Transmission of the Safflower Phyllody Mollicute by *Neolaiturus fenestratus*

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ABSTRACT

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An efficient transmission of the safflower phyllody mollicute by the leafhopper, *Neoaliturus fenestratus*, is reported. More than 50% of the insects given an acquisition feeding on phyllody-affected safflowers became infective. The duration of the latent period was found to be 20–25 days; the duration of vector inoculativity was about 38 days for mated insects and more than 25 for unmated insects. Females infected more plants per vector than did males, but the difference was not significant. Of the test plants presented to vectors, 50–60% became infected. Insects given an access feeding on infected safflower had a mean longevity 23 days shorter than that of leafhoppers feeding for the same time on healthy plants. Also, reproductive rate values were smaller for inoculative leafhoppers than for leafhoppers in the control. The possible effects of these factors on the efficiency of the vector is discussed.

Safflower phyllody is caused by a mollicute (8). The disease occasionally causes serious damage to safflower (*Carthamus tinctorius* L.), a crop that recently has become important in semiarid areas. The disease agent was reported to be transmitted by the leafhopper *Neoaliturus fenestratus* (8), but to date no detailed transmission studies have been reported. In the Mediterranean region, this leafhopper occurs on several host species, mainly in the Compositae, but also on plants of species in other botanical families (3,10).

The disease agent is persistently transmitted by *N. fenestratus*. Therefore, it was of interest to measure the efficiency of transmission throughout the vector's life. Also, possible effects of the mollicute on survival and fecundity of the vector were studied.

MATERIALS AND METHODS

Leafhopper culture. The *N. fenestratus* population used in our experiments was derived from a pair of insects collected on the weed *Carthamus tenuis* in the summer of 1978 at Bet Dagan. The leafhopper culture has been since maintained on *C. tinctorius* by weekly passages of adults to a new series of plants. The culture was maintained under constant illumination, at 25 ± 2 C. When nymphs of uniform age were needed, mothers were transferred to safflowers for 48 hr of oviposition. Nymphs usually hatched after 11 days and were uniform.

Safflower phyllody source plants. The original source of the disease agent was an infected *C. tenuis* plant found in a field in the vicinity of Bet Dagan. Periodic inoculations of new series of safflower plants by exposing them to infectious *N. fenestratus* were made to maintain or increase numbers of diseased plants. The infected plants were kept in an insect-proof compartment at 25 ± 2 C. For transmission studies, only diseased plants with fully developed symptoms were used.

Experimental procedures. Sylvester and Richardson (13) adopted the life-table technique (1) for transmission studies. This technique was also used here.

Acquisition was achieved by caging 4- to-5-day-old nymphs on an infected safflower plant for 72 hr. Thereafter, the nymphs were transferred to test plants for individual rearing. Seven-day-old safflower test plants grown in a greenhouse were changed twice each week. After the change, the test plants were fumigated and transferred to the test-plant compartment, where insecticides were applied when necessary. The test plants were kept there for 7–8 wk, but not less than 30 days after the first appearance of symptoms.

In one experiment, males and females that were not allowed to mate were used, and in another experiment mated insects were tested. Thus, after the completion of the acquisition feeding and immediately upon ecdysis to the adult stage, males and females were caged in pairs for a 48-hr mating period, then separated and placed in individual cages on test plants. Reproduction was determined by counting the hatched nymphs. Thus, test plants inoculated by mated females remained covered with cages up to hatching; then, nymphs were counted and the test plants were fumigated and transferred to the test-plant compartment. There was some mortality of nymphs during both the acquisition feeding and the parallel feeding on the healthy plant. Arbitrarily, we decided to begin mortality counts after 10 days, when acquisition and the first transfer were completed.

Survival and reproduction of leafhoppers, number of infected test plants, and the time that symptoms appeared were recorded.

RESULTS

The data on transmission of the safflower phyllody agent by *N. fenestratus* is presented in Table 1. The median latent periods (the time from the acquisition of the disease agent by leafhopper to the appearance of the first symptoms in the test plants for 50% of the vectors) were almost the same for both males and females, whether mated or unmated, and ranged between 20 and 25 days for the four groups (Table 1). Individual leafhoppers needed up to 53 days to become infective.

Of the 44 unmated leafhoppers given an acquisition access feeding, and completing the latent period, 24 became inoculative whereas 19 out of 36 of the mated ones became inoculative. Therefore, more than 50% in each group became infective.

The efficiency of transmission is expressed by the mean number of plants inoculated per vector throughout its life. This expression is equal to the product of the age-specific rate of survival and the age-specific transmission (Table 1). This expression shows a smaller number of test plants infected by males than by females, whether mated or unmated. The mean weighted duration of inoculativity (Table 1) was about 10 days longer for unmated insects than for the mated ones. If we take into account the latent period, it is apparent that most insects remain inoculative to the last day of their life. In all cases, about 50% of the plants exposed to vectors became infected. The incubation period for the disease in safflower lasted between 27–35 days. Table 2 summarizes the life-table statistics for inoculative (= insects that were given acquisition

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TABLE 1. Statistics of the safflower phyllody agent transmission by the leafhopper, *Neoaliturus fenestratus*

Treatment	Number of insects Used	Number of insects Infective	Latent period[w] (LP_{50}) (days)	Mean number of plants infected per vector[x]	Mean weighted duration of inoculativity (days)[y]	Rate of infection. Infected/exposed and percent in (X)
Unmated						
females	44	16	24.69	6.75	37.41	106/195 a[z] (56.4)
males		8	24.94	4.99	38.61	40/81 a (49.4)
Mated						
females	18	10	20.56	8.0	28.28	66/113 a (58.4)
males	18	9	21.1	6.1	25.96	55/112 a (49.1)

[w] Median latent period (LP_{50}) estimated by linear regression of time and percent transmission.
[x] This expression is equal to $\Sigma l_x i_x$ where l_x is the proportion of vectors surviving at age X, and i_x is the age-specific rate of transmission.
[y] This value is the mean weighted period, in days, that the vector remained inoculative, and is equal to $\Sigma l_x i_x X / \Sigma l_x i_x$, when the preinoculative period of life is deducted.
[z] Means followed by the same letter do not differ significantly, $P < 0.05$. Calculations described in footnotes x and y are after Sylvester and Richardson (13).

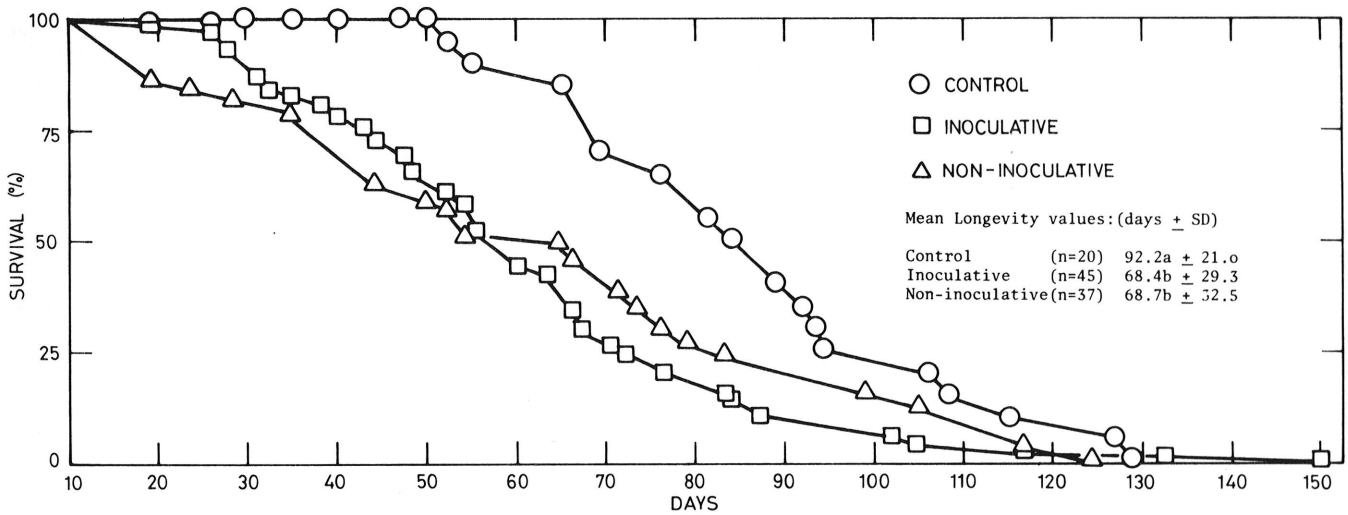

Fig. 1. Survival curves and longevity values for the leafhopper, *Neoaliturus fenestratus*, the vector of the safflower phyllody agent. Inoculative insects were those transmitting at least once. Noninoculative insects were given an acquisition feeding on infected safflower, but did not transmit. Control leafhoppers were fed on healthy plants only. Means followed by the same letter are not significantly different ($P < 0.01$) (n = number of insects used).

TABLE 2. Life-table statistics for inoculative, noninoculative, and control *Neolaiturus fenestratus*, the vector of the safflower phyllody agent[a]

Leafhoppers (status and no. tested)	Net reproductive rate (♀/♀/ generation)[b]	Generation time (days)[c]	Intrinsic rate of increase (♀/♀/day)[d]	Finite rate of increase (♀/♀/day)[e]
Inoculative				
10	24.94	43.93	0.073	1.075
Noninoculative				
9	37.68	44.68	0.0812	1.084
Control				
10	158.2	62.1	0.0815	1.085

[a] Inoculative leafhoppers are considered those that infected test plants at least once; noninoculative insects are considered those given an access feeding on a diseased plant but did not transmit the pathogen control leafhoppers were fed on healthy plants. Number of insects used in parentheses.
[b] The net reproductive rate is equal to $\Sigma l_x m_x$, where l_x is the age-specific rate of survival, m_x is the age-specific fecundity, and X is the age in days.
[c] The generation time is obtained using the formula: $\Sigma l_x m_x X / \Sigma l_x m_x$.
[d] The intrinsic rate of increase is equal approximately to ln $\Sigma l_x m_x$/ Generation time.
[e] The antilog for the intrinsic rate of increase.

feeding and became infective), noninoculative (= insects that were given acquisition feeding, but did not become infective), and control (= insects that were fed on healthy plants) leafhoppers. As seen from Table 2, the net reproductive rate of the inoculative insects was severely affected, being only 24.94 females per female per generation. The noninoculative leafhoppers were severely affected also, having only a slightly higher reproductive rate (37.68), while the net reproductive rate of the control insects was 158.2 females per female per generation. Figure 1 shows the effect of the plant pathogen on survival. The mean longevity of inoculative and noninoculative insects was more than 20 days shorter than that of control leafhoppers. Generation time was 16 days less in insects feeding on diseased plants (Table 2). The effect on net reproduction was clear, as five of the females that were inoculative produced none or only a few nymphs. All the noninoculative females tested were fertile, but they produced only a few nymphs. Thus, the intrinsic rate of increases (Table 2) was smallest for the inoculative females, and so was its reciprocal (Table 2), the finite rate of increase.

DISCUSSION

The leafhopper *N. fenestratus* was found to be an efficient vector of the safflower phyllody agent, as characterized by rates of transmission and duration of inoculativity. Similar parameters were studied for the transmission of the western X agent by *Colladonus montanus* (7). The latent period was longer than reported for aster yellows mycoplasma, which is transmitted by *Macrosteles fascifrons* (9), but less than for western X disease transmitted by *C. montanus* or by other vectors (7,12). The incubation of the safflower disease agent in plants was also long, an average of 4–5 wk. We did not observe significant differences in duration of inoculativity between mated and unmated insects, but there was a tendency toward a longer duration of inoculativity for

the unmated. It also appeared that females inoculated more plants per vector than males.

Since 1958, when the western X disease of peach was found to affect its leafhopper vector, *C. montanus* (5,6), several additional organisms were found to affect their vector: Aster yellows agent induced changes in fat body nuclei of *M. fascifrons* (11). *Spiroplasm citri* affected *M. fascifrons*, the vector of the aster yellows, but did not affect *Dalbulus elimatus*, the vector of the corn stunt (15), nor did it affect *Euscellis plebejus* in which it reproduces (14). On the other hand the corn stunt organism affected both *D. elimatus* and *D. maidis*, reducing the leafhopper longevity (4). A possible effect of the safflower phyllody agent on the vector was reported, showing longevity reduction. Thus this pathogen actually reduced the transmitting efficiency of the vector. The effect of the pathogen was apparent in insects that fed on diseased plants although they did not become infective. One explanation may be that the effect on the insect was indirect, acting through toxins acquired from the infected plant. Such toxins have been identified for *S. citri* (2), but they were not toxic to *E. plebejus*. Potential toxin production and other aspects of the disease caused by the safflower phyllody agent for its vector *N. fenestratus* deserve more intensive research in the future.

The long maintenance of infectivity by this vector makes it most important to keep safflower fields as free as possible of the weed *C. tenuis* in which the leafhopper breeds, because the leafhopper is frequently found to be carrying safflower phyllody pathogen. Control of this host could probably reduce the infection and spread of safflower phyllody.

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