Relation of Age, Sex, and Mating of Macrosteles fascifrons to Transmission of Aster Yellows

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ABSTRACT

The effects of sex, mating, and age when pathogen was acquired on transmission of aster yellows by *Macrosteles fascifrons* are reported. Fewer 1st-instar nymphs than adults became inoculative during 2 days on diseased plants. The nymphs that became inoculative transmitted as frequently, but no more so, than did adults. Males transmitted less frequently than did females. No effects on transmission due to mating were observed. Phytopathology 61:657-659.

Additional key words: leafhoppers, mycoplasma.

Many variables have been identified in the circulative transmission of plant viruses by insects (17). Sex, along with age and mating, are possible variables in disease transmission by leafhoppers that have not yet been adequately investigated. Several comparisons have been made of virus or mycoplasma transmission by male and female leafhoppers, when the pathogen was acquired either as nymphs or adults, or both. Results have been either inconclusive (10), have shown no difference between males and females (7, 16), or have shown that females were more efficient transmitters than were males (4, 5). Age of nymphs and adults, and whether or not insects had mated at the time of virus acquisition, were seldom specified in previous work. The results might have been generally more consistent among the viruses, or different results might have been obtained with specific viruses, if age and mating had been controlled. This paper reports a comparison of aster yellows transmission by males and females, by mated and unmated insects, and by insects that became infective as adults and 1st-instar nymphs.

MATERIALS AND METHODS.—The aster yellows used in these experiments was obtained from J. Raine, Canada Department of Agriculture, Vancouver, B. C. A mycoplasmalike organism was isolated from plants infected with this culture (1). Aster yellows is now regarded as of probable mycoplasma etiology (18), and will be referred to herein simply as aster yellows (AY). *Macrosteles fascifrons* cultures were maintained on barley.

Aster plants, *Callistephus chinensis* Nees 'Princess Imperial Wilt-Resistant Hybrids', were used as virus source plants and as test plants in all cases. Each insect was given a 2-day period on diseased plants, then transferred daily to a succession of healthy plants for 60 days or until death. Adults were fed on diseased plants during the first 2 days of adult life, and 1st-instar nymphs were fed on diseased plants during the first 2 days after they hatched. Mated insects were confined in pairs for the first 7 to 14 days after they became adults. Examination of plants on which 20 of the mated females were placed showed that they laid eggs, which hatched.

Aster plants were grown in a greenhouse over a period of 8 months, usually under supplementary fluorescent light to make 16-hr daily light periods. Temperatures were approximately 24 C during the day and night. Each plant was kept in a controlled environment chamber during the day when a leafhopper was confined on it. Thus, the leafhoppers were kept continuously in a controlled environment chamber from the time they acquired AY until the experiment was terminated. The daily light period was 16 hr; the day temperature was 24-25 C and the night temperature was 18-19 C.

Transmission tests were made with the following types of insects: unmated females, AY acquired as adults; unmated females, AY acquired as nymphs; mated females, AY acquired as adults; unmated males, AY acquired as adults; unmated males, AY acquired as nymphs; mated males, AY acquired as adults.

The transmission tests could not all be made simultaneously because of the number of insects (219) used. The time required to grow a suitable test plant was about 16 days after germination, and each plant was kept for about 21 days after inoculation, depending on the time of the year, so that about 37 plants were concurrently maintained in the greenhouse for each insect that was being tested. Variation in survival of insects did not permit an experimental design which would allow a rigorous statistical test, so the significance of small differences cannot be determined. Serial transmission tests were made in such a way that, at any time during the experimental period, each of the six types of insects was represented in the tests. This was done in order to average-out any variables not controlled in the growth chamber that might have resulted from relative position in the succession of serial transfers. The strain of aster yellows used affected aster yellows severely, so that virus source plants were always used within a few days after the appearance of symptoms. Several parameters were estimated (Table 1), including plants infected per insect day. This was determined for a group of insects by subtracting the mean latent period from the mean longevity. The mean number of plants infected per transmitting insect was then divided by this difference.

RESULTS.—The results are summarized in Table 1.

TABLE 1. Aster yellows transmission by *Macrosteles fascifrons* as related to sex, mating, and stage when pathogen was acquired

	Females			Males		
	Unmated adult	Unmated nymph	Mated adult	Unmated adult	Unmated nymph	Mated adult
Total insects	56	26	39	43	30	25
Transmitters	37 (66%)	6 (23%)	28 (72%)	24 (56%)	12 (40%)	16 (64%)
Longevity of transmitters[a]	51.5	57.3	46.9	52.4	51.2	58.2
Longevity of nontransmitters[a]	50.0	56.4	38.8	50.5	41.8	52.6
Plants infected/insect-day	0.57	0.67	0.61	0.61	0.49	0.45
Latent period[a]	26.0	26.5	24.3	29.2	27.8	24.0

[a] Mean no. of days.

One clear-cut difference is that fewer nymphs than adults transmitted. Among females, 68% of the adults transmitted vs. 23% of the nymphs; among males, the percentages were 59 and 40, respectively. Among all adults, 64% transmitted vs. 32% of the nymphs. Those nymphs that became infective transmitted as frequently, but not more so, than did adults. Frequency of transmission was expressed as the number of plants infected per day by individual insects. Another difference was that males consistently transmitted less frequently than did the comparable females, although the differences were not large. The consistently shorter longevity of nontransmitters may be an artifact of the experiments. Some insects did not survive as long as the minimum latent period, 18 days, in these experiments, and were not included in the summary of results. The latent period was commonly as long as 28-30 days. Any insect that survived only 20-30 days without transmitting was classed a nontransmitter, although some might have transmitted had they lived a few days longer. It would have taken only a few of these to bias the estimates of longevity.

The frequency of transmission by all males and all females in sequential daily transfers is shown in Fig. 1. Both sexes reached a peak of transmission efficiency on the 36th day. The more frequent transmission by females is evident, although transmission frequency decreased more rapidly from the peak level for females than for males.

DISCUSSION.—Experiments to compare transmission should be designed to estimate two parameters: (i) the number of insects that become inoculative; and (ii) frequency of transmission. These may depend on entirely different characteristics of the insects, just as establishment of virus infection and subsequent virus multiplication in plants may depend on entirely independent mechanisms (2).

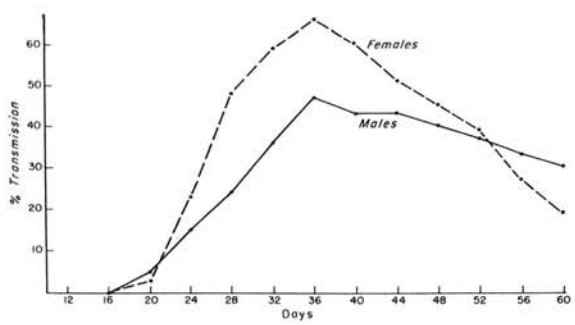

Fig. 1. Transmission of aster yellows by male and female *Macrosteles fascifrons* in sequential daily transfers.

The results of the sequential daily transfer tests (Fig. 1) differ from results of others who found that leafhoppers transmitted AY with a high frequency throughout their lives (8, 11). This probably reflects differences in the strain of AY used. The strain used in these experiments affected aster much more severely than other strains with which I have worked. Also, it was transmitted only very infrequently by *Scaphytopius delongi* (Young), and not at all by *Colladonus montanus* (Van D.), *Euscelidius variegatus* (Kirsch.) and *Fieberiella florii* (Stål), as reported for western AY (14, 15), and for eastern X-disease by *F. florii* (9).

The fact that markedly fewer nymphs than adults transmitted in these experiments appears to contradict the results of Sinha & Chiykowski (16) with aster yellows and *M. fascifrons*. They found that there was no essential difference between nymphs and adults, but they used 2nd- and 3rd-instar nymphs. It is probable that the newly hatched 1st-instar nymphs, having shorter stylets, could not feed in the same tissues as could older nymphs or adults. Also, Sinha & Chiykowski (16) used a 7-day access period, whereas I used a 2-day period. A longer access period would allow more of the less susceptible insects to become infective (11), and thus obscure differences in susceptibility of the insects. Similarly, if the period on the test plant is long enough, or the test plant is sufficiently susceptible, all infective insects may transmit, so that differences in transmitting ability between groups of insects may not be detected.

Female *M. fascifrons* transmitted clover phyllody virus more frequently than did males (4). Chiykowski (4) suggested that females spent a greater amount of their time feeding because of the demands of ovarial development (12). Thus, they would be more likely to acquire virus or to inoculate healthy plants. This may account for the differences between males and females in my experiments. It is surprising that sexual maturation does not affect transmission in more fundamental ways. The onset of reproductive processes in mature females produces tremendous physiological changes, characterized by the formation of female-specific blood proteins and their transfer from the blood to the ovaries (6, 13). One would not expect

the effect of ovarial development to be manifested in a differential transmission between mated and unmated females. The leafhoppers, *M. fascifrons* (K. G. Swenson, *unpublished data*) and *Scaphytopius delongi* (G. G. Kennedy, *unpublished data*), may develop and lay eggs without mating, as do some other insects (3).

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