Dual Transmission of the Aster Yellows Mycoplasmalike Organism and the Oat Blue Dwarf Virus and Its Effect on Longevity and Fecundity of the Aster Leafhopper Vector

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ABSTRACT

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Longevity and fecundity of the aster leafhopper were unaffected by acquisition-access feeding on aster yellows mycoplasmalike organism (AY)-infected asters; on oat blue dwarf virus (OBDV)-infected oats; or on AY- then OBDV-infected plants. Reproduction for all females that had acquisition access feeding on AY-, OBDV-, AY then OBDV-infected plants or kept on healthy plants averaged 22.1, 21.9, 23.6, and 20.8 nymphs, respectively, in a total of 67 days of oviposition. Mean survival of all leafhoppers was 44.9, 45.1, 45.0, and 40.0% at 40 days after acquisition access feeding on AY, OBDV, AY- then OBDV-infected or on healthy plants, respectively. Few leafhoppers in any group survived more than 95 days after acquisition access feeding. Analysis of the data suggested that interference between these pathogens in the aster leafhopper affected their transmission. Transmission means of 76.6 and 34.4% were observed for leafhoppers given 1 wk of acquisition access feeding on only AY- or OBDV-infected plants, respectively. Transmission means of 42.0 and 20.8% were observed for AY or OBDV, respectively, for leafhoppers, that had acquisition access feeding on AY-infected plants for 1 wk, then OBDV-infected plants for 1 wk. Transmission means of 29.7 and 31.0% of leafhoppers transmitted AY or OBDV, respectively, when given similar acquisition-access feeding times on the reverse sequence of hosts. Few leafhoppers simultaneously transmitted both pathogens.

Additional key words: interference, *Macrosteles fascifrons.*

Simultaneous transmission of viruses and mycoplasmalike organisms (MLO) to plants by leafhoppers or planthoppers has been reported infrequently (5,7,9, and Martinez-Lopez, *personal communication*), but little is known about the interactions between the pathogens in the vectors involved.

The aster leafhopper (*Macrosteles fascifrons* Stål [Homoptera: Cicadellidae]) is the vector for both the oat blue dwarf virus (OBDV) and aster yellows mycoplasmalike organism (AY-MLO) (2,3,7). Both pathogens have broad monocotyledonous and dicotyledonous host ranges and the virus, the AY-MLO, and their vector share several common hosts (1,2,6,17,21,23).

Simultaneous transmission of OBDV and AY-MLO to flax, *Linum usitatissimum* L., by individual leafhoppers was reported by Frederiksen (7). He found that few leafhoppers transmitted both pathogens simultaneously and concluded that acquisition of one pathogen did not prevent acquisition and transmission of the second. However, the survival of insects in his experiments was low.

In our experiments in which aster leafhoppers were given combined acquisition-access feeding on AY- and OBDV-infected plants there appeared to be interference between these pathogens in the vector. A second possibility, although longevity of leafhoppers was not recorded, was that combined acquisition of AY and OBDV increased mortality of the vectors which diminished transmission of both pathogens. Although there appeared to be no deleterious effects on leafhoppers that transmitted OBDV alone, no data were available for comparing longevity or fecundity of viruliferous and healthy insects.

The OBDV is a 28–30 nm, RNA-containing phytarbovirus that persists and multiplies in *M. fascifrons* (4). Approximately 30% of individual insects in nonselected populations transmitted the virus to oat seedlings after acquisition access feeding on oats and a minimum 6-day incubation (3).

The longevity and fecundity of aster leafhoppers that transmit AY-MLO alone apparently are unaffected, however certain cytological abnormalities have been observed in the fat bodies of infective insects (14). The AY-MLO also persists and multiplies in *M. fascifrons* (15). Males (59%) were less efficient vectors of AY-MLO than were females (68%) and a mean of 64% of individual adult leafhoppers transmitted the pathogen to aster seedlings after an acquisition access feeding of 2 days on infected asters and a minimum incubation period of 18 days (19).

The objectives of the following experiments were to determine: whether OBDV alone affected longevity or fecundity of the aster leafhopper; whether combined acquisition of the OBDV and AY-MLO affected longevity or fecundity of the aster leafhopper; and whether combined acquisition of AY-MLO and OBDV affected percentage of transmission of either or both pathogens.

MATERIALS AND METHODS

Aster leafhoppers from two sources were used: an HT-6 line of leafhoppers was obtained from R. T. Timian, ARS-USDA, Fargo, ND (20); and a second line of leafhoppers that originally was collected from the field at St. Paul in 1966 and had since been maintained in the greenhouse. Each line of leafhoppers was maintained separately on healthy oat plants. Periodic assays of these insects were made by caging some insects of each line on healthy aster and oat plants to be sure that they were free from AY-MLO and OBDV. In experiments, leafhoppers were caged individually on seedling plants in polyvinyl chloride cages 5 cm diameter × 30 cm high with screened tops and ventilation holes on the sides. The experiments were made in the greenhouse at 20–24 C with periodic wider temperature fluctuations in summer and winter.

The OBDV strain ATCC PV-151 and a zinnia-infecting strain of AY (13) were used in all experiments. OBDV and AY were propagated in oat plants and asters, *Callistephus chinensis* Nees, respectively.

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RESULTS

Effect of acquisition access to OBDV and AY-MLO separately or together on longevity and fecundity of the aster leafhopper. To determine if acquisition-access of OBDV alone or in combination with AY affects longevity or fecundity of the aster leafhopper, the two lines of leafhoppers were allowed to feed separately on plants in the following sequences: (i) OBDV-infected oat plants 1 wk, then healthy oats 1 wk; (ii) AY-infected asters 1 wk, then healthy oat plants 1 wk; (iii) AY-infected asters 1 wk, then OBDV-infected oat plants 1 wk; (iv) healthy oat plants 2 wks. After the leafhoppers had completed the respective acquisition-access feedings, they were transferred individually to flax seedlings for 3 days; then alternately to oat and aster seedlings, at 12-day intervals. These transfers were made until the insect died or until eight transfers were completed. Reproduction was measured by counting nymphs at 3–4 day intervals in cages containing females, and as nymphs were counted, they were removed from each plant. Four separate experiments were made and the data from the two leafhopper lines were combined in Tables 1 and 2.

The data indicate that the longevity of aster leafhoppers apparently is not diminished by acquisition-access to either pathogen alone or to both pathogens. Approximately half of each population survived 40 days and small numbers survived for 3 mo regardless of the pathogens carried.

Fecundity was measured by nymph production; the data are shown in Table 2. Acquisition-access feeding by *M. fascifrons* on plants infected with either or both pathogens did not markedly affect the reproduction of this vector. These data represent only a part of the total reproduction (67 days of oviposition) because the insects were adults when they were selected for these experiments and they were kept on infected or healthy plants for 2 wk during acquisition access feeding prior to transfer to the plants on which counts of nymphs were made. Approximately 85% of all nymphs were produced within 40 days of oviposition.

Effect of single or combined acquisition-access of OBDV and AY-MLO on transmission. To test for the possibility of interference between AY and OBDV in the *M. fascifrons,* two experiments were conducted in which leafhoppers were given acquisition-access feeding treatments in the following sequences: (a) OBDV-infected oats 1 wk, then AY-infected asters 1 wk; (b) AY-infected asters 1 wk, then OBDV-infected oats 1 wk; (c) AY-infected asters 1 wk, then healthy oats 1 wk; (d) OBDV-infected oats 1 wk, then healthy oats 1 wk; and (e) healthy oats 2 wk. In two other experiments, only treatments a and b were used. The data from these experiments were combined and are shown in Table 3. Transmission of either pathogen was diminished when leafhoppers were given acquisition access feeding for both pathogens regardless of the sequence of acquisition. Transmission of the pathogen acquired second in the sequence of acquisition access feeding was less efficient than that of the one acquired first. If the ability to transmit either pathogen were independent and there were no interference between the pathogens, according to these data one might expect approximately 26% of the insects to transmit both pathogens. However, with apparent interference between them, the expected value for transmission of both pathogens is 9.2% (observed = 4.9) when OBDV precedes AY-MLO and 8.7% (observed = 6.0) when AY-MLO precedes OBDV in acquisition-access feeding.

TABLE 1. Longevity of *Macrosteles fascifrons* after acquisition-access feeding on aster yellows mycoplasmalike organism (AY)-infected asters, oat blue dwarf virus (OBDV)-infected oats, or both

Acquisition Access	Survival (%)[a] after post-acquisition-access feeding				
	1 day	15 days	40 days	67 days	95 days
OBDV	100	70.0	45.1	12.4	1.4
AY	100	68.4	44.9	15.9	2.1
AY + OBDV	100	71.7	46.0	20.6	2.4
None	100	66.3	40.0	14.3	3.2

[a] Figures are based on at least 100 insects in each of two experiments. Differences in percent survivors among treatments for any days post-acquisition-access feeding are not statistically significant, $P = 0.05$.

TABLE 2. Reproduction of *Macrosteles fascifrons* after acquisition-access feeding on aster yellows mycoplasmalike organism (AY)-infected asters, oat blue dwarf virus (OBDV)-infected oats or both

Acquisition access	Progenies per female[a]
OBDV	21.9
AY	22.1
AY + OBDV	23.6
None	20.8

[a] Values based on at least 48 females per treatment and a total of 76 days of oviposition in each of two experiments. Differences are not statistically significant, $P = 0.05$.

TABLE 3. Transmission of aster yellows mycoplasmalike organism (AY) and the oat blue dwarf virus (OBDV) by aster leafhoppers acquisition-access fed on oat plants infected with either or both pathogens

Acquisition Access	Transmission[x]		
	OBDV (%)	AY (%)	OBDV + AY (%)
OBDV	34.4[y] A	...	...
AY	...	76.6[y]	...
OBDV, then AY	31.0[z] A	29.7[z] B	4.9[z] C
AY, then OBDV	20.8[z] A	42.0[z] B	6.0[z] C
None (control)	0.0[z]	0.0[z]	0.0[z]

[x] Values in each column not followed by common letters are statistically different, $P = 0.10$.

[y] Based on a total of at least 86 leafhoppers in two experiments.

[z] Based on a total of at least 250 leafhoppers in four experiments.

DISCUSSION

Some plant viruses and mycoplasmalike agents are reported to diminish the longevity and fecundity of their leafhopper vectors (10–12,22,25). However, neither longevity nor reproduction of *M. fascifrons* are affected by acquisition and transmission of AY, OBDV, or both. Thus, it appears that infrequent simultaneous transmission is not due to poor survival of insects that have had acquisition access to both pathogens.

The data suggest that there is interference between AY and OBDV in *M. fascifrons*. Few studies have been made of interactions of MLOs and viruses in leafhopper vectors (7,9); however, cross protection between strains of MLO in leafhopper vectors has been reported (8,13,16). In our experiments, transmission of both pathogens by *M. fascifrons* was diminished regardless of order of acquisition access. However, transmission of the pathogen acquired second was reduced more than the one acquired first. The length of acquisition-access to each pathogen also may affect the degree of interference (8,13). Varying the length of acquisition-access to AY-MLO and OBDV was not tested in our experiments.

Explanations for interference between AY-MLO and OBDV in *M. fascifrons* are obscure. The hypotheses that have been proposed to explain cross protection or interference between virus or MLO strains in plants may not apply to interference between distinctly different pathogens in insects. However, competition for replicative sites or substrates has been suggested to explain interference between certain virus/MLO interactions in vertebrate tissue cultures (18). Another hypothesis is that infection by the first agent stimulates a "resistance mechanism" in the leafhopper that limits replication of the challenge agent. Although insects are not known to produce antibodies, apparently they have primitive mechanisms for resisting invading microorganisms (24). Whether AY and OBDV activate those types of responses in *M. fascifrons* is unknown.

In our experiments as well as in these cited reports it is possible that at least some leafhoppers may have been infected with both pathogens but did not transmit them and, therefore, they remained undetected.

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