Selective Inbreeding of Macrosteles fascifrons for Increased Efficiency in Virus Transmission

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ABSTRACT

The efficiency of transmitting oat blue dwarf virus (OBDV) was more than doubled in some lines of *Macrosteles fascifrons* through selection for transmission and inbreeding for three generations. The ability to transmit at a high rate was retained through four additional generations in which selection was not involved. Leafhopper lines selected for transmission of OBDV transmitted at 69%, whereas those selected for nontransmission transmitted at a rate of 6% after the same number of inbreeding generations. Inbreeding did not change the incubation time of the virus in the vector, and there was no apparent effect on reproduction in most lines of inbred leafhoppers.

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Blue dwarf of oats was first reported in 1952 (8). The causal agent of blue dwarf was identified as a virus transmitted by the aster leafhopper *Macrosteles fascifrons* (Stål) and reported in 1962 (1) as being present in Minnesota and the North Central states. Oat blue dwarf virus (OBDV) has been present in nursery plots at Fargo, No. Dak., for several years, and during the last 2 years it has seriously hampered selection and seed production in early generation barley breeding lines.

The vector of OBDV, *M. fascifrons*, transmits the virus at a relatively inefficient rate (1). An efficient vector is needed for study of characteristics of the virus and host-vector-virus relationships.

Several workers (3, 4, 5, 7, 9. 10) have shown that insects with high vectoring capabilities can be developed through selective breeding or inbreeding. Therefore, a program was initiated to select lines of leafhoppers that were efficient and nonefficient in transmitting OBDV.

Since the incubation period of OBDV in the aster leafhopper and in the plant host may be as long as 35 days, it was impractical for us to utilize the methods of increasing virus-transmitting efficiency previously reported for *Sogata orizicola* Muir (5, 7). Therefore, a method of selecting and inbreeding leafhoppers needed to be developed.

MATERIALS AND METHODS.—A wild population of *M. fascifrons* collected in the Fargo area during 1969 was used as the initial source of leafhoppers. These leafhoppers were maintained in cages under greenhouse conditions through several generations with no selection for virus-transmitting ability. They were known to have the ability to transmit OBDV and the inciting agent for aster yellows.

Forty-nine adult female leafhoppers were selected in December 1970 from the general population and caged individually on barley plants for egg deposition. After 4 days the leafhoppers were placed on OBDV-infected oat plants for an acquisition feeding of 6 days. Thereafter they were serially transferred to individual test plants for feeding periods of either 3 or 4 days for a total of 30 days or until they died.

Progeny numbers were assigned to each female and to the nymphs in each succeeding generation. F_1 progeny from virus transmitting females were allowed to mature and mate. The males from these progenies were tested and compared with females for virus transmission ability. Individual females were first allowed a period of 5 days for egg deposition and were then allowed an acquisition feeding of 5 days before testing for transmitting ability (Fig. 1).

The F_2 nymphs from the above F_1-transmitting females were handled in the same manner as the F_1 populations, except that the males were discarded without further testing because of limited facilities.

The F_1 nymph progeny from nontransmitting females in the initial test were also selected. After maturation and mating, the females were allowed to deposit eggs. The procedure for inbreeding and testing was the same as for the transmitting lines, except that selection was for nontransmitting insects.

The above program was carried through the F_4 generation of inbreeding with testing and selection in each generation. The F_4 generation was not tested or selected, and the population was allowed to proceed to the F_5. At that time, a test for virus transmission was made with selected females and males from each family.

The F_5 families were allowed to proceed to the F_7 generation, at which time a portion of each family was again tested for transmitting ability.

Selection of females for further inbreeding favored those females that were most consistent in transmitting as well as transmitting over a longer period of time. Because of the large populations involved, it was necessary to limit the number of females selected for further inbreeding to ten in each generation. After testing, this number was reduced to two or three/generation. A total of four of the original transmitting females and three of the original nontransmitting females were represented in the F_7 generation.

Cages used in these studies were constructed from

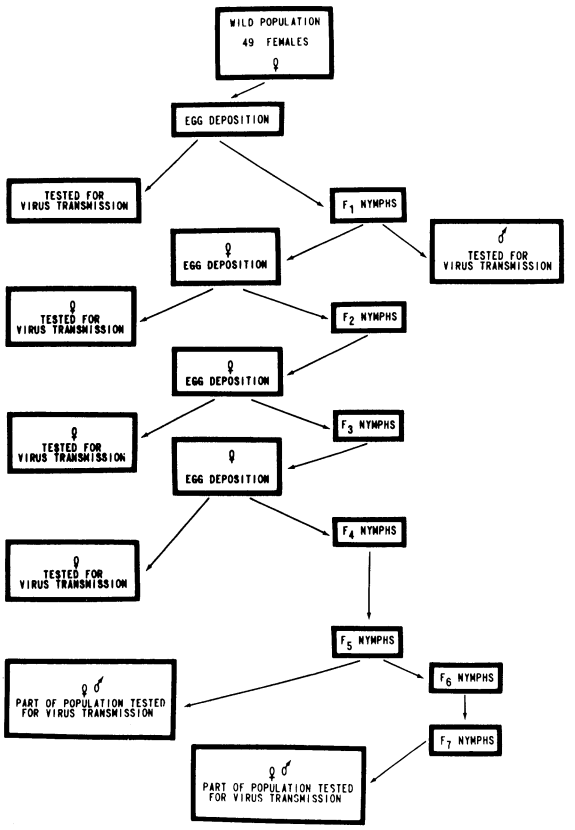

Fig. 1. Schematic diagram of the method of inbreeding *Macrosteles fascifrons* for virus transmission.

5-cm-diam polyethylene tubing. A square 5-cm opening cut in the side of the tubing and covered with nitex screen provided ventilation. The tubes capped with plastic closures were inserted into the soil around plants grown in 9-cm pots.

Black Hulless barley and Rodney oats were used throughout the tests. Three or four seeds were planted/9-cm pot. Plants were 1-8 cm high at the time of use. Greenhouse temperatures varied from 18 to 30 C depending on the time of day and year. In the winter months, supplemental fluorescent lights (about 1,200 ft-c) were used to extend the (light) period to 16 hr.

The methods used in membrane feeding were as reported previously (6). OBDV that had been clarified by cycle centrifugation and stored for 1 month in liquid nitrogen served as the inoculum. The leafhoppers were allowed a 24-hr acquisition feeding on the virus preparation through a parafilm membrane. Their transmission efficiency was determined in the same manner as that used in other tests.

RESULTS AND DISCUSSION.—Results from these tests showed that the ability to transmit oat blue dwarf virus is apparently a genetically controlled characteristic in *M. fascifrons*.

Thirty-nine percent of the original wild leafhopper population transmitted OBDV (Table 1). Sixty-nine percent of the leafhoppers of one inbreed F_7 family transmitted the virus, whereas none of the inbred F_5 family selected for no transmission transmitted the virus.

There was a 41% increase in efficiency of virus transmission in F_1 progeny of transmitting females over their parents. The F_2 progeny from virus-transmitting females transmitted OBDV at 79%, a rate double that of the wild population. All of the progeny in some of the F_3 families transmitted OBDV, but the over-all transmission rate of the F_3 progeny was not greater than that of the F_2 progeny. At this time there is no explanation for this apparent plateau.

No further selection for transmitting ability was done in succeeding generations of leafhoppers, but tests in the F_5 generation of leafhoppers showed that a bulk sample of all leafhoppers from families in which selection for transmission was practiced through the F_3 generation transmitted at a rate of 48%. In a single family in the F_7 generation in which selection for transmission was practiced through the F_3 generation (Table 1), the transmission rate was 69%.

The transmission rate was down to 6%, with some families not transmitting at all in leafhopper lines selected on the basis of no transmission through the F_3 generation and then carried to the F_5 generation without further selection.

The mechanism of inheritance in *M. fascifrons* for the ability to transmit or not to transmit OBDV is not known, but is apparently a relatively simple one. Further inbreeding followed by crossing between high and low transmitting lines will be necessary for an understanding of the genetic mechanism.

There was an apparent loss in reproduction rate in

TABLE 1. Oat blue dwarf virus transmitting ability in various inbreeding generations of *Macrosteles fascifrons*

Leafhopper generation	Total no. leafhoppers tested	% Leafhoppers transmitting
Original wild stock	36	39
F_1 progeny (T)[a]	31	55
F_1 progeny (NT)	21	38
F_2 progeny (T)	56	79
F_2 progeny (NT)	22	14
F_3 progeny (T)	45	73
F_3 progeny (NT)	12	0
F_5 progeny (T)	259	48
F_5 progeny (NT)	107	6
F_7 progeny (T) (memb.)[b]	18	50
F_7 progeny (T) (HT)[c]	77	69

[a] T = lines selected for transmission; NT = lines selected for no transmission.

[b] Leafhoppers were fed through a membrane on a partially purified, nonconcentrated, buffered sucrose solution of virus.

[c] Single lines of leafhoppers that transmitted at greatest efficiency.

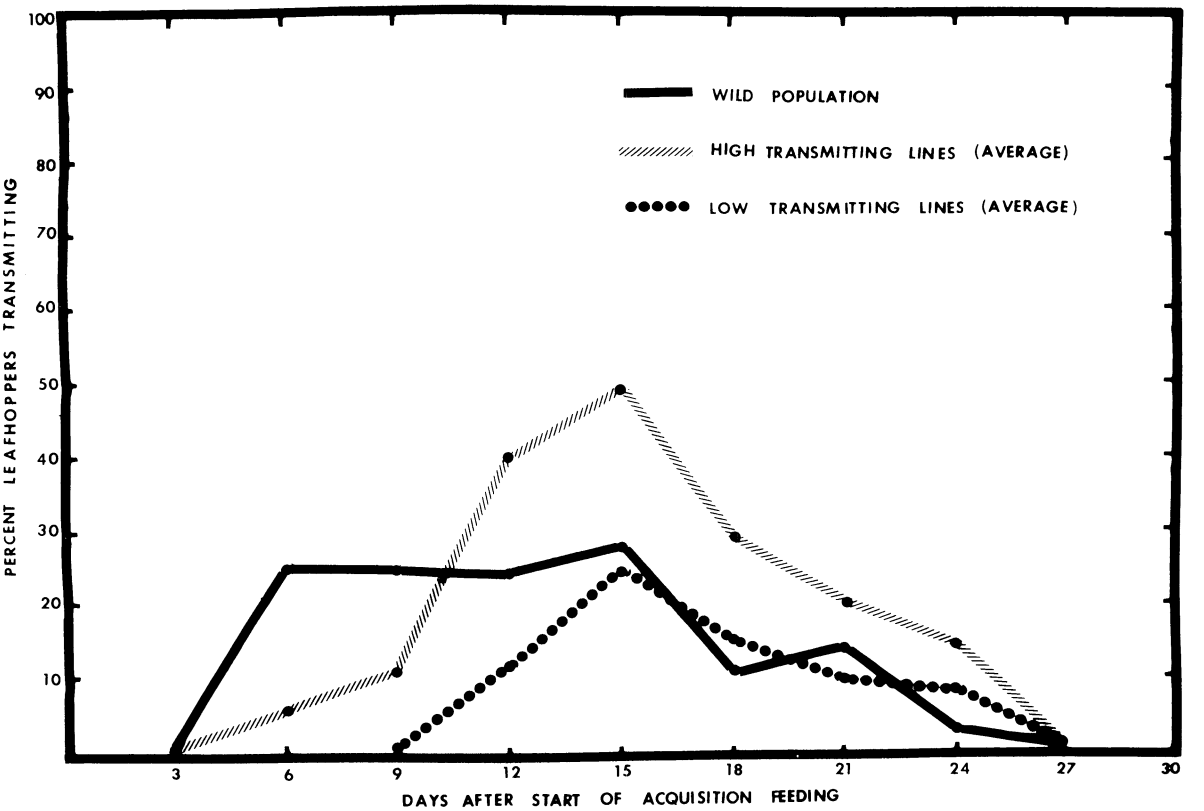

Fig. 2. Percentages of leafhoppers from three sources that transmitted oat blue dwarf virus over a period of 30 days following acquisition feeding on virus-infected host plants.

some lines as a result of inbreeding, but others did not show a decline. This would indicate that there is perhaps little if any genetic linkage between OBDV-transmitting ability and reproductive vigor, and that perhaps lines of the leafhopper can be developed that will transmit the virus at nearly a 100% rate.

Studies are being initiated to determine morphological or other characteristics that might be responsible for the ability or inability of the leafhoppers to transmit OBDV. One test in which F_7 progeny were tested for their ability to acquire the virus through artificial membranes showed that the transmission rate was 50% (Table 1). The number of leafhoppers involved in this test was not large, but the indication is that no changes in normal feeding and/or acquisition abilities were involved. The virus in vitro on which the leafhoppers fed had been stored in phosphate buffer frozen in liquid nitrogen for a period of 1 month. Perhaps the transmission rate would have been even higher on freshly expressed and clarified plant sap.

Previous workers reported peak transmission of OBDV at 15 days (1) and 28 days (2) after initiation of acquisition feeding on virus-infected plants. In our studies, maximum transmission occurred at 15 days after the start of acquisition feeding (Fig. 2). The selection for high and low transmitting lines had no apparent effect on the time for peak transmission in the various groups of leafhoppers. Taken as a group, 48% of the leafhoppers from lines selected for transmitting ability transmitted OBDV 15 days after acquisition-incubation started. Leafhoppers selected for nontransmission transmitted at a peak of 24% at 15 days after the start of acquisition. Twenty-eight percent of the wild population transmitted OBDV at 15 days after initial acquisition.

From these results one may conclude that there are no apparent differences for peak transmission among the wild population, selected high transmitting inbred, or selected low transmitting inbred lines of leafhoppers. There may be differences among wild populations of *M. fascifrons* regarding incubation and peak transmission times as indicated by previous reports.

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. 1970. Transmission of oat blue dwarf virus by the aster leafhopper following natural acquisition or inoculation. Phytopathology 60:399-402.
3. BENNETT, C. W., & H. E. WALLACE. 1938. Relation

of the curly top virus to the vector, Eutettix tenellus. J. Agr. Res. 56:31-51.

4. BLACK, L. M. 1943. Genetic variation in the clover leafhopper's ability to transmit potato yellow-dwarf virus. Genetics 28:200-209.
5. HENDRICK, R. D., T. R. EVERETT, H. A. LAMEY, & W. B. SHOWERS. 1965. An improved method of selecting and breeding for active vectors of hoja blanca virus. J. Econ. Entomol. 58:539-542.
6. LONG, DONNA L., & R. G. TIMIAN. 1971. Acquisition through artificial membranes and transmission of oat blue dwarf virus by Macrosteles fascifrons. Phytopathology 61:1230-1232.
7. MC MILLIAN, W. E., J. V. MC GUIRE, & H. A. LAMEY. 1960. Hoja blanca studies at Camaguey, Cuba, p. 21. Rice Tech. Working Group Proc.
8. MOORE, M. B. 1952. The cause and transmission of blue dwarf and red leaf of oats. Phytopathology 42:471 (Abstr.).
9. NAGARAJ, A. N., & L. M. BLACK. 1962. Hereditary variation in the ability of a leafhopper to transmit two unrelated plant viruses. Virology 16:152-162.
10. STOREY, H. H. 1932. The inheritance by an insect vector of the ability to transmit a plant virus. Roy. Soc. (London) Proc. B112, 46-60.