

## Transmission of *Spiroplasma citri* from Citrus to Citrus by *Scaphytopius nitridus*

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### ABSTRACT

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Laboratory-reared *Scaphytopius nitridus*, a leafhopper that reproduces on citrus in southern California, transmitted *Spiroplasma citri* from stubborn-diseased citrus to healthy sweet orange seedlings. Transmission occurred after leafhoppers were fed either 3 wk on a source plant, or 5 days on a source plant, then 16 days on celery. After either feeding regimen, transmission to healthy citrus occurred very infrequently when plants were exposed to 15 insects each.

Transmission occurred more frequently when *S. nitridus* were fed 3 wk on the source plant and then were given access to healthy sweet orange seedlings at the rate of 100/plant. Sweet orange seedlings exposed only to *S. nitridus* (15/plant and 100/plant) that had fed only on celery or healthy citrus did not develop stubborn, nor did several hundred other such seedlings that were not exposed to *S. nitridus*.

*Additional key word:* mycoplasma.

The organism found in phloem cells of stubborn-diseased citrus was isolated and cultured in cell-free media (2, 11) and subsequently characterized and described as *Spiroplasma citri*, n. sp., n. g. (12). *Spiroplasma citri* has also been isolated recently from field-collected *Circulifer tenellus* (Baker) (6) and transmitted to *Vinca rosea* L. and citrus by field-collected *C. tenellus* (9), a cicadellid previously known to transmit curly top virus but not known to transmit any cellular pathogen. Likewise, Daniels et al. (1) cultured spiroplasma from citrus affected by little leaf disease (= stubborn disease) (8), injected it into the European leafhopper, *Euscelis incisus* (Kirschbaum) = *plebejus* (Fallen), and demonstrated its transmission to white clover plants. Subsequently, others reported transmission of *S. citri* to citrus and several other plants by *E. incisus* injected with the organism (7, 13). Also, in California, Rana et al. (10) reported transmission of *S. citri* to citrus by both *C. tenellus* and *Scaphytopius nitridus* (DeLong) after they acquired it by feeding through a stretched Parafilm membrane, and Kaloostian et al. (4) reported that *S. nitridus* acquired *S. citri* by feeding on stubborn-diseased citrus and subsequently transmitted it to *V. rosea*. Of the Cicadellidae, only *S. nitridus* has been found reproducing on citrus in Southern California (5). The present paper reports the results obtained in attempts to

transmit *S. citri* from diseased to healthy citrus by *S. nitridus*.

### MATERIALS AND METHODS

The source of inoculum for *S. nitridus* was greenhouse-grown Madam Vinous sweet orange seedlings, graft-inoculated with tissue from citrus harboring isolates of *S. citri* known to have spread recently in the field. The isolates, hereinafter called the Moreno and West Side isolates, had been recently recovered from citrus trees at the University of California farm at Moreno, Riverside County, and at the University of California West Side Field Station at Five Points in Fresno County. Isolation of the organism from citrus was performed as described by Fudl-Allah et al. (3). The insects used in the tests were descendants of *S. nitridus* collected from citrus groves near Riverside and colonized for several generations on celery grown from seed in the greenhouse. Transmission tests were performed in a greenhouse maintained at 26-30 C.

### EXPERIMENTS AND RESULTS

**Initial transmission of two isolates.**—Groups of *S. nitridus* were allowed to acquire *S. citri* using the following feeding regimens: (i) 5 days on a citrus plant infected with the Moreno isolate followed by 16 days on celery; (ii) 5 days on a citrus plant infected with the West Side isolate, followed by 16 days on celery; or (iii) 24 days

on a citrus plant infected with the Moreno isolate. Following each of these regimens, insects were given access to healthy sweet orange seedlings for 3 wk at the rate of 15/plant.

After about 2 mo, four of 47 plants exposed to leafhoppers developed symptoms of stubborn disease (Table 1). Subsequently, the organism, *S. citri*, was cultured from tissue of these four plants, three exposed to leafhoppers fed 24 days on a source plant harboring the Moreno isolate, one exposed to leafhoppers fed 5 days on a source plant harboring the West Side isolate. Other indicator plants were successfully graft-inoculated with *S. citri* from each of the four plants. No other indicator seedlings exposed to leafhoppers developed symptoms of stubborn and attempts to isolate *S. citri* from these plants were unsuccessful.

**Confirmation of transmission of the Moreno isolate by *Scaphytopius nitridus*.**—Since transmission occurred to a low percentage of indicator plants when they were exposed to 15 insects each, we performed a second test to verify transmission by *S. nitridus* and compare the rate of transmission with different numbers of insects. Thus, we allowed *S. nitridus* to feed for 3 wk on a citrus plant infected with the Moreno isolate of *S. citri*, then exposed indicator seedlings to either 15 or 100 *S. nitridus* for 3 wk. Other indicator seedlings were exposed for 3 wk to either 15 or 100 *S. nitridus* reared only on celery.

After about 2 mo, five of 10 indicator seedlings exposed to 100 *S. nitridus* given access previously to a *S. citri*

source plant developed symptoms of stubborn (Table 2) but none exposed to 15 *S. nitridus* treated similarly nor none exposed to either 15 or 100 *S. nitridus* reared solely on celery developed stubborn. Subsequently, we attempted to isolate *S. citri* from each indicator plant exposed to leafhoppers. Only the five plants exhibiting stubborn symptoms harbored the organism.

## DISCUSSION

Our tests revealed that *S. nitridus* is able to acquire at least two isolates of *S. citri* from citrus and subsequently transmit them to other citrus. One, the West Side isolate, was acquired from citrus, retained while the leafhoppers fed 16 days on celery, then transmitted to citrus. Although we did not extensively test transmissibility of the West Side isolate, it, as well as the Moreno isolate, was transmitted infrequently when small numbers of *S. nitridus* were fed on indicator plants. The Moreno isolate was transmitted to a higher percentage of indicator plants when larger numbers of *S. nitridus* were used. Whether this rate of transmission to citrus is typical for other combinations of populations of *S. nitridus* and isolates of *S. citri* remains to be investigated.

This is the first report of insect transmission of *S. citri* from citrus to citrus. The isolation of *S. citri* from *C. tenellus* (6), and the report that field-collected *C. tenellus* transmitted *S. citri* to *V. rosea* and citrus (9) indicate that *C. tenellus* may play some role in the epidemiology of stubborn disease in California. The relationship of *S. nitridus* and *C. tenellus* to the spread of stubborn disease is under further investigation.

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TABLE 1. Citrus to citrus transmission of Moreno<sup>a</sup> and West Side<sup>a</sup> isolates of *Spiroplasma citri* by *Scaphytopius nitridus* fed 5 or 24 days on source plant then fed on indicator plants at 15/plant

Isolate from:	Access time (days)	Ratio of plants infected to total plants exposed
Moreno	5 <sup>b</sup>	0/11
West Side	5 <sup>b</sup>	1/13
Moreno	24	3/23

<sup>a</sup>The "West Side" isolate was obtained from citrus trees growing at the University of California West Side Field Station at Five Points which is located in Fresno County on the west side of the southern San Joaquin Valley. The "Moreno" isolate was obtained from citrus trees growing at the University of California farm at Moreno in Riverside County.

<sup>b</sup>After access time, insects fed on healthy celery 16 days then fed on indicator plants.

TABLE 2. Transmission of the Moreno isolate<sup>a</sup> of *Spiroplasma citri* to indicator citrus plants by groups of 15 or 100 *Scaphytopius nitridus* fed 21 days on infected citrus plant or fed only on healthy celery

Access time (days)	(No./indicator plant)	Ratio of plants infected to total plants exposed
24	15	0/20
24	100	5/10
0	15	0/25
0	100	0/10

<sup>a</sup>The "Moreno" isolate was obtained from citrus trees growing at the University of California farm at Moreno in Riverside County.

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