Storage of Preparations of Barley Yellow Dwarf Virus

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ABSTRACT

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No loss of infectivity of two barley yellow dwarf virus isolates was detected during 4 years' storage of virus concentrates under a range of conditions. Treatments included preservation by freezing in liquid nitrogen or lyophilization together with storage at 4, −70, or −196 C. Results show that preserved virus collections need not be restricted to plant viruses that are sap-transmissible.

Additional key words: aphid transmission of viruses, *Rhopalosiphum padi, Macrosiphum avenae,* American Type Culture Collection.

Disadvantages of long-term plant virus maintenance by serial transfers in plants have been discussed by others (2, 3, 4). Many viruses can be preserved in dried or lyophilized plant tissue, but such efforts usually have involved sap-transmissible viruses (3, 5, 6). Little is known about long-term storage of vector-dependent viruses, such as barley yellow dwarf virus (BYDV), because of difficulties in recovering viruses that have not been transmitted mechanically to plants. Since BYDV can be recovered from virus preparations by letting aphids feed through membranes on samples or by injecting inocula into aphids (8, 9), we are doing a long-term study of methods for preservation of two distinct, vector-specific isolates of BYDV. Here we report results of tests during the first 4 years of storage by the American Type Culture Collection (ATCC).

The MAV (ATCC Cat. No. PV93) and RPV (ATCC Cat. No. PV95) isolates of BYDV were used in these studies (8). Concentrated preparations of each virus (suspended in 0.1 M potassium phosphate buffer, pH 7.0) were made in Ithaca by chloroform clarification and differential centrifugation as previously described (9). The MAV preparation originated from 5,173 g of infected tissue of oats (*Avena byzantina* C. Koch 'Coast Black'), concentrated about 68-fold relative to the volume of clarified juice. The RPV preparation, which was made from 3,101 g of tissue, was concentrated about 100-fold. During shipment from Ithaca, New York, to Rockville, Maryland, the concentrates were kept at about 15 C. The 25 ml of MAV concentrate contained about 9 μg of virus per ml. The 20 ml of RPV preparation contained about 14 μg of virus per ml. Recent work suggests that these estimates of virus concentration probably are high (1, 9).

When the preparations were received at the laboratories of the ATCC, each sample was divided to permit mixing with an equal volume of 20% glycerol (for liquid nitrogen preservation), with 24% sucrose (for lyophilization), or with distilled water as controls. The mixtures then were divided into samples of 0.3 ml for lyophilization or for freezing in liquid nitrogen (−196 C). For lyophilization, the samples were frozen in a bath of dry ice and ethyl cellosolve at −70 C and kept frozen for about 2 hours. After a 48-hour drying cycle, the vials were sealed and stored either at −70 C or 4 C. For treatment with liquid nitrogen, the vials were sealed and placed directly into the liquid. Some vials were kept there during storage; others were moved to −70 C for storage.

We made infectivity assays of the original virus preparations and of samples returned to Ithaca after intervals of storage for 1 month, 1 year, and 4 years. During the usual 1-day transit to Ithaca, samples were packed in dry ice. The frozen samples were thawed at 37 C. The lyophilized samples were resuspended in 20% sucrose in neutral 0.1 M potassium phosphate buffer. All then were kept at 4 C until used. Two series of assays were made in all cases (7, 8, 9). In one series, transmission by means of both *Rhopalosiphum padi* (L.) and *Macrosiphum avenae* (F.) was tested to compare the vector specificity of each virus sample. Results of all of these assays were in agreement. Isolate RPV was transmitted specifically by *R. padi;* isolate MAV was transmitted specifically by *M. avenae.* In the various assays for RPV made by means of membrane feeding, *R. padi* transmitted virus to 127 of 138

Coast Black oat plants, but *M. avenae* transmitted RPV to none of 138 plants. In the corresponding assays for RPV made by means of injection (using five injected aphids per plant), 44 of 48 plants infested with injected *R. padi* became infected, but only one of 60 plants infested with injected *M. avenae* became infected. In the tests of MAV, 173 of 174 plants became infected following acquisition feeding by *M. avenae* through membranes on the virus preparations, but none of 174 plants became infected in parallel tests with *R. padi*. Similarly, 58 of 60 plants became infected in tests of MAV injected into *M. avenae;* none of 72 plants became infected in tests with injected *R. padi* . None of 196 control plants in these experiments became infected. Thus, there is no evidence for any alteration in biological specificity of the virus samples during storage.

The second series of assays was designed to detect possible major changes in infectivity of the stored samples

TABLE 1. Bioassay of preparations of the MAV isolate (ATCC No. PV-93) and RPV isolate (ATCC No. PV-95) of barley yellow dwarf virus at intervals during storage by American Type Culture Collection for four years

Virus isolate	Original additive and treatment	Storage temp. (C)	Storage time (months)	Virus transmission in membrane or injection assays at virus concentration (μg/ml) shown[a]					
				Membrane[b]					Injection[c]
				0.50	0.10	0.02	0.004	0.0008	0.50
MAV	Water	−196	1	11	12	12	. . .	. . .	31
	−196 C		12	12	12	9	6	0	27
			48	12	12	11	7	1	33
MAV	20% glycerol	−196	1	12	12	12	. . .	. . .	40
	−196 C		12	12	12	9	3	0	31
			48	12	12	11	6	3	40
MAV	20% glycerol	− 70	12	12	12	9	5	1	29
	−196 C		48	12	12	10	7	2	39
MAV	Water	− 70							
	−196 C		48	12	12	12	4	1	37
MAV	Water	− 70	1	12	12	11	. . .	. . .	23
	Freeze-dry		12	12	12	8	4	2	28
			48	12	12	3	0	0	23
MAV	24% sucrose	+ 4	12	12	12	11	8	1	24
	Freeze-dry		48	12	12	9	2	4	32
MAV	24% sucrose	− 70	1	12	12	12	. . .	. . .	36
	Freeze-dry		12	12	12	12	7	1	26
			48	12	12	12	3	2	20
RPV	Water	−196	1	12	12	12	. . .	. . .	34
	−196 C		12	12	12	12	10	1	26
			48	12	11	7	4	1	17
RPV	20% glycerol	−196	1	12	12	12	. . .	. . .	32
	−196 C		12	11	12	10	7	5	15
			48	11	12	10	5	1	26
RPV	Water	− 70	1	12	12	10	. . .	. . .	24
	Freeze-dry		12	9	12	11	6	0	27
			48	10	12	10	2	1	7
RPV	24% sucrose	+ 4	12	5	8	11	6	0	28
	Freeze-dry		48	11	10	10	2	1	13
RPV	24% sucrose	− 70	1	12	12	12	. . .	. . .	23
	Freeze-dry		12	11	11	9	2	0	22
			48	12	12	9	8	2	8

[a]Concentration of RPV in assays was twice that shown for each sample.

[b]Number of plants that became infected of 12 infested, each with 10 aphids (*Macrosiphum avenae* for MAV; *Rhopalosiphum padi* for RPV) that fed through membranes on virus in 20% sucrose for about 18 hours at 15 C before feeding on Coast Black oat seedlings for 5 days at 21 C. None of 216 plants infested as controls became infected.

[c]Number of plants (of 48) that became infected following feeding for 5 days at 21 C by single *M. avenae* (for MAV) or *R. padi* (for RPV) that had been injected with about 0.02 μl of virus preparation. None of 900 noninjected control aphids transmitted virus.

and any major differences among the various treatments. Samples stored under different conditions were assayed in a series of dilutions with vectors that had acquired virus by feeding through membranes (Table 1). Each preparation was also assayed by injecting diluted virus into 48 aphids, and then testing each injected aphid singly on a test plant (Table 1). Virus was readily recovered from all stored samples; no major differences were apparent during the 4 years of storage of the samples. Despite the limited precision of such bioassays, we think any major trends, or any major differences among treatments, would have been identified. The three lowest values in the injection assays of RPV in tests made after 48 months appeared to suggest some loss of infectivity for these samples (Table 1). But we think these results merely illustrate variation in the bioassays because all three values came from one experiment in which survival of the injected aphids was below the usual 100% rate.

The data of Table 1 are in striking contrast to results of bioassays of samples of the original preparations stored in commercial freezers in Ithaca. Portions of the original preparations stored in lots of 0.3 ml for 1 month at 4 C or at about −10 C had infectivities similar to those shown in Table 1 for the same kinds of assays. After we had assayed samples stored for 4 years (Table 1), however, we removed some portions of the original preparations that had been kept in a commercial freezer. Although RPV or MAV were recovered from all of these samples, consistent transmission occurred only in tests of the most concentrated dilution in the membrane assay, and only a few of the 48 injected aphids transmitted virus in two tests for each of the virus isolates. For example, in the four injection assays, only 2, 4, 2, and 7 of the aphids transmitted virus. None of 48 control plants became infected. Thus, storage at the low temperatures used by the ATCC laboratory appears to be worthwhile and probably necessary for long-term preservation of BYDV.

Storage of crude concentrates in commercial freezers is a routine method we use to offset limitations of preparing purified BYDV (9). A recent test of the adequacy of this procedure revealed no serious disadvantages for MAV concentrates stored nearly 3 years. In the experiment a preparation concentrated 36-fold was divided into six equal portions of 25 ml for storage in the freezer at about −10 C. At intervals of 4, 6, 7, 17, 24, and 31 months, a portion of the concentrate was removed from the freezer and used to make a purified preparation (9). The yield of virus for each of the six preparations was 115, 96, 78, 104, 76, and 81 μg of MAV, respectively. Results of bioassays of the preparations showed variations, but no general decline of infectivity during storage. Our experience suggests that storage of crude concentrates beyond about 3 years might not be desirable. These observations are in agreement with the contrast mentioned above between infectivity of samples stored under various conditions by ATCC, and untreated samples of the same preparations kept in commercial freezers for 4 years.

The present results, those of previous studies (7, 8, 9), and lyophilization tests made by Gill (2) all show that the vector-dependent BYDV can be handled as readily as sap-transmissible viruses in preserved samples. Although recovery of BYDV from frozen or lyophilized samples is more difficult than rubbing virus on leaves, workable and reliable procedures are available not only for BYDV, but also for other similar viruses. Thus, preserved collections of plant viruses need not be limited to those that are sap-transmissible.

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