Acquisition Through Artificial Membranes and Transmission of Oat Blue Dwarf Virus by Macrosteles fascifrons

Donna L. Long and R. G. Timian

Graduate Research Assistant, Plant Pathology Department, North Dakota State University; and Research Plant Pathologist, Plant Science Research Division, USDA, North Dakota State University, Fargo 58102, respectively.

Cooperative investigations of the Plant Science Research Division, ARS, USDA, and the North Dakota Agricultural Experiment Station, Fargo.

Accepted for publication 19 May 1971.

ABSTRACT

Oat blue dwarf virus (OBDV) was transmitted by *Macrosteles fascifrons* (aster leafhopper) after an acquisition feeding on clarified plant sap through Parafilm M membranes. Transmission was successful only if the plant sap was refrigerated during feeding. OBDV acquisition and transmission through membranes was slightly less efficient than acquisition and transmission from infected plants. Phytopathology 61:1230-1232.

Symptoms of oat blue dwarf virus (OBDV) and transmission by the aster leafhopper, *Macrosteles fascifrons* (Stål.), to oats, barley, and flax have been described (1, 3). Partial purification of OBDV has been accomplished, and the morphological characteristics have been studied with the electron microscope (2, 7). The physical properties of the virus have not been determined. Due to the obligatory relationship among the virus, plant, and leafhopper, the study of some of the physical properties involves the treatment and introduction of the virus-containing plant sap into the leafhopper either by injection or membrane feeding. Compared to injection, membrane feeding has advantages in that larger numbers of insects can be treated in shorter periods of time, and immediate mortality of leafhoppers is negligible. This technique was used successfully for transmission of other viruses by leafhoppers (4, 5) and aphids (6). This paper reports the technique involved in the successful acquisition of OBDV by the aster leafhopper feeding through artificial membranes.

The nonviruliferous leafhoppers used in these studies were maintained on barley, cultivar Black Hulless (Cereal Investigations [C.I.] No. 666). The OBDV was obtained from field-collected infected oats, transferred to and maintained in oats, cultivar Rodney (C.I. 6661) in the greenhouse.

The plant extract was prepared as follows: Healthy or infected oats (30-35 days old) were ground in a food grinder with an equal quantity (w/v) of 0.01 M phosphate buffer, pH 7.0, and the crude extract was expressed through four layers of cheesecloth. The crude extract was centrifuged at 8,720 *g* for 15 min; the supernatant was centrifuged at 65,950 *g* for 3 hr. The resulting pellet was resuspended to the original volume with phosphate buffer and centrifuged at 8,720 *g* for 15 min.

The feeding chamber was of tripartite construction with a Nitex No. 295 (Smico Sales Co., Minneapolis, Minn.) screen cage to contain the leafhoppers and butyrate reservoir which held the extract above a Parafilm M (Marathon Corp., Menasha, Wisc.) membrane. The Nitex screen was cut to a 40-mm height, glued to form a 23-mm diam cylinder, and closed at one end with clear plastic. A 5-mm hole reinforced with clear plastic was made in the side of the Nitex cylinder at three-fourths the distance from the bottom to facilitate leafhopper handling. The reservoir was made of clear butyrate tubing, 22 mm in diam and 20 mm in height (Fig. 1-A). After assembling the feeding chamber, 2 ml of the clarified plant sap containing 5% sucrose were added.

The plant extract was kept at 9 C by a Force Flow Liquid Coolant Circulator, Model R5-240 (A. Daigger & Co., Chicago, Ill.), connected to glass manifolds attached to copper tubing (1 mm inside diam) bent to fit into the plant extract as illustrated in Fig. 1-B. Four adult leafhoppers which had been fasted for 2-4 hr were placed in each chamber and allowed an acquisition feeding period of 24 hr. Individual leafhoppers were removed to seedling Rodney oats for test feeding, and transferred every 5 days for a total of eight transfers.

In testing for transmission following natural acquisition from infected oats, adult leafhoppers were al-

TABLE 1. The per cent of *Macrosteles fascifrons* transmitting oat blue dwarf virus during six test feeding periods following an acquisition feeding period of 5 days on virus-free and virus-infected oats

Test feeding periods in days[a]	Transmission following acquisition feeding on			
	Virus-free plants		Virus-infected plants	
	No.	%	No.	%
0-4	0/30[b]	0	9/39	23.0
5-8	0/25	0	8/36	22.2
9-12	0/25	0	9/35	25.7
13-16	0/16	0	3/32	9.4
17-20	0/11	0	4/31	12.9
21-24	0/5	0	1/28	3.6

[a] An 8-day incubation period preceded the first test feeding period.

[b] Number of leafhoppers transmitting over total number of leafhoppers fed.

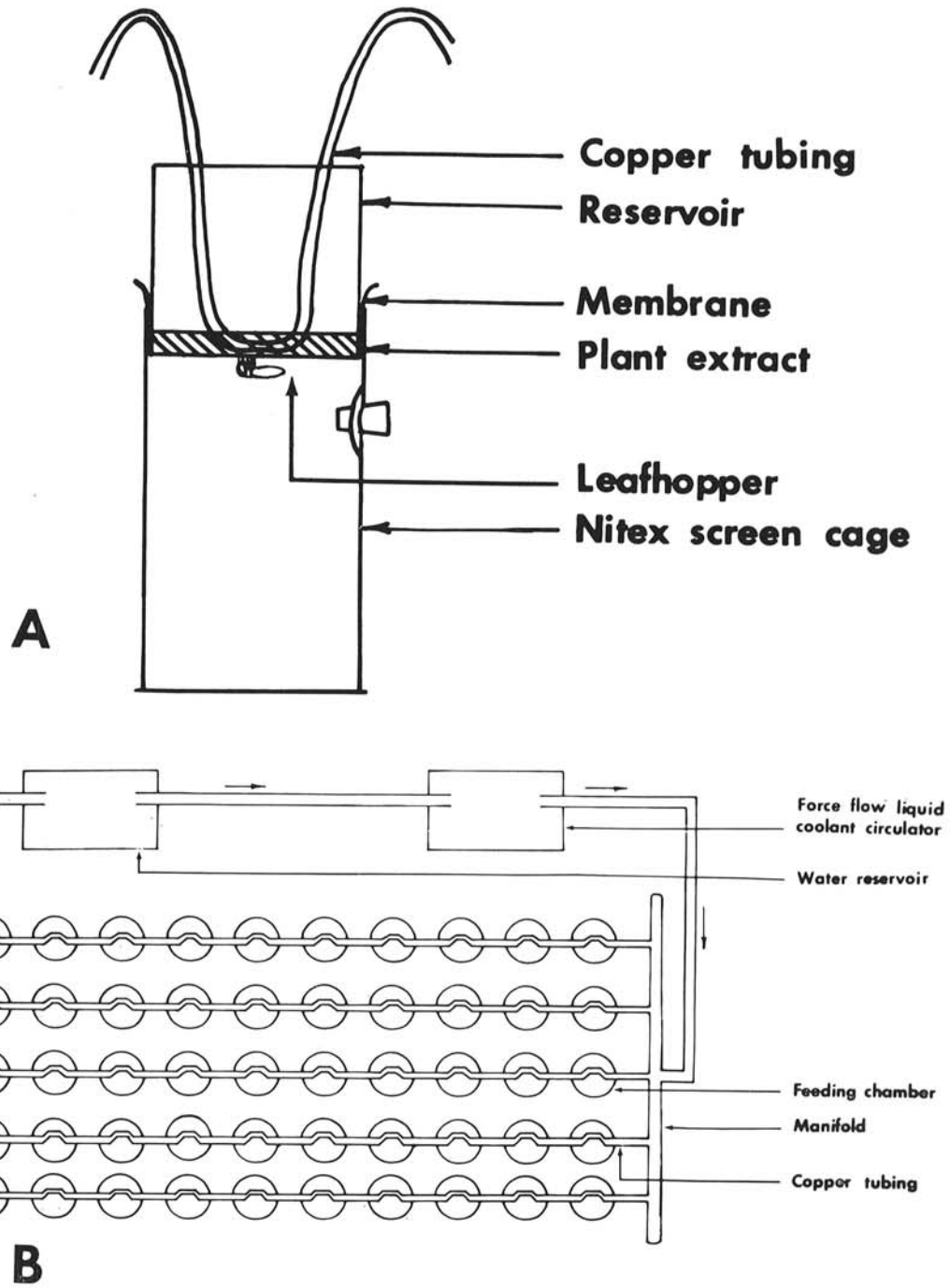

Fig. 1. Refrigerated membrane feeding apparatus. **A)** Individual chamber showing leafhopper feeding on cooled plant extract through membrane, and **B)** diagram of entire unit.

lowed an acquisition feeding period of 5 days on infected Rodney oats, and an incubation period of 8 days on Black Hulless barley. Following the incubation period, the leafhoppers were test-fed for six transmission feeding periods of 4 days each.

Following a 5-day acquisition period and an 8-day incubation period, leafhoppers transmitted with the greatest efficiency for 12 days, after which transmission decreased. Peak transmission (25.7%) occurred during the 9- to 12-day test feeding period after incubation (Table 1).

In preliminary studies, leafhopper survival following acquisition feeding through membranes was ca. 95% if three conditions were met: (i) The leafhoppers were

TABLE 2. The per cent of *Macrosteles fascifrons* transmitting oat blue dwarf virus during eight test feeding periods following an acquisition feeding period of 24 hr on plant extract through artificial membranes[a]

Test feeding periods in days after acquisition[b]	Transmission following acquisition feeding on virus-infected plant extract[c]					
	1	2	3	4	5	Avg
	%	%	%	%	%	%
0-5	0.0	3.3	0.7	0.0	0.0	0.8
6-10	31.1	3.6	10.0	3.8	4.0	8.5
11-15	29.1	3.9	13.3	20.3	9.3	13.6
16-20	28.8	9.5	9.0	16.7	11.8	13.6
21-25	24.4	18.9	8.9	9.4	6.3	14.9
26-30	3.3	8.5	0.0	2.6	9.6	5.1
31-35	10.0	5.5	0.0	0.0	11.1	5.2
36-40	0.0	0.0		0.0	5.7	1.8

[a] Control data: No transmission occurred following feeding on virus-free plant extract. Number of leafhoppers which fed for each test feeding period ranged from 9 to 120.

[b] Leafhoppers were not held on virus-free plants for an incubation period.

[c] Average number of leafhoppers for each test feeding period for each trial was 73, with a range of 15-145.

not fasted more than 8 hr; (ii) the plant extract was clarified; and (iii) at least 2.5% sucrose was added to the plant extract prior to feeding.

Successful acquisition and subsequent transmission of OBDV were accomplished only when the plant extract was kept cool. The system designed made it possible to keep the leafhoppers at room temperature for normal body functioning while they fed on the refrigerated extract.

Using the membrane feeding technique, leafhoppers transmitted at a maximum between 11 and 25 days after the acquisition feeding period (Table 2). Peak transmission was recorded at 14.9% during the 21-25 day test feeding period, although higher transmission was recorded in individual trials. Transmission in the second to fifth test feeding periods (6-25 days) in Trial 1 was more than double the average rate of all trials combined for those periods. Higher than average transmission also occurred in other test feeding periods in Trials 2 and 5. Differences between trials could be attributed to acquisition and transmission efficiency differences among the vector population. Other unpublished data from this laboratory, as well as work from another laboratory (1, 3), show considerable variability among transmission efficiencies in trials involving natural acquisition. Comparing the two methods of acquisition, membrane acquisition appears to be slightly less efficient than natural acquisition from infected oats.

LITERATURE CITED

1. BANTTARI, E. E., & M. B. MOORE. 1962. Virus cause of blue dwarf of oats and its transmission to barley and flax. Phytopathology 52:897-902.
2. BANTTARI, E. E., & R. J. ZEYEN. 1969. Chromatographic purification of the oat blue dwarf virus. Phytopathology 59:183-186.
3. BANTTARI, E. E., & R. J. ZEYEN. 1970. Transmission of oat blue dwarf virus by the aster leafhopper following natural acquisition or inoculation. Phytopathology 60:399-402.
4. CARTER, W. 1927. A technic for use with homopterous vectors of plant disease, with special reference to the sugarbeet leafhopper, Eutettix tenellus (Baker). J. Agr. Res. 34:449-453.
5. CARTER, W. 1928. An improvement in the technique for feeding homopterous insects. Phytopathology 18: 246-247.
6. ROCHOW, W. F. 1960. Transmission of barley yellow dwarf virus acquired from liquid extracts by aphids feeding through membranes. Virology 12:223-232.
7. ZEYEN, R. J., & E. E. BANTTARI. 1969. Electron and light microscopy of oat tissue infected with the oat blue dwarf virus. Phytopathology 59:1059 (Abstr.).