Transmission of Oat Blue Dwarf Virus by the Aster Leafhopper Following Natural Acquisition or Inoculation

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ABSTRACT

A few nymphs and a few adult aster leafhoppers transmitted the oat blue dwarf virus (OBDV) to oats after a 15-min acquisition feeding period on diseased oats. Highest numbers of nymphs became viruliferous after an acquisition-feeding period on diseased oats of 1 week, and adults became so after 2 days. The shortest incubation time of the virus in leafhoppers that fed on diseased oats was 7 days, and the majority of insects transmitted virus between the 17th and 34th days. With few exceptions, they ceased transmitting OBDV after the 66th day. The shortest incubation time for insects inoculated with extracts from infected plants or viruliferous leafhoppers was 1 day, and the majority of insects transmitted the virus between the 6th and 20th days. With few exceptions, the inoculated leafhoppers ceased transmitting OBDV after the 40th day. Patterns of serial transmission by both inoculated leafhoppers and by leafhoppers that acquired the virus by feeding were erratic. A higher percentage of leafhoppers transmitted OBDV when inoculated with extracts from ground viruliferous insects than when inoculated with extracts from diseased plants. Inoculation of leafhoppers by placing virus extracts on the severed tarsus or tibia was unsatisfactory for transmission. Phytopathology 60:399-402.

The oat blue dwarf virus (OBDV) is a small sphere or polyhedron 28-30 mμ in diam (3). The only reported vector for OBDV is the aster leafhopper (*Macrosteles fascifrons* Stål) (2), and no transmission mechanically or by aphids has been detected. The acquisition, incubation, and persistence of the virus in the leafhopper have not been determined, although abdominally inoculated leafhoppers became viruliferous (1).

The studies reported here were undertaken to (i) determine the optimum acquisition-feeding time; (ii) determine the shortest incubation time; (iii) compare serial transmission by leafhoppers that acquired the virus naturally with those that acquired it by abdominal inoculation; and (iv) to attempt inoculation of leafhoppers through leg wounds.

MATERIALS AND METHODS.—The virus used for these experiments was originally transmitted from infected oats (*Avena sativa* L.) from a field at St. Paul, Minnesota, to seedling oats in the greenhouse, and was propagated in the cultivar Rodney. The field strain(s) caused severe stunting in at least five oat varieties, and was readily transmitted by the aster leafhopper. Virus-free leafhoppers were reared on Rodney oats, and were periodically assayed on seedling barley, oats, or asters to assure that they were free from any viruses detectable in these hosts. Rodney oats were used for propagation of OBDV and as the assay host in transmission studies. During winter, supplemental light was used to extend daily light periods to 16 hr for plants and insects. Individual cages used for all acquisition assays were transparent 5 × 30-cm polyvinyl chloride tubes with screened tops and ventilation holes. Plastic cages cut from 1.5-cm diam tubing, screened at one end, with an iron washer fastened on the opposite end, were used for the serial transfer experiments. A leaf from the assay plant was placed between the washer and a pot label-supported magnet that held the cage in place.

RESULTS.—*Acquisition-feeding by nymphs or adults.*—Although transitory yellowing virus of rice may be acquired in as little as 5 min and wheat striate mosaic virus in as short as 30 sec by their respective leafhopper vectors (5, 10), usually substantially longer acquisition-feeding times are necessary to obtain highest numbers of viruliferous insects with these as well as with other leafhopper-virus combinations (4, 5, 6, 9, 10). To test the optimum acquisition-feeding time for aster leafhoppers on OBDV-infected oats, 2nd, 3rd, and 4th instar nymphs and adult leafhoppers were used. To induce them to feed quickly, the nonviruliferous leafhoppers were fasted for 2 hr before being placed on diseased plants. The leafhoppers were then placed in cages on infected oats and allowed to feed for the following acquisition periods: 15 or 30 min, 1, 3, 8, 24, 48, 96 hr, and 1 week. Leafhoppers reared and maintained continuously on infected oats were also tested. Of the insects allowed 15 or 30 min of acquisition-feeding, only those that appeared to be in a feeding position were transferred for assay. Individual insects were transferred to cages over seedling oats for 12-14 days, after which they were again individually transferred to seedling oats for another 12-14 days. This allowed all insects a total of 24-28 days in which to transmit the virus. They were then killed with a water suspension of malathion (25% wettable), and the plants were placed in the greenhouse. Plants developed blue dwarf symptoms as early as 10 days after the insects were first placed on them. A few insects in the 15- or 30-min acquisition-feeding groups became viruliferous. Thirty-four per cent of the adults and 34% of the nymphs became viruliferous in the 2-day and 1-week acquisition-feeding periods, respectively (Table 1). The percentage transmission of OBDV by leafhoppers that had been maintained on infected plants was no greater than that of leafhoppers that were given 2-day or 1-week acquisition periods (Table 1).

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TABLE 1. Transmission of oat blue dwarf virus by the aster leafhopper after different periods of acquisition feeding

Acquisition-feeding time	Insects transmitting virus					
	Nymphs[a]			Adults		
	Transmissions	Total		Transmissions	Total	
	no.	*no.*	*%*	*no.*	*no.*	*%*
15 min	3	150	2	5	150	3
30 min	2	74	3	1	24	3
1 hr	0	85	0	2	131	2
3 hr	4	95	4	7	150	5
8 hr	4	86	5	7	128	6
24 hr	13	101	13	19	130	15
48 hr	14	98	14	41	119	34
96 hr	16	74	22	32	102	31
1 week	90	266	34	91	284	32
Continuous[b]				41	137	30

[a] Included second, third, and fourth instars.
[b] These leafhoppers hatched and fed continuously on blue dwarf oats until they were selected for the experiment.

Abdominal inoculation versus feeding on infected plants for serial transmission of OBDV.—Serial transmission by individually inoculated leafhoppers was compared with serial transmission by leafhoppers that had acquired OBDV naturally. Furthermore, these experiments would determine the shortest incubation of the virus in leafhoppers that had acquired the virus by either means. Adult leafhoppers were anesthetized with CO_2 in an inoculation chamber at 3-4 C, and then inoculated through the ventral surface of the abdomen in the area of the third or fourth abdominal segment. The sources of OBDV inoculum were clarified extracts from oats in 0.01 M phosphate buffer (pH 7) or clarified extracts of triturated viruliferous leafhoppers in 0.01 M phosphate buffer (pH 7). The proportions of virus material to buffer used were: 1 g of plant material to 3 ml of buffer, and 1 g of leafhoppers to 100 ml of buffer. Both ground plant material and triturated leafhoppers were suspended in buffer and centrifuged at 7,000 *g* for 10 min. The supernatant fluids were filtered through cotton and used as inoculum. After inoculation of the leafhoppers, they were individually placed on oat seedlings and transferred daily, except on Sundays or holidays, to new seedlings. The data for serial transmission of OBDV by leafhoppers inoculated with either plant or leafhopper extracts are combined and shown in Fig. 1. Although a few insects transmitted the virus the 1st day after inoculation, the majority transmitted virus between the 6th and 20th days. After the 20th day, transmission diminished until the 40th day, when

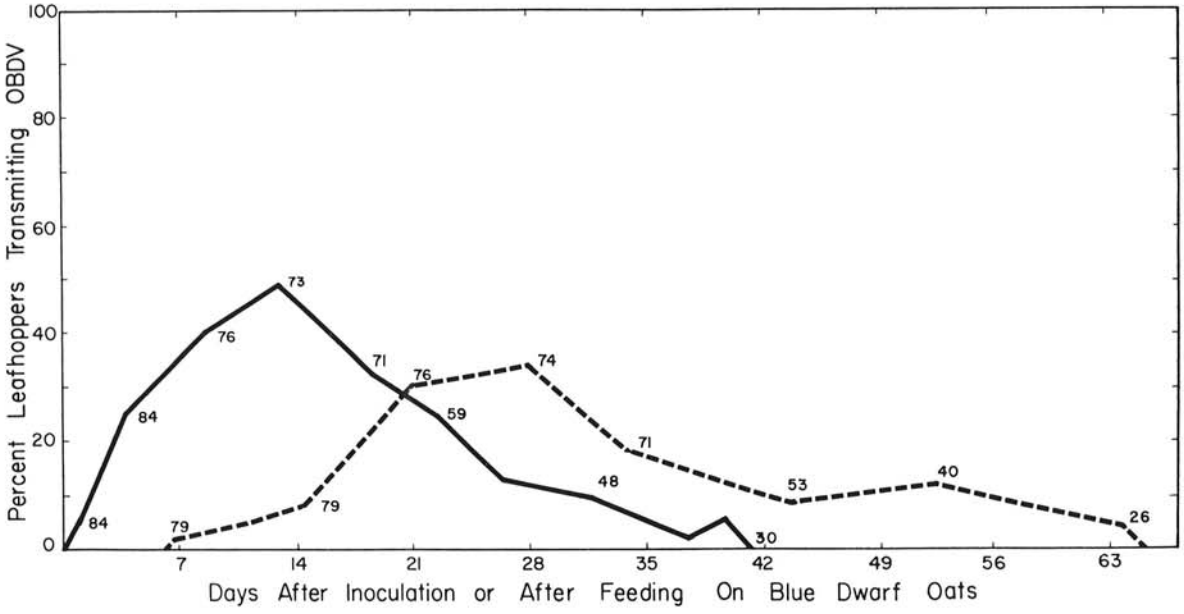

Fig. 1. Serial transmission of oat blue dwarf virus (OBDV) by leafhoppers that were abdominally inoculated (————) or by leafhoppers that acquired the virus by feeding on diseased oats (---------). A total of 153 leafhoppers in four experiments were abdominally inoculated with virus, and a total of 223 leafhoppers in six experiments acquired the virus naturally as nymphs or adults. The numbers along each curve indicate surviving leafhoppers that transmitted virus at least once during the experiments.

all but a few insects ceased to transmit virus. The daily patterns of transmission by individual insects were erratic. Some insects transmitted virus fairly consistently for periods up to 2 weeks after inoculation; however, other insects transmitted virus only at intervals of several days to 1 week or more. An average of 64% of the leafhoppers inoculated with extracts of viruliferous insects transmitted the virus at least once during the period of serial transfers. These insects transmitted OBDV on 24% of the total days in the experiments. An average of 42% of the leafhoppers inoculated with diseased plant extracts transmitted virus at least once during the period of serial transfers. These insects transmitted virus on only 11% of the total days in the experiments. Males and females were equally efficient vectors when inoculated with the extracts.

Serial transmission of OBDV by leafhoppers which acquired the virus by feeding on infected oats was done using nymphs in the second, third, or fourth instar stages or adult insects. The leafhoppers were given a 3-day acquisition-feeding in one experiment and a 1-week acquisition-feeding in five experiments. Individual leafhoppers were transferred daily to seedling oats, except on Sundays and holidays. Leafhoppers that acquired the virus as nymphs did not transmit it until they matured to adults; then their transmission pattern was no different from those that acquired the virus as adults. Therefore, the data for serial transmission of OBDV by those leafhoppers that acquired it as nymphs and those that acquired it as adults are combined (Fig. 1). A few adults transmitted the virus as early as 7 days after feeding; however, the majority of insects transmitted virus between the 17th and 34th days. After 34 days, the number of insects that transmitted gradually declined until the 66th day, after which only three leafhoppers transmitted the virus. These three individuals transmitted the virus occasionally until the 87th day after natural acquisition. Leafhoppers which acquired OBDV naturally also transmitted it erratically but less frequently than did inoculated insects. Certain insects transmitted virus only once or twice during their lifetime, and others missed several days or as much as 3 weeks between days in which they transmitted virus. An average of 35% of the leafhoppers that acquired the virus by feeding transmitted OBDV at least once during their lifetime, and they transmitted it on 12% of the total days of the experiments. There was no significant difference in percentage transmission between males and females in two experiments where the sexes were noted.

Inoculation of leafhoppers through a severed tarsus or tibia.—In two experiments, third and fourth instar nymphs or adults were anesthetized with CO_2 in a cold chamber. The tarsus or tibia of the front, middle, or hind leg was severed, and a drop of clarified extract from OBDV-infected plants or viruliferous leafhoppers was placed on the wound with a glass needle. It appeared that some of the virus suspension was drawn into the wounds. These leafhoppers were then assayed individually on oat seedlings. In two experiments, only 2 of 60 insects inoculated in this manner transmitted the virus. During molting, the exoskeleton remained attached to the wound site of numerous nymphs, and they subsequently died.

DISCUSSION.—Leafhoppers occasionally acquired OBDV from diseased oats within 15 min, and subsequently transmitted the virus to oat seedlings, but short acquisition feeding periods were usually inadequate. Since each insect was not individually observed to determine if its stylets were inserted in plant tissue, we could not be sure that each had fed. However, each insect appeared to be in a feeding position at the time it was chosen for assay. At least a 2-day acquisition-feeding for adults and a 1-week feeding period for nymphs were necessary to produce the highest number of OBDV viruliferous leafhoppers (Table 1). The long acquisition-feeding time required to obtain the most OBDV viruliferous leafhoppers appears similar to that reported for other viruses and leafhoppers (4, 6, 9, 10). It may be that the insects are unable to obtain OBDV from all the tissues they feed on, and that some leafhoppers require numerous different feedings.

The range of incubation times from 7 to 47 days before leafhoppers which acquired the virus from diseased plants could subsequently transmit OBDV indicates considerable variability among these insects in this aspect of transmission. It indicates that the virus is circulative, and may undergo multiplication in the leafhoppers. Insects that were inoculated with virus extracts began to transmit virus earlier and reached a higher incidence of transmission than did insects acquiring virus by feeding on plants. Because the virus was forced directly into the hemolymph, it reached the salivary glands more rapidly and was discharged earlier. Leafhopper extracts appeared to be superior to plant extracts as a source of virus for inoculation studies. Insects inoculated with extracts from viruliferous leafhoppers transmitted OBDV more than twice as frequently, within a 50-day period, as those inoculated with plant extracts. The concentration of OBDV is probably greater in the leafhopper than in infected oats. Peters (8) concluded that extracts from the aphid vector, *Myzus persicae*, yielded higher titers of potato-leafroll virus than did extracts from diseased *Physalis floridana*.

The erratic patterns of transmission characteristic of insects that acquired OBDV either naturally or by inoculation appear to be similar to that reported to occur in other serial transmission studies with leafhopper-transmitted viruses (10). Almost all insects ceased to transmit OBDV several days before they died. However, one insect transmitted virus seven times between the 19th and 35th days of serial transfer, five more times between the 64th and 81st days, but did not transmit virus again until it died on the 85th day. A few other leafhoppers behaved similarly. This erratic transmission and cessation of transmission prior to death of the insect may not mean that the virus is not propagative. Paliwal (7) found that *Endria inimica* ceased to transmit wheat striate mosaic virus prior to the death of the insect, although there was evidence for virus multiplication.

Our evidence indicating that OBDV is propagative in the leafhopper is that (i) there is a relatively long latent period following natural acquisition of virus; (ii) some leafhoppers transmit virus for an extended time; and (iii) leafhoppers appeared to be a better source of virus than plants in inoculation studies. If the virus does multiply in the aster leafhopper, there are factors which limit the insect's ability to transmit the virus regularly. It may be that the virus cannot multiply in certain individuals, multiplies poorly in others, and multiplies abundantly in still others. This may explain why some of them transmit virus in a manner that suggests the virus is circulative but non-propagative, whereas others transmit it in a manner that suggests it is circulative and propagative.

LITERATURE CITED

1. BANTTARI, E. E. 1967. Leafhopper transmission of the oat blue dwarf virus extracted from oats or barley. Phytopathology 57:802 (Abstr.).
2. 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.
3. BANTTARI, E. E., & R. J. ZEYEN. 1969. Chromatographic purification of the oat blue dwarf virus. Phytopathology 59:183-186.
4. BENNETT, C. W., & H. E. WALLACE. 1938. Relations of the curly top virus to the vector, *Eutettix tenellus*. J. Agr. Res. 56:31-51.
5. CHIU, R., J. JEAN, M. CHEN, & T. LO. 1968. Transmission of transitory yellowing virus of rice by two leafhoppers. Phytopathology 58:740-745.
6. MARAMOROSCH, K. 1950. Influence of temperature on incubation and transmission of the wound-tumor virus. Phytopathology 40:1071-1093.
7. PALIWAL, Y. C. 1968. Changes in relative virus concentration in *Endria inimica* in relation to its ability to transmit wheat striate mosaic virus. Phytopathology 58:386-387.
8. PETERS, D. 1967. The purification of potato leafroll virus from its vector *Myzus persicae*. Virology 31:46-54.
9. SINHA, R. C., & D. V. R. REDDY. 1964. Improved fluorescent smear technique and its application in detecting virus antigens in an insect vector. Virology 24:626-634.
10. SLYKHUIS, J. T. 1963. Vector and host relations of North American wheat striate mosaic virus. Can. J. Bot. 41:1171-1185.