# Some Properties of Oat Blue Dwarf Virus

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ABSTRACT

Some of the properties of oat blue dwarf virus (OBDV) (a ~30-nm diameter, spherical, vector-propagated virus) in crude juice were determined by membrane-feeding adults of *Macrosteles fascifrons* (aster leafhopper) on treated, refrigerated plant extract at 9 C. Refrigeration of the virus suspension during acquisition feeding greatly decreased leafhopper mortality. The dilution end point of OBDV was slightly over 1:512. The longevity in vitro was between 16 and 32 days. The thermal inactivation point was between 60 and 70 C. The virus remained viable after 3 months of storage (longest period tested) in clarified plant sap in liquid nitrogen, and after 1 month storage (longest period tested) in leaf tissue in liquid nitrogen. The virus was somewhat tolerant of a range of hydrogen ion concentrations and retained viability best when extracted at pH 6.85. There was (with one exception) a minimum 5-day incubation period before the leafhoppers could transmit OBDV after acquisition through membranes, and transmission was maximum 21-25 days after acquisition feeding.

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Since oat blue dwarf virus (OBDV) was first reported in 1952 (6) methods for studying the virus and some of the characteristics of the virus have been reported. Banttari and Zeyen (2) have reported on a purification method for the virus and the morphology and size of the virus have been determined (10). The nucleic acid has been analyzed (7) and the host range (9) and some of the relationships of the virus and its vector have been determined (3).

The purpose of this study was to define the dilution end point, thermal inactivation point, longevity in vitro, effect of hydrogen ion concentration on the virus, and storage characteristics of this virus. Preliminary results on some of these properties have been reported by the authors (5).

MATERIALS AND METHODS.—The nonviruliferous aster leafhoppers, *Macrosteles fascifrons* (Stål), used in these studies were maintained on barley, *Hordeum vulgare* L. 'Black Hulless' (C.I. 666), in a controlled environment (16-hour day, 1700 lx, 23 ± 2 C). The OBDV was obtained from field-collected, infected oats and identified by comparisons with published symptom descriptions (1, 5) and transmission studies. The virus was transferred to and maintained in oats, *Avena sativa* L. 'Rodney' (C.I. 6661), in the greenhouse.

The virus was extracted from infected oat plants (25-30 days after infection) by grinding the plant tissue in a food chopper with an equal (1:1, w/v) quantity of 0.01 M phosphate buffer (pH 6.85).

The crude sap was treated for each physical property studied and then clarified by cycle centrifugation except for the dilution end point determination in which clarification preceded treatment (dilution). Clarification of the virus suspension was done by centrifuging at 8,720 *g* for 15 minutes, followed by centrifuging at 65,950 *g* for 3 hours. The resulting pellet was resuspended to the original volume with phosphate buffer and centrifuged at 8,720 *g* for 15 minutes and the pellet discarded. The clarified plant extract, to which five percent sucrose was added, was assayed for infectivity by allowing the leafhoppers to acquire the virus through stretched Parafilm M membranes. Four or five adult leafhoppers that had fasted 2-4 hours were then placed in the feeding unit and allowed a 24-hour period of acquisition feeding through membranes. The number of trials used to make each determination varied, and the number of trials is given under specific methods for each of the properties being determined. Freshly prepared virus suspensions were used in each trial. The number of leafhoppers used in each trial was initially between 25 and 50, but mortality during acquisition feeding and incubation reduced the number tested for transmission.

Because of the erratic transmission of OBDV, we thought initially that the virus was unstable at room temperature. To minimize this instability, a system as previously described (4) was used to cool and hold the plant extract at 9 C during the acquisition-feeding period in the membrane-feeding process.

After acquisition feeding, individual leafhoppers from each acquisition-feeding chamber were transferred to caged oat seedlings for a series of five transfers at 5-day intervals. After the leafhoppers were removed, the test plants were sprayed with insecticide to prevent later contamination. Plants were examined for virus symptoms 15 days after the leafhoppers had been removed, and the number of leafhoppers that transmitted the virus was determined.

For the dilution end point (DEP) determination a twofold dilution series up to 1:512 was made with the clarified virus suspension and buffer. Five trials were made for each dilution using between 25 and 50 leafhoppers for each trial.

To determine the thermal inactivation point (TIP), 2-ml aliquots of the crude extract were heated for 10 minutes at temperatures of 40, 50, 60, 70, and 80 C in thin-walled test tubes submerged in a constant-temperature water bath. The samples were immediately cooled in running tap water, clarified by centrifugation, and fed at a

1:16 dilution to leafhoppers as described earlier. After acquisition feeding, leafhoppers were transferred to oat seedlings as described above. One trial was used for the 40, 50, and 80 C treatments, and two trials were used for the 60 and 70 C treatments. In each trial, an equivalent number of leafhoppers were carried as checks. (Leafhoppers fed on unheated virus suspensions and tested for transmission).

To determine longevity in vitro (LIV), crude juice samples (1:1 w/v of plants and buffer) were held at 23 C for 0-32 days in a stoppered Erlenmeyer flask. After the prescribed test period in vitro, "of 0, 1, 2, 4, 8, 16, and 32 days", samples were clarified by centrifugation and membrane-fed at a 1:16 dilution to leafhoppers as described earlier. After acquisition feeding, leafhoppers were transferred to oat seedlings for transmission tests. Nothing was added to suppress bacterial or fungal growth during the aging process in crude sap. Two trials were made on the sap aged for 1, 2, and 32 days and three trials were made for all other treatments. Aged sap from virus free plants served as the checks in all trials.

To determine the effect of hydrogen ion concentration on the virus, oat tissue was ground in 0.01 M phosphate buffer at pH values of 5.5, 6.0, 6.5, 6.85, 7.5, and 8.0 and clarified by cycle centrifugation. Each solution was diluted to 1:16 with the appropriate buffer and fed to leafhoppers as previously described. A single trial for each treatment was used.

To determine storage characteristics, OBDV-infected oat tissue was frozen in liquid nitrogen for 1 month, or clarified plant sap from infected tissue was frozen in liquid nitrogen for 1 and 3 months. The oat tissue was ground while it was frozen, then clarified as previously described. The frozen plant sap was first thawed at 4 C and then membrane-fed to leafhoppers as previously described. A single trial was used in determining storage characteristics.

RESULTS AND DISCUSSION.—*Dilution end-point.* —The DEP of OBDV was greater than 1:512 (Table 1). Dilutions higher than 1:512 were not tested; but because only one leafhopper of 143 was able to acquire the virus at this dilution, we can assume that the end point would be less than 1:1024. In these studies, transmission differences which occurred through the 1:32 dilution cannot be considered significant. The first definite effect of dilution is evidenced at the 1:128 dilution treatment. There was a small decrease in the transmission rate at the 1:64 dilution, but such decreases can be attributed to inherent variation in the system.

*Thermal inactivation point.*—In these studies the thermal inactivation point was between 60 and 70 C (Table 2). In two trials at the 60 C temperature using sap from two different sets of infected plants, the infectivity was about the same. In two trials, there was no transmission with different extracts at the 70 C level, which indicates that the thermal inactivation point in raw sap is below 70 C.

*Longevity in vitro.*—When aged in buffered crude extract at room temperature (23 C), OBDV lost all infectivity between 16 and 32 days (Table 3). Transmission dropped gradually during aging. The LIV of OBDV was surprising, because we initially reported poor success with this system, unless the plant extract was refrigerated during the feeding process.

Since this initial work, we have used an inbred line of leafhoppers developed at this station (8). When OBDV was acquired from infected plants, this inbred line had a higher rate of transmission (69%) than did the wild population (39%). In later membrane-feeding tests with the inbred line on refrigerated and nonrefrigerated plant extract, transmission was slightly higher from extract at 9 C (43%) based on 148 leafhoppers tested in three trials than from extract at 23 C (39%) based on 140 leafhoppers tested in three trials. Although there was a relatively small increase in percentage of transmission of the virus when a 9 C temperature was used, the advantage of using a refrigerated system becomes apparent when one considers the survival data on the leafhoppers.

About 360 leafhoppers in three trials were checked for mortality following acquisition feeding, and again at the end of five five-day test feeding periods. Mortality at the end of the 24-hour acquisition period was 2% in

TABLE 1. The number of *Macrosteles fascifrons* leafhoppers that transmitted oat blue dwarf virus after a 24-hour acquisition feeding period through stretched Parafilm M membranes on infected plant extract diluted in a twofold dilution series with phosphate buffer (pH 6.85)

| Sap dilution | Number of leafhoppers that transmitted after feeding on sap from: | | |
|---|---|---|---|
| | Infected plants | | Healthy plants |
| | (No.) | (%) | (No.) |
| 1:2 | 16/119[a] | 13 | 0/53 |
| 1:4 | 27/111 | 24 | 0/49 |
| 1:8 | 18/121 | 15 | 0/48 |
| 1:16 | 21/98 | 21 | 0/45 |
| 1:32 | 18/128 | 14 | 0/41 |
| 1:64 | 19/209 | 9 | 0/71 |
| 1:128 | 6/155 | 4 | 0/50 |
| 1:256 | 6/146 | 4 | 0/50 |
| 1:512 | 1/84 | 1 | 0/50 |

[a]Number of plants infected per total number tested. The figures given were obtained from a total of five trials in which different extracts were used.

TABLE 2. Number of *Macrosteles fascifrons* leafhoppers that transmitted oat blue dwarf virus to oat *Avena sativa* seedlings after a 24-hour acquisition-feeding period on plant extract previously exposed to different temperatures for 10 minutes

| Treatment temperature (C) | Leafhoppers transmitting | |
|---|---|---|
| | No.[a] | (%) |
| 40 | 5/27 | 19 |
| 23 (check) | 11/24 | 46 |
| 50 | 20/45 | 44 |
| 23 (check) | 5/45 | 11 |
| 60 | 16/72 | 22 |
| 23 (check) | 32/66 | 48 |
| 70 | 0/79 | 0 |
| 23 (check) | 28/79 | 35 |
| 80 | 0/46 | 0 |
| 23 (check) | 26/46 | 57 |

[a]Number transmitting/total number tested. The 60 and 70 C treatment figures were obtained from two trials in which different extracts were used.

TABLE 3. Number of *Macrosteles fascifrons* leafhoppers that transmitted oat blue dwarf virus to oat *Avena sativa* seedlings after 24-hr acquisition-feeding periods on plant extracts that had been aged in vitro for various periods of time

| Age of plant extract (days) | Leafhoppers transmitting[a] from sap from: Infected plants Trial 1 | Trial 2 | Trial 3 | Total | % | Healthy plants Number |
|---|---|---|---|---|---|---|
| 0 | 21/42[a] | 21/45 | 21/46 | 63/133 | 47 | 0/35[a] |
| 1 | 24/40 | 17/37 | . . . | 41/77 | 53 | 0/45 |
| 2 | 9/39 | 16/44 | . . . | 25/83 | 30 | 0/44 |
| 4 | 8/39 | 7/41 | 12/43 | 27/123 | 22 | 0/37 |
| 8 | 7/43 | 2/41 | 6/48 | 15/132 | 11 | 0/42 |
| 16 | 5/36 | 0/42 | 1/46 | 6/124 | 5 | 0/44 |
| 32 | . . . | 0/45 | 0/37 | 0/82 | 0 | 0/39 |

[a]Number transmitting per total number tested. Different extracts were used in each trial.

TABLE 4. Number of *Macrosteles fascifrons* leafhoppers that transmitted oat blue dwarf virus to oat *Avena sativa* seedlings after a 24-hour acquisition-feeding period on plant extracts in 0.01 M phosphate buffer at various pH levels

| pH | Transmission (No.) | (%) |
|---|---|---|
| 5.5 | 2/35[a] | 6 |
| 6.0 | 6/31 | 19 |
| 6.5 | 5/38 | 13 |
| 6.85 | 16/34 | 47 |
| 7.5 | 16/43 | 37 |
| 8.0 | 12/35 | 34 |

[a]Number transmitting per total number tested.

TABLE 5. Number of *Macrosteles fascifrons* leafhoppers that transmitted oat blue dwarf virus to oat *Avena sativa* seedlings after a 24-hour acquisition-feeding period on clarified plant extract stored in liquid nitrogen by various methods, varying periods of time, or both

| Condition | (No.) | (%) |
|---|---|---|
| Fresh sap | 15/31[a] | 48 |
| Clarified sap in liquid N: | | |
| 1 month | 3/13 | 23 |
| 3 month | 8/30 | 27 |
| Leaf tissue in liquid N: | | |
| 1 month | 3/22 | 14 |
| Healthy check | 0/45 | 0 |

[a]Number transmitting per total number tested.

TABLE 6. Number of *Macrosteles fascifrons* leafhoppers that transmitted oat blue dwarf virus to oat *Avena sativa* seedlings at least once during five successive 5-day feeding periods after a 24-hour acquisition-feeding period through stretched Parafilm M membranes

| Test feeding period (days after acquisition) | Leafhoppers transmitting (No.) | (%) |
|---|---|---|
| 0-5 | 1/347[a] | 0 |
| 6-10 | 21/371 | 6 |
| 11-15 | 52/309 | 17 |
| 16-20 | 67/229 | 29 |
| 21-25 | 63/212 | 30 |

[a]Number transmitting per total number tested.

leafhoppers that acquired virus from sap at 9 C, but 28% in leafhoppers that acquired virus from sap at 23 C. At the end of the five test feeding periods, the mortality in leafhoppers that had acquired the virus at 9 C was 17% but it was 44% for leafhoppers that had acquired the virus at 23 C. At present, the reasons for these differences in mortality are unknown.

*Hydrogen ion concentration.*—OBDV was found to have a relatively broad range of tolerance to pH, in that the virus was not completely inactivated over the pH range tested. The optimum pH for extraction of the virus was between 6.85 and 7.5 (Table 4). Transmission was low from plant tissue extracted in pH values below 6.85. The virus appeared to be quite tolerant to neutral or slightly alkaline conditions, because transmission was quite high at pH 7.5 (37%). No discernible effects were observed on leafhopper mortality during the acquisition-feeding period on plant extract at various pH levels or on leafhopper mortality during the feeding periods after acquisition feeding.

*Storage characteristics.*—OBDV remained viable when stored frozen in liquid nitrogen, either in fresh leaf tissue or in clarified plant extract, for up to 3 months, the longest storage time tested (Table 5). A single trial was made on the viability of the virus by using a 1:16 dilution of the stored sap or sap extracted from the frozen tissue. The only indication of loss of infectivity of the virus in storage was for that stored in leaf tissue. Since only one trial was made, it is not known if this is a real difference. We assumed that no further loss in infectivity would have occurred over longer storage periods, because most plant viruses stored in liquid nitrogen by other workers did not decrease in viability (Jerry Blizzard, *personal communication*).

*Transmission patterns.*—The incubation period of OBDV in leafhoppers and the length of time required to reach peak transmission in a population of leafhoppers that acquired the virus through membranes is shown in Table 6. Results presented here are a compilation of the data obtained from the trials on the physical property determinations above. Each leafhopper was tested for transmission during each of five successive test-feeding periods after acquisition. Only one leafhopper of 347 transmitted OBDV during the first test-feeding period after acquisition. During the second period, 6-10 days after acquisition, 21 of 371 leafhoppers transmitted the

virus. During the third, fourth, and fifth test-feeding periods, 52 of 309, 67 of 229, and 63 of 212 leafhoppers respectively, transmitted the virus. In these studies, maximum transmission occurred during the last two test-feeding periods. Previous studies (8) showed that transmission was maximum 15 days after the start of acquisition feeding on infected plants. The concentration of virus in extracted sap as used in these experiments was lower than it would have been in infected plants, because buffer was added in extraction and virus was lost in clarification. Therefore one might expect a longer incubation time in leafhoppers, because of the correspondingly lower virus concentration in the extract ingested by the leafhoppers during acquisition.

These studies show that OBDV can withstand considerable physical stress without inactivation. The DEP at more than 512 shows that the virus can be purified in fairly high quantity. The virus can withstand temperatures up to 60 C without excessive infectivity loss, and the pH tolerance is quite high, with an optimum near neutrality. The most surprising property of this virus is its tolerance to aging and the little or no loss of infectivity after 24 hours at room temperature. Viral retention of good infectivity after storage in liquid nitrogen for 3 months lets us maintain virus cultures with relative ease.

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