Transmission Characteristics of the Beet Leafhopper Transmitted Virescence Agent

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ABSTRACT

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Beet leafhopper transmitted virescence agent (BLTVA), which causes a disease with a presumed etiology by a mycoplasma-like organism, is vectored by *Circulifer tenellus* in a pattern consistent with other leafhopper-borne plant pathogenic mollicutes. The minimum acquisition access period (AAP) was 5 min; more than 50% of test plants developed symptoms after exposure to insects that had undergone a 4-hr AAP. Transmission increased with increasing AAP until 100% of test plants became infected after an AAP of 1 or 2 days. The minimum demonstrable latent period in *C. tenellus* was 12 days. Maximum inoculation efficiency was reached at 26–27 days after which a gradual decline in efficiency occurred. An inoculation access period (IAP) of 5 min rarely resulted in transmission. IAPs of 2 days provided high rates of infection in test plants. Single *C. tenellus* transmitted to about 50% of young plants of *Apium graveolens, Brassica geniculata, Catharanthus roseus,* and *Raphanus sativus*. Groups of 5 or 10 inoculative insects gave nearly 100% transmission to the same hosts. Transmission efficiency was not related to sex; groups of 10 male and female leafhoppers transmitted to 82 and 81% of test plants, respectively.

Additional key words: MLO, *Spiroplasma citri*, wall-less prokaryotes.

A disease exemplified by virescence and rosetting in Madagascar periwinkle (*Catharanthus roseus* (L.) G. Don) after exposure to field-collected beet leafhoppers (*Circulifer tenellus* (Baker)) was reported by Oldfield et al in California (9). Mycoplasma-like organisms (MLO) were seen in the phloem of infected plants under electron microscopic examination. In addition to periwinkle, a number of common brassicaceous plants were found to be both natural and experimental hosts of the beet leafhopper transmitted virescence agent (BLTVA) (7).

BLTVA may of itself be an economically important pathogen. It has a wide host range (Sullivan, *unpublished*), and its vector is prevalent in much of the western United States (3). In addition, it may also be important in the epidemiology of citrus stubborn disease. The beet leafhopper is an efficient laboratory vector of the citrus stubborn disease agent, *Spiroplasma citri* Saglio et al (6). Reports of its natural inoculativity in diverse areas of western United States indicate a primary role in spreading *S. citri* (8,10). When plants of *C. roseus* are infected with both BLTVA and *S. citri*, the normally lethal effects of *S. citri* are in some way prevented; the plants harboring both agents live as long or longer than healthy controls (7). We have observed (*unpublished*) the same phenomena in field situations where *C. roseus* singly infected with *S. citri* will wilt and die in a matter of weeks, whereas dually infected plants survive for months.

The extended survival of *S. citri* in herbaceous host plants by dual infection with BLTVA may play an important role in the ecology of stubborn disease. A number of plant species have been demonstrated to serve not only as hosts of BLTVA and *S. citri*, but also as reproductive hosts of *C. tenellus*; these include *Brassica geniculata* (Desf.) J. Ball, *Raphanus sativus* L., and *Sisymbrium irio* L. (1,3,7). It seems logical that such plants would be of particular importance in the epidemiology of citrus stubborn disease.

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Because of the potential economic importance of BLTVA and its interaction with the stubborn agent, we have undertaken detailed studies of the transmission biology of this organism. This report elucidates the vector relationship of BLTVA with *C. tenellus* relative to acquisition, latency, and inoculation.

MATERIALS AND METHODS

The pathogen. One line of BLTVA, FC-83-13, was used in these studies. It has been transmitted to a young, greenhouse-grown periwinkle plant by a single *C. tenellus* collected from Buena Vista, Kern County, CA, in the fall of 1983. This line of BLTVA produced typical symptoms in several hosts and was efficiently transmitted by *C. tenellus*. The agent was maintained in our greenhouse in periwinkle and radish plants; it was frequently transmitted by leafhoppers to fresh host plants to preserve its insect transmissibility.

We were unable to culture *S. citri* using the techniques of Fudl-Allah et al (5) from the original periwinkle infected with FC-83-13 BLTVA. Because BLTVA may mask the symptoms of infection by *S. citri*, our BLTVA stocks were tested several times a year for *S. citri* contamination; *S. citri* was never detected in any of our BLTVA infected plants.

Samples of *C. roseus* infected with FC-83-13 BLTVA were examined by electron microscopy using procedures described elsewhere (4), and mycoplasma-like bodies were seen in the phloem of infected tissues but not in control plants (Fig. 1). Infected periwinkle were treated twice weekly with 100 μg/ml of antibiotic solutions applied as a foliar spray to the point of runoff and a 100-ml root drench to each 4-in.-square pot. Plants developed normal flowers after applications of tetracycline and oxytetracycline but not penicillin or water. When treatments were discontinued, virescence symptoms reappeared. These findings are consistent with the presumed MLO etiology of BLTVA.

Plants and insects. All plants were grown from seed in a separate greenhouse fumigated biweekly with DDVP (2,2-dichlorovinyl

dimethyl phosphate). These plants included: short-pod mustard (*B. geniculata*, field collected), celery (*Apium graveolens* L. var. *dulce* (Mill.) 'Giant Pascal'), Madagascar periwinkle (*C. roseus* 'Little Pinkie'), radish (*R. sativa* 'Scarlet Turnip White Tipped'), and sugar beet (*Beta vulgaris* L. 'V5H10').

C. tenellus colonies were reared on sugar beet plants maintained at 20–30 C under a light:dark regimen of 16L:8D. The original colony was initiated from leafhoppers collected in Buena Vista, Kern County, CA, in the spring of 1982. Leafhoppers were fed monthly on healthy indicator plants to ensure that the population remained free of mollicute contamination. No contamination with BLTVA or *S. citri* was detected in healthy stocks of *C. tenellus*.

Acquisition access period. The amount of time needed by the leafhopper to acquire BLTVA by feeding, that is, the acquisition access period (AAP), was determined by allowing young adult leafhoppers to feed on radish plants showing symptoms typical of early infection with BLTVA. Leafhoppers in groups of 100 were starved for 1/2 hr and then allowed access to the infected radish for 5, 15, or 30 min; 1, 2, 4, 6, or 12 hr; or 1 or 2 days. Tests of times under 12 hr were conducted in the morning to avoid potential differences in acquisition rates because of circadian variation in leafhopper feeding behavior. Insects were then transferred to healthy sugar beet plants for a 2-wk holding period. Groups of 10 insects per plant were then placed on young healthy periwinkle, 6- to 10-cm high, in cylindrical cages (11) and allowed to feed for 2 wk in the greenhouse. Afterwards, the caged plants were fumigated with DDVP, removed from cages, and placed in a greenhouse for the development of symptoms. Plants that failed to develop disease symptoms within 8 wk were rated as negative for infection. This experiment was repeated four times and the data were pooled for a total of at least 20 plants per acquisition time.

Latent period. Fourth- and fifth-stage nymphs of *C. tenellus* were fed for 24 hr on a BLTVA-infected radish plant and then placed in groups of 10 on small radish test plants. Thereafter, every 2 days the insects were transferred to a fresh radish plant. Dead leafhoppers were replaced from a pool of insects that had received identical handling so that each plant was exposed to the same number of leafhoppers. All transmission feeding was done in a constant temperature cabinet set for a 16-hr day with a day:night temperature regime of 24:20 ± 3 C. After the test exposure to leafhoppers, radish plants were sprayed with acephate and placed in the greenhouse for observation. Radish plants were kept up to 12 wk in the greenhouse to allow development of floral symptoms. This experiment was repeated three times so that a total of 30 plants was exposed to insects during each 2-day interval.

Fig. 1. Transmission electron micrograph of a phloem sieve element of a Madagascar periwinkle, *Catharanthus roseus*, infected with the beet leafhopper transmitted virescence agent showing mycoplasma-like organisms. Scale line = 0.5 μm.

Inoculation. Preliminary observations showed that highly inoculative *C. tenellus* were produced when adult leafhoppers were allowed to oviposit in infected radish plants, and the resulting mature progeny, which had been reared on diseased host material, were collected and placed on test plants. In all of our inoculation tests, leafhoppers were produced by this method.

The inoculation access period (IAP) was determined by starving inoculative young adults for 1/2 hr, then placing them in groups of 10 on individual periwinkle plants for 5, 15, or 30 min; 1, 2, 4, 6, or 12 hr; or 1 or 2 days. As in the case of the AAP experiments, IAPs of less than 12 hours were determined during the morning. In each test, insects were fed on 10 plants for each time, and the entire experiment was repeated four times. Plants were held at least 8 wk for the development of symptoms.

The inoculation efficiencies of single insects, 5 insects, and 10 insects to a number of different host plants were compared by placing inoculative young adults on very young plants for 2 days. The plants were then fumigated and held in the greenhouse for observation. Celery, *B. geniculata*, periwinkle, and radish test plants were all exposed in this manner. Periwinkle plants were held at least 8 wk; the other plant species were held several weeks longer, as needed for symptom development.

The potential difference in the inoculation rate by sexes was also determined. Males and females were separated from groups of inoculative leafhoppers after anesthetization with carbon dioxide gas. Groups of 10 leafhoppers were placed on periwinkle for a 2-wk IAP. Test plants were fumigated and transferred to a greenhouse for observation.

RESULTS

Acquisition access period. Leafhoppers acquired BLTVA from infected radish plants in as little as 5 min. This was the shortest AAP tested, but only one of the 36 plants exposed to 10 leafhoppers after a 5-min AAP developed disease symptoms (Fig. 2). Similar inefficient transmission occurred with groups of insects allowed 15 and 30 min access to inoculum. A 1-hr AAP resulted in a 25% transmission rate to test plants. With a 4-hr AAP a majority of test plants became infected. An AAP of either 24 or 48 hr on radish resulted in transmission of BLTVA by 10 insects to 100% of the plants in each of the four replicates of this experiment.

Latent period. The relationship between the length of the postacquisition access period and transmission is shown in Figure 3. No plants developed disease symptoms when exposed to leafhoppers that had undergone a latent period (LP) of less than 12 days. One of 30 plants developed symptoms after exposure to insects with an LP of 12–13 days. Transmission increased quickly after this minimum LP was satisfied; by 20–21 days postacquisition about 50% transmission was recorded.

Maximum transmission, which approached 100%, occurred between 26 and 27 days postacquisition and declined gradually thereafter. By 38–39 days only 50% of plants developed disease symptoms. At this time, leafhopper survival was too low to continue two of the three runs of this experiment. In one replicate, however, there were sufficient surviving leafhoppers to continue the experiment for 52 days. The decline in transmission was less pronounced in this run; transmission rates of 70% were observed both 40- to 41-days and 50- to 51-days postacquisition.

Inoculation. As with acquisition, inoculation with BLTVA can occur in as little as 5 min. As seen in Table 1, transmission during the IAPs of 30 min or less did not occur frequently. After a 12-hr

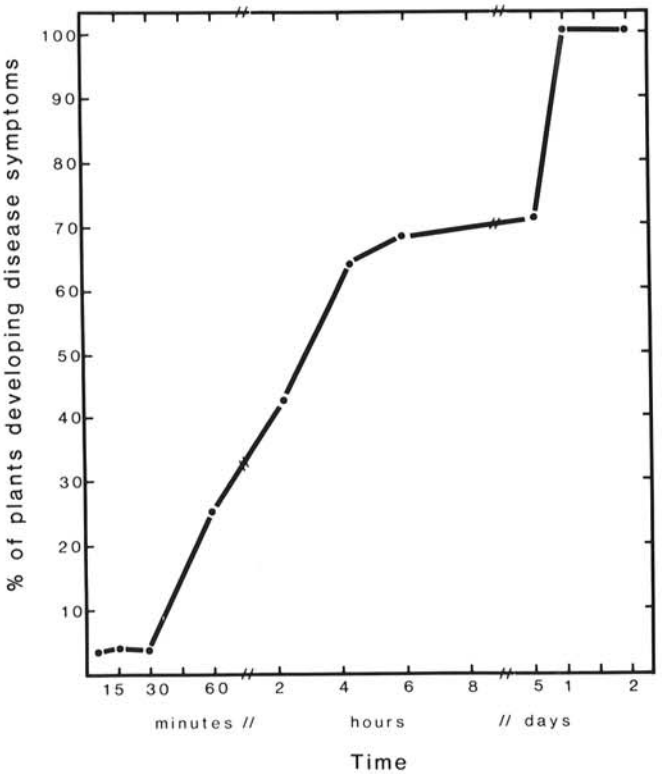

Fig. 2. The effect of length of acquisition access period upon the percentage of test plants developing symptoms of infection by the beet leafhopper transmitted virescence agent.

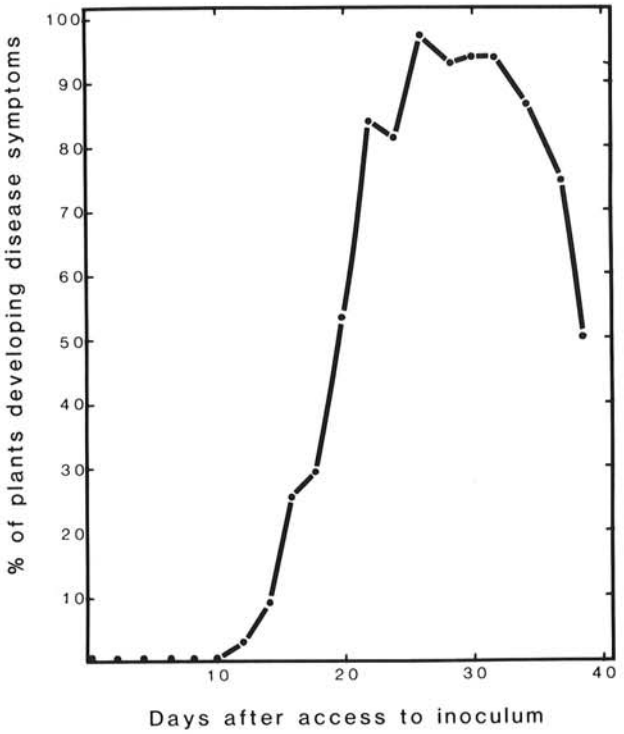

Fig. 3. Percentage of plants developing symptoms of beet leafhopper transmitted virescence agent after exposure to beet leafhoppers, *Circulifer tenellus*, fed on diseased plants. Leafhoppers were given a 24-hr access to an infected plant and then moved to fresh plant material every 2 days.

TABLE 1. The effect of the length of inoculation access period (IAP) on the number of periwinkle plants developing symptoms of beet leafhopper transmitted virescence after exposure to inoculative *Circulifer tenellus*[a]

Inoculation access period	No. symptomatic plants/ No. exposed plants				
	Exp. 1	Exp. 2	Exp. 3	Total	% infected
0	0/10	0/10	0/10	0/30	0
5 min	0/10	0/10	1/10	1/30	3.3
15 min	2/10	0/10	1/10	3/30	10.0
30 min	2/10	2/10	1/10	5/30	16.6
1 hr	3/10	2/10	2/10	7/30	23.3
2 hr	3/10	1/10	1/10	5/30	16.6
4 hr	2/10	4/10	4/10	10/30	33.3
6 hr	2/10	5/10	5/10	12/30	40.0
12 hr	2/10	5/10	7/10	14/30	46.6
24 hr	6/10	9/10	7/10	22/30	73.3
48 hr	8/10	10/10	9/10	27/30	90.0

[a]Each periwinkle was exposed to 10 leafhoppers.

IAP, groups of 10 leafhoppers transmitted to 50% of exposed test plants. Maximum inoculation followed IAPs of 24 and 48 hr.

About 50% of plants exposed to individual *C. tenellus* for 2 days became infected with BLTVA (Table 2). This held true for *A. graveolens, B. geniculata, C. roseus*, and *R. sativus*. Almost all plants exposed to groups of 5 or 10 leafhoppers developed symptoms of infection.

The percentage of periwinkle plants that developed disease after exposure to groups of male *C. tenellus* was 82% (43/53), whereas that which developed disease after exposure to female leafhoppers was 81% (38/46). We noted no significant sex-linked difference in BLTVA transmission by *C. tenellus*.

DISCUSSION

Detailed studies of the transmission biology of a number of leafhopper-borne mollicutes by specific vectors in different disease systems already exist in the literature (2,12,13). This study of the interaction between *C. tenellus* and BLTVA would indicate that the transmission processes of acquisition, latency, and inoculation

TABLE 2. Transmission efficiency[a] of groups of *Circulifer tenellus* inoculative for beet leafhopper transmitted virescence agent (BLTVA) when fed on different plant hosts[b]

Test plant	No. plants infected/No. plants exposed		
	1 insect	5 insects	10 insects
Apium graveolens	5/12	10/12	12/12
Brassica geniculata	7/12	11/12	11/12
Catharanthus roseus	21/48	31/31	28/28
Raphanus sativus	26/35	20/20	16/16

[a]Symptom development was used as the criteria for infection.
[b]Transmission access period was 2 days.

occur in much the same pattern that has been observed for other mollicutes such as *S. citri*, the aster yellows agent, and the clover phyllody agent.

The 12-day latent period that we observed before *C. tenellus* was able to transmit BLTVA is consistent with an MLO etiology. The wall-less prokaryotes are believed to undergo circulation and multiplication in their insect hosts before inoculation is possible. Similarly, the decline in transmission efficiency detected after about 30 days is similar to that generally observed in other mollicute-leafhopper systems (12).

Transmission of BLTVA occurred quite readily when single insects were fed for 2-day periods on any of several species of host plants. Any future comparisons of the susceptibility of different plant varieties or species should be done with single insects. The high efficiency of transmission with even small groups of insects would obscure any subtle differences in host susceptibility.

The numbers of plants that eventually developed disease in the IAP tests were lower and the data more variable than in any of the other experiments we conducted under similar environmental conditions. We attribute this anomaly to the frequent manipulation of insects required to achieve a uniform population of 1,000 insects with which to perform each replication of this experiment. Adult insects were removed from the infected plants on which they had been reared, pooled and mixed, and then held in subgroups for starvation periods before they were placed on individual test plants. Transmission rates by groups of male and

female insects were also less than anticipated but these leafhoppers were anesthetized before sorting under a dissecting microscope. In both these cases, repeated handling or anesthesia may have resulted in an unusually large number of damaged leafhoppers and, subsequently, relatively low rates of transmission.

In conclusion, these data on the transmission of the FC-83-13 line of BLTVA by *C. tenellus* provide additional evidence that the agent is of MLO etiology. They also provide a framework of information that will be useful to investigations of the nature of the relationship between BLTVA and *S. citri* in individual plants and in an ecological context.

LITERATURE CITED

1. Calavan, E. C., and Oldfield, G. N. 1979. Symptomatology of spiroplasmal plant disease. Pages 37-64 in: The Mycoplasmas. Vol. 3. R. F. Whitcomb and J. G. Tulley, eds. Academic Press, New York. 351 pp.
2. Chiykowski, L. N. 1981. Epidemiology of diseases caused by leafhopper-borne pathogens. Pages 106-161 in: Plant Diseases and Vectors. K. Maramorosch and K. F. Harris, eds. Academic Press, New York. 368 pp.
3. Cook, W. C. 1967. Life history, host plants, and migrations of the beet leafhopper in the western United States. U.S. Dep. Agric. Tech. Bull. 1365. 122 pp.
4. Eastman, C. E., Schultz, G. A., Fletcher, J., Hemmati, K., and Oldfield, G. N. 1984. Virescence of horseradish in Illinois. Plant Dis. 68:968-971.
5. Fudl-Allah, A. E-S., Calavan, E. C., and Igwegbe, E. C. K. 1972. Culture of a mycoplasmalike organism associated with stubborn disease of citrus. Phytopathology 62:729-731.
6. Liu, H.-Y., Gumpf, D. J., Oldfield, G. N., and Calavan, E. C. 1983. Transmission of *Spiroplasma citri* by *Circulifer tenellus*. Phytopathology 73:582-585.
7. Oldfield, G. N. 1982. A virescence agent transmitted by *Circulifer tenellus* (Baker): Aspects of its plant host range and association with *Spiroplasma citri*. Rev. Infectious Dis. (Suppl.) 4:S279.
8. Oldfield, G. N., and Kaloostian, G. H. 1979. Vectors and host range of the citrus stubborn disease pathogen, *Spiroplasma citri*. Proc. R.O.C.-United States Cooperative Science Seminar on Mycoplasma Diseases of Plants. NSC Symposium Series No. 1:119-125.
9. Oldfield, G. N., Kaloostian, G. H., Pierce, H. D., Granett, A. L., and Calavan, E. C. 1977. Beet leafhopper transmits virescence to periwinkle. Calif. Agric. 31(6):14-15.
10. Oldfield, G. N., Sullivan, D. A., and Calavan, E. C. 1984. Inoculativity of leafhopper vectors of stubborn disease in California. Proc. 9th Conf. Int. Org. Cit. Virol., pp. 125-130.
11. Pierce, H. D. 1976. A new, lightweight transparent insect cage. USDA-ARS West. Reg. Publ. ARS-W34. 4 pp.
12. Purcell, A. H. 1982. Insect vector relationships with prokaryotic plant pathogens. Annu. Rev. Phytopathol. 20:397-417.
13. Rosenberger, D. A. 1982. Fastidious Prokaryotes: Epidemiology of the hidden pathogens. Pages 71-98 in: Phytopathogenic Prokaryotes. Vol. 2. M.S. Mount and G. H. Lacy, eds. Academic Press, New York.