Aspects of Transmission of the Citrus Stubborn Pathogen by Scaphytopius nitridus (DeLong)

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ABSTRACT. An isolate of *Spiroplasma citri* Saglio, *et al.* (designed —215), obtained from a naturally infected sweet orange seedling at Moreno, Riverside County, California, was acquired by nymphs and adults of *Scaphytopius nitridus* (DeLong) and transmitted by adults to indicator plants. Males and females transmitted the pathogen but no transovarian transmission occurred. Acquisition occurred during access periods of 2 hours or longer; transmission required access periods of at least 6 hours. Adults began transmitting 16-18 days after initiation of the acquisition access period and transmission continued at least 20 days. Adults fed 2 days on an infected plant, then 19 days on celery, subsequently transmitted the —215 isolate and two isolates from turnip and from *Circulifer tenellus* (Baker), respectively, from the state of Washington, at similar rates when fed on Madagascar periwinkle plants at the rate of 25, 50 or 100 adults per plant.

Scaphytopius nitridus (DeLong), a leafhopper that reproduces on citrus in southern California (3), transmits *Spiroplasma citri* Saglio *et al.* from citrus to Madagascar periwinkle (5) and from citrus to citrus (11) in the laboratory. The report of transmission of *S. citri* to several nonrutaceous plants (8) led to further investigations of its plant host range (1, 4, 10, 12, 13) in which *S. nitridus* and *Circulifer tenellus* (Baker) were used experimentally. As *S. nitridus* is easily reared on celery and is easily manipulated in the laboratory, it was considered an excellent species to use in host range studies. Herein, we report investigations on several aspects of transmission of *S. citri* by *S. nitridus*. This information should facilitate the use of this species in studies of the host range of *S. citri* and in elucidating the role of *S. nitridus* as a natural vector.

MATERIALS AND METHODS

Test insects were from laboratory colonies initiated from *S. nitridus* collected from citrus near Riverside, California in 1971, and maintained for many generations on greenhouse-grown celery plants. Insects in stock colonies and those used in experiments were maintained at 30 ± 3°C under a 16:8-hour light: dark regimen in a greenhouse. Sweet orange (var. Madam Vinous) and Madagascar periwinkle plants (both circa 30 cm high) were grown from seed in the greenhouse. They were inoculated with an isolate of *S. citri* (designated —215) obtained from a naturally infected orange tree at Moreno, Riverside County, in 1973 and were used as acquisition access plants. Leafhoppers were fed on celery plants (circa 30 cm high) between acquisition access and transmission access periods. Madagascar periwinkle (var. Little Pinkie) plants (circa 10 cm high) were used as indicator test plants. Test plants exhibiting disease symptoms were cultured for *S. citri* using the technique of Fudl-Allah *et al.* (2). The in vitro culturing technique of Lee *et al.* (6) was used to test leafhoppers from stock colonies and from selected experiments for the presence of *S. citri*. Two isolates of *S. citri* obtained from the state of Washington were used in comparative transmission studies of this pathogen. The cultures are designated as WACT, Washington isolate from *Circulifer tenellus* (Baker), and WATU, Washington isolate from turnip, *Brassica rapa* L.

RESULTS

Acquisition of *S. citri* by nymphs. Leafhoppers in the 1st and 2nd nymphal stages were given a three-day acquisition access period on an infected sweet orange, fed for 15 days on celery, then given a transmission access period of seven days on test plants in three successive tests to ascertain whether acquisition occurred during the nymphal stage. By the time transmission access began, leafhoppers were in the late nymph or adult stage. These were fed on test plants at the rate of 54-95 insects/plant. Insects from each test transmitted **S. citri** (table 1), indicating that transmission can occur late in the nymphal stage or in early adulthood following acquisition by young nymphs.

Transmission by sexes of adult leafhoppers. Male and female adults were fed together for 21 days on an infected sweet orange, then separated and given a transmission access period of seven days on different sets of test plants (50/plant). Males and females transmitted *S. citri* to four of five and three of five test plants, respectively, indicating that either sex transmits this organism.

Transmission by progeny of inoculative adults. In one test, several hundred adult leafhoppers were fed on an infected sweet orange plant for three weeks. Then, 100 leafhoppers were fed on a Madagascar periwinkle test plant for one week, while the rest were transferred to successive celery plants every five days. Progeny produced on celery plants by the remaining adults were collected periodically during a 2-month period and fed on test plants. In all, 1725 progeny were tested (100/test plant). Leafhoppers transferred to a test plant at the end of the three-week acquisition access period transmitted *S. citri*; none of the progeny transmitted.

In a second test, late stage nymphs were fed one day on an infected Madagascar periwinkle plant and allowed to mature and reproduce on a series of celery plants. Three weeks after transfer to celery (before any progeny matured), 50 parent leafhoppers were transferred to each of two test plants to verify inoculativity. Progeny of these adults were collected as late nymphs and were given a seven-day transmission access period on test plants (100/plant). In all, 2348 progeny were tested. As in the first test, the original parent leafhoppers transmitted *S. citri* to two of two test plants, but none of the progeny transmitted.

Acquisition and transmission access periods. Acquisition access was studied by allowing adult leafhoppers, starved at 15°C for six hours, various acquisition access periods on an infected sweet orange plant, then feeding them on celery for the remainder of a three-week period. Thereafter, they were given a one-week transmission access period on test plants.

Transmission access was studied by giving adult leafhoppers a two-day acquisition access period on an infected Madagascar periwinkle plant, then a 19-day feeding period on celery. Thereafter, leafhoppers

TABLE 1
TRANSMISSION OF SPIROPLASMA CITRI BY SCAPHYTOPIUS NITRIDUS FOLLOWING ACQUISITION AS 1st-2nd STAGE NYMPHS

Test no.	No. insects used	Avg./plant	No. infected plants/no. exposed plants
1	286	95	3/3
2	240	80	2/3
3	162	54	3/3

were starved for six hours at 15°C, then were given various transmission access periods on test plants (100/plant).

As indicated in table 2, transmission occurred after acquisition access periods of two hours or longer (except at six hours) and no transmission occurred following acquisition access periods of 1.5 hours or shorter. In the case of transmission access, more than 40% of the plants exposed to leafhoppers for 12 hours or longer developed infection by *S. citri*. Only one of 14 plants exposed to leafhoppers for six hours became infected, and none of 99 plants exposed to leafhoppers for periods of four hours or less became infected.

TABLE 2
TRANSMISSION OF SPIROPLASMA CITRI BY *SCAPHYTOPIUS NITRIDUS* GIVEN VARIOUS ACQUISITION ACCESS PERIODS ON INFECTED SWEET ORANGE, OR TRANSMISSION ACCESS PERIODS ON HEALTHY MADAGASCAR PERIWINKLE*

Period (hr)	No. infected plants/no. exposed plants following indicated access: Acquisition**	Transmission***
96	2/4	10/11
48	2/4	4/11
24	1/5	7/15
12	2/3	4/9
6	0/4	1/14
4	—	0/15
2	2/10	0/20
1.5	0/6	—
1	0/6	0/38
0.5	—	0/9
0.25	0/5	0/7
0.08	0/5	0/10

*100 adults per test plant.

**Leafhoppers starved at 15°C for 6 hours, given indicated acquisition access period on infected sweet orange, fed on celery for remainder of three week period, and then fed one week on Madagascar periwinkle.

***Leafhoppers allowed two-day acquisition access on infected Madagascar periwinkle, 19-day feeding period on celery, and then indicated transmission access period on Madagascar periwinkle.

Latent period. Several hundred young adult leafhoppers were given access to an infected sweet orange plant for four days, then they were given successive two-day transmission access periods on groups of test plants (100/plant) starting on the fifth day. At selected times thereafter, two groups of 15 insects were assayed for *S. citri*.

S. citri was cultured from the bodies of leafhoppers as early as the 14th day, and at two-day intervals thereafter until this assay was terminated on the 22nd day (table 3). During the first six-two-day transmission access periods,

TABLE 3
TRANSMISSION OF SPIROPLASMA CITRI AND ITS ISOLATION FROM BODIES OF SCAPHYTOPIUS NITRIDUS AT INDICATED TIME AFTER INITIATION OF FOUR-DAY ACQUISITION ACCESS PERIOD*

Day(s)	No. infected plants/no. plants exposed	No. positive cultures/no. cultures attempted
5-6	0/5	—
7-8	0/5	—
9-10	0/5	—
10	—	0/2
11-12	0/5	—
13-14	0/5	—
14	—	1/2
15-16	0/5	—
16	—	2/2
17-18	1/5	—
18	—	1/2
19-20	3/5	—
20	—	1/2
21-22	2/5	—
22	—	1/2
23-24	2/5	—
25-26	4/5	—
27-28	1/5	—
29-30	2/5	—
31-32	2/5	—
33-34	2/5	—
35-36	3/5	—
37-38	0/4	—
39-40	1/3	—
41-42	0/1	—

*100 adults/plant.

i.e. from the fifth until the 16th day after initiation of the acquisition access period, no transmission occurred. Starting 17-18 days after initiation of the acquisition access period, transmission occurred during each two-day transmission access period until the 37th day. Transmission occurred during days 39-40. The test was terminated after the 42nd day because most leafhoppers had died.

Comparative transmission of three isolates of S. citri. Transmission rates of three isolates of *S. citri* were compared by giving adult leafhoppers a two-day acquisition access period on Madagascar periwinkle plants infected with one of the three isolates. The leafhoppers were transferred to celery plants for 19 days, followed by a one-week transmission access period on test plants. Thus, leafhoppers were given access to plants infected with, respectively, the —215 isolate from citrus or either of the two isolates from the state of Washington, WACT, from *C. tenellus* or WATU from turnip.

Rates of transmission of the three isolates by 25 insects/plant were similar (table 4). When 50 insects/plant were used, rates of transmission were similar among the three isolates and similar or

TABLE 4
TRANSMISSION OF THREE ISOLATES OF SPIROPLASMA CITRI BY DIFFERENT NUMBERS OF SCAPHYTOPIUS NITRIDUS

Isolate	Numbers of leafhoppers 25	50	100
—215*	22/34**	110/175	8/8
WACT	20/34	65/78	8/8
WATU	10/16	129/174	—

*Two-day acquisition access period on infected Madagascar periwinkle, 19-day feeding period on celery, then one-week transmission access period on Madagascar periwinkle.

**Plants infected/plants exposed.

higher rates observed using 25 insects/plant. All of the relatively few plants exposed to 100 insects few plants exposed to 100 insects carrying the —215 isolate or the WACT isolate became infected. Transmission of the WATU isolate was not investigated using 100 insects.

DISCUSSION

This study demonstrated that early stage nymphs of *S. nitridus* can acquire *S. citri*. After an appropriate latent period, *S. citri* was transmitted during either the late nymph stage or the adult stage. No attempt was made to ascertain whether the latent period was completed before nymphs molted to the adult stage. However, the ability of early nymphs to acquire the organism suggests that the latent period (between two and three weeks in adults) can be completed before adult emergence if acquisition occurs early in nymphal life. A recent report on development of *S. nitridus* (9) at lower temperatures than that used in our tests indicates a minimum of 40 days for the nymphal stage. Whether or not the nymphal stage is sufficiently long under our experimental conditions to allow completion of the latent period of *S. citri* must await further study. That nymphs of *S. nitridus* survive to transmit *S. citri* is interesting in light of a report that adults of *C. tenellus* are adversely affected by this organism (7).

The knowledge that female adults transmit *S. citri* (from our tests, apparently about as readily as males) allowed pursuit of the question of transovarian transmission with the confidence that females can transmit. The absence of transovarian transmission was expected as no transovarian transmission of MLOs or spiroplasmas has been reported to our knowledge. Under the conditions of this study, the minimum demonstrable latent period in adults of *S. nitridus* was 17-18 days. The successful isolation of *S. citri* from the bodies of adults a few days before transmission occurred probably indicates increasing titre in insect tissue before the insect becomes inoculative. The effect of environmental factors (especially temperature) may considerably shorten or lengthen the demonstrable latent period. As all tests in this study were conducted at temperatures expected to prevail during the warm season in most areas where stubborn exists, the latent period, acquisition access period and transmission access periods probably approximate those that occur at that season. In this regard, it is interesting to speculate that such periods might be lengthened greatly by lower temperatures and, indeed, the latent period might last several months during the winter.

In this study, the minimum access time required for acquisition of *S. citri* was two hours and the minimum transmission access time was six hours. Whether or not acquisition or transmission occasionally occurs in shorter access periods can only await further investigation. Yet, the establishment of these relatively short access periods as sufficient for acquisition and transmission by *S. nitridus* is of practical value in understanding conditions required for such phenomena to take place in the field. A comparison of minimum acquisition access and transmission access periods for *S. nitridus* and other vectors of *S. citri* under different environmental conditions could further contribute to an understanding of the epidemiology of *S. citri*.

Finally, tests reported here indicate the *S. nitridus* is capable of transmitting three isolates from diverse areas and hosts at similar rates of efficiency. This is noteworthy since two of the isolates are from areas far removed from citrus and from outside the known geographical range of *S. nitridus*.

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