Transmission of a Celery-Infecting Strain of Aster Yellows by the Leafhopper Aphrodes bicinctus

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ABSTRACT

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Aphrodes bicinctus was shown to be a new leafhopper vector of a celery-infecting strain of the aster yellows agent (CAYA). The number of *A. bicinctus* that transmitted CAYA after ingestion or injection (over 80%) was approximately the same as that for *Macrosteles fascifrons*. The average incubation period of CAYA in *A. bicinctus* (43.9 days) was considerably longer than in *M. fascifrons* (25.6 days). Transmission by most individuals of *A. bicinctus* became inconsistent as the leafhopper aged although some still were capable of transmitting CAYA 5-6 mo after the start of the acquisition access period.

Aphrodes bicinctus (Schrank) is a widely distributed polyphagous leafhopper species that has been reported from Europe, Russia, and North America (9). In Europe and Russia it is a vector of stolbur (1), clover dwarf (8), and clover phyllody (6, 10), diseases suspected to be of Mycoplasma etiology. Heinze and Kunze (7) obtained transmission of a yellows disease agent with *A. bicinctus* in Germany but they could not confirm that it was European aster yellows because the field-collected leafhoppers used may have been naturally infected with a yellows disease agent and the disease symptoms produced on aster were less distinct than those produced by the European aster yellows agent transmitted by *Macrosteles laevis* (Rib.). In North America, *A. bicinctus* has been shown to be a vector of the agents of clover phyllody (2, 4) and clover yellow edge (5). The present paper is the first report of *A. bicinctus* as a vector of a celery-infecting strain of the aster yellows agent (CAYA) in North America and compares some of the transmission characteristics with those of *Macrosteles fascifrons* (Stål).

MATERIALS AND METHODS

The isolate of the aster yellows agent was originally obtained from field-infected aster, *Callistephus chinensis* Nees, found in field plots at the Central Experimental Farm, Ottawa in 1972 and subsequently maintained in aster through transmission by *M. fascifrons*. This isolate readily infects celery, *Apium graveolens* L., and produces symptoms in aster that generally are associated with the western or celery-infecting strain of the aster yellows agent.

All healthy leafhoppers used in these experiments were reared in the laboratory. Continuous colonies of *M. fascifrons* were maintained on oats, *Avena sativa* L. *Aphrodes bicinctus,* however, could not be reared continuously and required special treatment in the egg stage (3). Cold treatment (4-7 C) of eggs oviposited in Ladino clover (*Trifolium repens* L.) plants for several weeks, followed by incubation at room temperature (24 C), produced sufficient numbers of nymphs for experimental purposes. Eggs treated in this manner began hatching approximately 2 wk after being placed at room temperature.

All procedures involving leafhopper feeding were carried out in an artificially lighted room (16-hr day at approximately 10,000 lx) at 21-24 C. Source plants for acquisition access feeding consisted of infected celery, cultivar Utah 15, or aster, cultivar Shell Pink. After exposure to infected plants or after injection with CAYA inocula, *A. bicinctus* and *M. fascifrons* were transferred weekly in groups to healthy Ladino clover and aster, respectively. For determining percentage transmission, the insects were tested singly on seedlings of the same hosts. After being subjected to leafhopper feeding, test plants were held for symptom development in a greenhouse regulated near 23 C with supplementary light to provide a 16-hr day.

Inocula for injection tests with both leafhopper species were prepared from extracts of *M. fascifrons* that had been exposed to infected asters for 2 wk and then maintained on healthy aster for at least 1 wk. The exposed leafhoppers were ground in a selected volume of PBS (phosphate-buffered saline, 0.01 M potassium phosphate, 0.15 M NaCl, pH 7.0) in a 1-ml capacity tissue grinder, the homogenate was centrifuged for 10 min at 3,000 *g*, and the resulting supernatant liquid used for injecting healthy leafhoppers. In calculating the dilution of inoculum, it was assumed that 1 g of insects had a volume of 1 ml.

RESULTS

Transmission by Aphrodes bicinctus and Macrosteles fascifrons after ingestion.—Twenty-five early instar nymphs of each species were given a 7-day acquisition access period on each of three aster and celery plants. Four weeks later the insects were caged singly on healthy seedlings and their sex recorded. At the end of 2 wk *M. fascifrons* was discarded. *Aphrodes bicinctus,* however, was transferred to a new set of single plants because preliminary results had suggested that a long incubation period was involved. The insects were left for 5 wk at which time they were transferred to a third set of plants for approximately 4 wk.

On the basis of numbers of insects that became inoculative, *A. bicinctus* was found to be as efficient as *M. fascifrons* in transmitting CAYA from both aster and celery (Table 1). No difference in transmission was found between males and females of either leafhopper species.

Transmission by Aphrodes bicinctus and Macrosteles fascifrons after injection.—Nymphs of both species were injected with 10^{-1} or 10^{-2} dilutions of inocula. Five weeks after injection, *A. bicinctus* was tested singly for three 2-wk periods on clover seedlings while *M. fascifrons* was tested on only one set of aster seedlings for 2 wk.

Percentages of *A. bicinctus* that became inoculative after injection with CAYA inocula diluted 10^{-1} and 10^{-2} were 96% (24/25) and 83% (25/30), respectively. Comparable percentages of *M. fascifrons* were 90% (28/31) and 83% (20/24).

Incubation period in insects.—Second- and third-instar nymphs of *A. bicinctus* were given a 7-day acquisition access period on infected aster, maintained in groups for 7 days on healthy Ladino clover, and finally caged singly on clover seedlings. The insects were transferred to new plants every 7 days for 10 wk. A similar experiment was conducted with *M. fascifrons* at the same time so that the incubation period of CAYA could be compared in the two vector species. *Macrosteles fascifrons* singles were tested on aster seedlings for only 6 wk.

The incubation period of CAYA, based on 18 of 30 *A. bicinctus* and 20 of 23 *M. fascifrons* that became inoculative, was considerably longer in *A. bicinctus* than in *M. fascifrons* (Fig. 1). At the end of the 28- to 35-day period, 80% of the *M. fascifrons,* but only 11% of the *A. bicinctus,* had transmitted at least once. The average incubation periods were 43.9 and 25.6 days in *A. bicinctus* and *M. fascifrons,* respectively.

Transmission pattern.—Early instar nymphs of *A. bicinctus* were given a 7-day acquisition access period on infected clover, held in groups on healthy clover for 6 wk, then transferred singly to healthy seedlings. The insects were transferred to new sets of seedlings at irregular intervals until the last insect had died.

The transmission records of 14 female leafhoppers that became inoculative showed that most insects transmitted

TABLE 1. Transmission of aster yellows agent by *Aphrodes bicinctus* and *Macrosteles fascifrons* after a 7-day acquisition access period on infected aster and celery plants

	Transmission by:							
	A. bicinctus[a]				*M. fascifrons*[a]			
Source	♀	♂	Total	(%)	♀	♂	Total	(%)
Aster	29/32[b]	10/12	39/44	89	27/31	21/24	48/55	87
Celery	15/18	1/1	16/19	84	26/29	21/25	47/54	87

[a]*A. bicinctus* were tested singly on clover; *M. fascifrons* on aster.
[b]Numerator = number transmitting; denominator = number tested singly.

TABLE 2. Transmission pattern of a celery-infecting strain of aster yellows agent by inoculative *Aphrodes bicinctus* leafhoppers at various intervals from the start of a 7-day acquisition access feed

	Day from start of acquisition access period										
Insect no.	49 to 63	63 to 100	100 to 114	114 to 128	128 to 142	142 to 159	159 to 173	173 to 187	187 to 201	201 to 216	216 to 230
1	+[a]	−	−	−	−	D					
2	+	+	−	−	D						
3	+	+	+	+	−	D					
4	+	+	−	D							
5	+	−	−	D							
6	+	−	+	+	−	−	+	+	−	D	
7	+	+	+	D							
8	+	+	+	+	−	−	−	−	D		
9	+	+	+	+	+	+	−	+	+D		
10	+	+	−	+	−	D					
11	+	−	+	D							
12	+	−	−	D							
13	−	+	−	−	D						
14	+	+	−	+	+	−	+	−	−	−	D

[a]Symbols: + = transmission; − = no transmission; D = insect died.

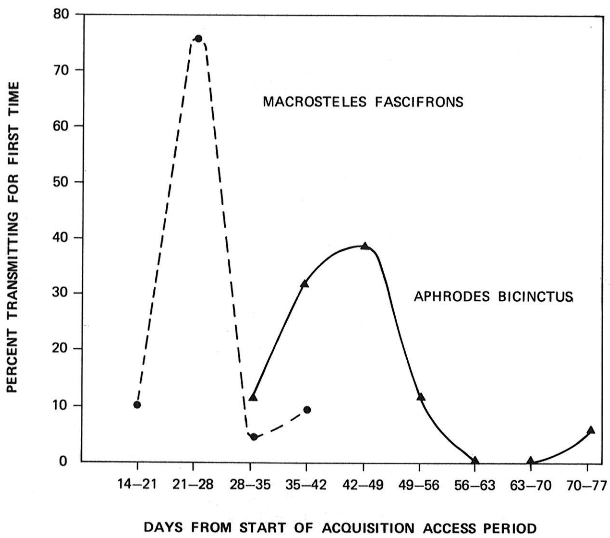

Fig. 1. Percentage of insects transmitting aster yellows agent for the first time at various intervals from the start of a 7-day acquisition access period; insects were transferred to new plants every 7 days.

in an inconsistent or erratic manner (Table 2). One insect (no. 9) transmitted in a relatively consistent manner, failing to infect only one of nine plants over a period of 187 days; two insects (nos. 6 and 14) displayed intermittent transmission and four transmitted to only one plant. Most insects ceased transmitting 2 wk or more before death although some, such as nos. 1 and 8, lived for at least 59 days following their last transmission. Of interest also was the fact that some insects, such as nos. 9, 6, and 14, were still capable of transmitting CAYA 5 to 6 mo after acquisition. Overall, excluding plants on which insects died, 41 of 73 (56%) plants fed on became infected.

DISCUSSION

The percentage of *A. bicinctus* that became inoculative after either ingestion or injection was equal to that of *M. fascifrons,* probably the most efficient known vector of CAYA. Both species are polyphagous feeders and have a relatively wide distribution throughout the southern areas of most provinces of Canada. Unlike *M. fascifrons,* however, *A. bicinctus* is a rather inconspicuous leafhopper in nature, being easily missed when standard collection procedures with a sweep net are used. The green-colored, slow-moving nymphs normally feed on plant stems near ground level, whereas the straw-colored adults have a habit of falling to the ground feigning death when disturbed. This inconspicuous nature may partially explain why *A. bicinctus* has until now not been recognized as a vector of CAYA. The potential importance of *A. bicinctus* in nature would appear to be in biennial and perennial crops rather than annual crops since the former provide an overwintering source for both leafhopper eggs and the causal agent. Large populations of leafhoppers emerging and feeding on infected overwintered host plants could result in a high incidence of disease.

Although over 80% of the population of *A. bicinctus* in these tests became inoculative, the ability of inoculative insects to transmit CAYA decreased as the insects aged, a situation similar to that observed in the transmission of the clover phyllody agent by *A. bicinctus* (4). However, in the case of clover yellow edge, once individuals of *A. bicinctus* became inoculative they continued to transmit until death (5). Thus it would appear that failure of *A. bicinctus* to transmit as it ages is not a general characteristic of this leafhopper species, but is dependent on the specific causal agent involved.

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