/ no. exposed plants
Name	Mean no./plant	
Aceratagallia curvata	49	0/10
Circulifer tenellus	36	27/35
Collodanus montanus	22	0/12
Graminella sonora	53	0/20
Euscelidius variegatus	38	0/29
Macrosteles fascifrons	72	0/27
Ollarianus strictus	62	0/20
Scaphytopius acutus delongi	15	2/7
Scaphytopius nitridus	30	23/36
Texananus spatulatus	23	0/59

*Fed two days on infected Madagascar periwinkle and 19 days on respective rearing host plants.

TABLE 5
TRANSMISSION OF —215 ISOLATE OF SPIROPLASMA CITRI BY LEAFHOPPERS AFTER FEEDING TWO DAYS ON INFECTED PLANT AND 5-8 WEEKS ON REARING HOST PLANT

Leafhoppers		Incubation feeding on rearing plant (weeks)	No. infected plant/ no. exposed plants
Name	Avg. no./ plant		
Colladonus montanus	15	5	0/4
Euscelidus variegatus	50	5	0/5
Macrosteles fascifrons	91	5	0/7
M. fascifrons	50	6	0/3
Ollarianus strictus	95	5	0/11
O. strictus	50	8	0/7
Texananus spatulatus	30	5	0/12

spiroplasmas were isolated from field-collected specimens on several occasions (table 2) failed to transmit after three weeks (table 4) and after five or eight weeks (table 5). *T. spatulatus,* for which no evidence of laboratory acquisition was obtained (table 3), failed to transmit after three weeks (table 4) and five weeks (table 5).

DISCUSSION

The frequency at which field-collected *C. tenellus* transmitted *S. citri* to plants and harbored *S. citri* in their bodies, when compared with the frequency at which other field-collected leafhoppers transmitted or harbored *S. citri,* indicates an important role for *C. tenellus* in the epidemiology of *S. citri* in California. The extremely low frequency rates at which field-collected *S. nitridus* transmitted or harbored *S. citri,* and the absence of *S. citri* in association with *S. acutus delongi* in this study, indicate a relatively unimportant epidemiological role for this species. Differences in host preference of *S. nitricidus* and *S. acutus delongi* may account for the paucity of *S. citri* associated with field-collected specimens of these two species. Both species were usually encountered on mature citrus. Although *C. tenellus* was occasionally collected from mature citrus, most specimens were collected from weed hosts including *Sisymbrium Irio* L. and *Brassica geniculata* (Desf.) which are also hosts of *S. citri* (1, 10).

The failure to detect spiroplasmas in most leafhopper species collected in the field, when considered in the light of the ability of most of the same species to acquire *S. citri,* may indicate that these species also prefer hosts other than those most often infected with *S. citri.* Most collections included samples from several plant species, consequently an estimate of the number of each species taken from each host plant was impossible. However, the rearing hosts for several species were monocotyledonous plants; only two monocotyledonous hosts of *S. citri* have been reported (7, 11). The lack of transmission by *M. fascifrons* and the relatively infrequent culturing of *S. citri* after feeding on infected plants bears special note in light of a recent report from Illinois (6) of transmission of an isolate of *S. citri* from brittleroot-diseased horseradish by *M. fascifrons.* That *O. strictus* should naturally harbor spiroplasmas occasionally and apparently readily acquire and retain *S. citri* is interesting since it was unable to transmit this pathogen under the conditions of this study. Field-collected specimens failed to transmit naturally acquired spiroplasmas. The question of the identity of spiroplasmas harbored by *O. strictus* needs to be resolved. None of the cultures from field-collected *O. strictus* was verified to be *S. citri* before discard.

ACKNOWELDGMENTS

The authors wish to thank M. W. Nielson, USDA, ARS, Forage Insects Research Laboratory, Tucson, Arizona, and R. A. Flock, Imperial County Agriculture Commissioner's Office, El Centro, California for identifying various leafhoppers. We are indebted to Bethany Brown, D. J. Gumpf, H. Rahimian, and R. F. Whitcomb for assistance with the serological deformation test. H. D. Pierce contributed valuable technical assistance.

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