The Latent Period of Beet Curly Top Virus in the Beet Leafhopper, *Circulifer tenellus,* Mechanically Injected with Infectious Phloem Exudate

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ABSTRACT

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The latent period of beet curly top virus (BCTV) was investigated by injecting beet leafhoppers, *Circulifer tenellus*, with infectious phloem exudate from spinach plants. The probability of transmission to the first test plant usually was less than that to subsequent plants, but this may have been a behavioral artifact. Transmission efficiency during short (8-9 hr) inoculation access periods (IAP) was greater per unit of time than that during long (48 hr) IAP. This suggested that only part of the feeding cycle was involved in inoculation. The cumulative proportion of first transmissions during the first six successive inoculation access periods of equal length (8 or 9 hr) following injection approximated a binomial function. The median latent period (LP_{50}), estimated to vary from 16.3 to 18.8 hr at 27 C and ~27,000 lux of continuous light, suggests that although there may be a constant probability of inoculating a plant during any one of a sequence of IAP, it is less than 1.0.

The possible multiplication of plant viruses in insect vectors has been the subject of intensive research and speculation since the turn of this century. It is now evident that some plant viruses do multiply in their insect vectors, and that when this happens, vector transmission usually occurs only after completion of a long latent period (12).

The vector-virus relationships of beet curly top virus, which is confined to the phloem cells of various plants and transmitted by the leafhopper *Circulifer tenellus* (Baker), is characterized by a short latent period, prolonged retention of inoculativity, and by transstadial passage (1). Previous investigations with both individual and large groups of insects indicated that the minimum latent period of beet curly top virus (BCTV) varied from less than 1 hr to a number of days (2,3,8–10). Using needle injection of the beet leafhopper, Maramorosch (6) reported transmission only after a latent period in the insect and concluded that BCTV may multiply in its insect vector.

To obtain further evidence on the latent period, we examined the median latent period (LP_{50}) (13) in the beet leafhopper following injection with highly infectious phloem exudate from spinach plants (5). Two advantages to this experimental approach were: the virus concentration in the phloem exudate presumably was similar to that picked up by the vector when naturally feeding on phloem cells and other than dialysis, the test material was not subjected to any purification procedure that might affect the vector and thus interfere with transmissibility of the virus or increase mortality of the insect. A preliminary account of this investigation has been published (5).

MATERIALS AND METHODS

The original isolate of the virus (Fresno II) was maintained on sugar beets (4). Spinach (*Spinacia oleracea* L. 'Resistoflay') plants were raised in U. C. mix (7) and 20 days after planting were inoculated by attaching to each plant a leaf cage containing five viruliferous leafhoppers. After five days, the leaf cages were removed and the plants with vein clearing on the primary leaves were put in saucers and kept on benches under normal greenhouse conditions. Plants were sub-irrigated to avoid undesirable water droplets on veins and petioles of the leaves. About 10 μl of amber phloem exudate that appeared after eight to 13 days, after removal

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of leaf cages, was collected in micropipettes and pooled from 50 infected spinach plants.

Untreated droplets of exudate were injected into beet leafhoppers in preliminary experiments. In later experiments, however, because of high viscosity, the exudate (which contained about 9% sugar) was dialyzed overnight against 3% sucrose in 10 mM Tricine (N-tris hydroxymethyl-methyl glycine) buffer, pH 7.6. In a typical experiment, glass needles were used to inject ~0.02 μl of phloem exudate into each of fifty 5-day-old nonviruliferous adult beet leafhoppers (13). Injected insects were placed singly in small leaf cages, each clipped on a 10-day-old sugarbeet test plant (cultivar 742), and transferred every 8 hr (9 hr in two experiments) to a new 10-day-old sugarbeet plant. In some of the tests, additional transfers continued at 48-hr intervals. During the inoculation access periods (IAP), the plants were kept in a growth chamber at 27 C under continuous light of ~27,000 lux at plant level.

After each transfer, the test plants were placed in the greenhouse, sprayed with Dimethoate (O,O-dimethyl S-phosphorodithioate; Cygon® 25 WP), and checked daily for symptoms for 15 days.

The median latent period (LP_{50}) (ie, the time required for 50% of the infective insects to have transmitted at least once) was estimated using a log function of the probability of transmission plotted against time and calculating a least squares regression line (11).

RESULTS AND DISCUSSION

Analysis indicated that the probability of transmission was similar during the first six transfers in four of the seven trials; ie, during the initial series of 8 or 9-hr IAP (Table 1). However, transmission in the first IAP tended to be below the average of the first six transfers. This may have been a behavioral artifact resulting from collecting, injecting, and then caging the insects on an unfamiliar test plant. When the entire sequence of transfers was used, including the terminal series of 48-hr IAP (with a final 72-hr access period in trial VII), similar rates of transmission per transfer occurred only in two of the seven trials. Transmission in the prolonged access periods never was equivalent to the cumulative multiple of that during an 8-hr unit.

TABLE 1. The latent period and rate of transmission of beet curly top virus by injected beet leafhopper, *Circulifer tenellus*[a]

Trial	Transfer number[b]													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
I	2/30[c]	11/30	11/30	10/28	8/27	5/26	12/25							
II	4/38	18/38	14/38	17/38	8/37	7/37								
III	6/48	7/48	13/46	8/46	17/46	13/46	33/45	18/34	8/16	8/9	2/8	4/6	3/4	2/4
IV	9/51	16/51	9/51	10/49	10/49	11/47	11/45	14/45	7/44	4/44	13/44			
V	6/50	9/48	9/47	8/45	6/45	9/41	10/40	13/33	7/30	5/29				
VI	6/50	11/49	13/48	9/48	8/46	7/46	16/45	9/42	18/41	10/36				
VII	8/51	16/51	6/48	12/48	9/47	9/46	12/46	11/46	15/46	12/46	28/46			

[a] Adult insects were injected with an estimated 0.02 μl of infectious phloem exudate from spinach, caged on sugar beet test seedlings, and transferred under conditions of 27 C and constant light of ~27,000 lux.

[b] In trials I and II, all transfers were at 9-hr intervals. In trials III through VII, the initial six transfers were at 8-hr intervals, and the remaining transfers were at 48-hr intervals. The final inoculation access period in trial VII was 72-hr in duration.

[c] In the ratios listed, the numerator is the number of plants infected; the denominator, the number tested by exposure to single insects.

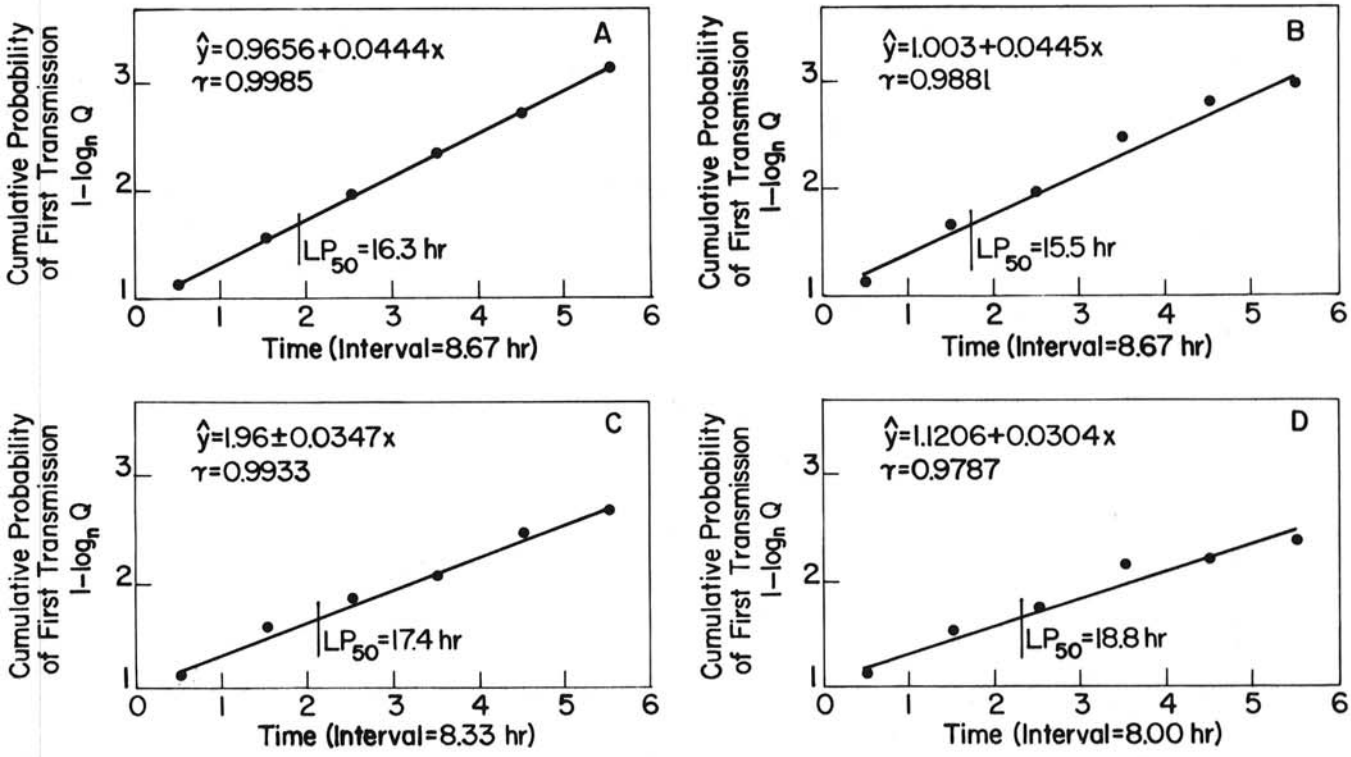

Fig. 1. Latent period estimates of curly top virus transmitted by beet leafhoppers, *Circulifer tenellus*, injected with an estimated 0.02 μl of infectious phloem exudate and transferred to sugar beet seedlings at 27 C and constant light. Among seven trials, those having similar probabilities of transmission were combined into subsets. The proportion of transmitting vectors realized in the first six transfers used to estimate the latent period were 87, 86, 74, and 75% in subsets **A, B, C,** and **D,** respectively.

Thus, transmission may not be a function of continuous feeding, but rather a phenomenon primarily associated with specific parts of the feeding cycle, for example with initiation and establishment of the feeding penetration. There was a tendency for the injected insects, during the initial six transfers, to be equally inoculative within a trial, with the exception of trials II and IV.

The trials were compared to form subsets with similar probabilities of transmission. The entire usable data obtained in each trial was examined, and the following subsets were formed: A (trials I, II, and III), B (trials I, II, and VII), C (trials II, VI, and VII), and D (trials IV, V, and VI), with probabilities of transmission of 0.32, 0.28, 0.26, and 0.22, respectively.

The data from the first six transfers in each subset were plotted (Fig. 1) as a binomial function ($1 - \log_n Q$, in which $Q = 1 - P$, and P was the cumulative probability of first transmissions) of time. The estimated LP_{50} ranged from 16.3 to 18.8 hr, with some tendency for shorter estimates to be associated with higher vector inoculativity.

There was little evidence that a true latent period (ie, an obligatory delay, after acquisition, before infective insects begin to transmit) had occurred. Rather, it seems in the transmission of BCTV by beet leafhoppers, that there is an "apparent" latent period, reflecting the fact that every access period, or feeding, by an infective insect does not result in transmission.

It has been suggested that the presence of a latent period is evidence of a possible propagative relationship between BCTV and the beet leafhopper (6). Our evidence would not support such a hypothesis.

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