Brome Mosaic Virus Isolates Naturally Infecting *Commelina diffusa* and *C. communis*

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ABSTRACT

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Brome mosaic virus (BMV) was isolated from *Commelina communis, C. diffusa*, and five Gramineae species growing in a lawn. Three isolates, designated BMV-1, BMV-2, and BMV-3, were readily distinguished by their reaction in *Datura stramonium*. The isolates were serologically identical to each other and to the type isolate. The chrysomelid beetle *Lema sexpunctata* transmitted BMV-1 to *C. communis*.

Commelina diffusa Burm. and *C. communis* L., dayflower of the family Commelinaceae, showing mosaic symptoms were noted in a lawn in Fayetteville, AR. Three isolates of virus producing different symptoms on several hosts were obtained. Routine serological testing proved all three to be related to brome mosaic virus (BMV). This study compared these three isolates with the type isolate of BMV in host reaction, serology, and RNA components. Their relative abundance in naturally infected hosts near the original area of isolation and the transmission of one of the isolates to *C. communis* by a chrysomelid beetle were also evaluated.

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MATERIALS AND METHODS

After three single local lesion transfers on *Chenopodium album* L., the three lawn isolates of BMV, designated BMV-1, BMV-2, and BMV-3, and BMV-type (ATCC PV47) were mechanically inoculated to various plant species using 0.01 M phosphate buffer, pH 7.2.

The three isolates and BMV-type were purified from barley (*Hordeum vulgare* L. 'Pike') harvested 3 wk after mechanical inoculation, using the low-speed centrifugation method described by Lane (4).

Table 1. Differential host reactions to three brome mosaic virus (BMV) isolates and to the type isolate following mechanical inoculation

Host[a]	BMV isolates			
	BMV-type	BMV-1	BMV-2	BMV-3
Commelina diffusa	S[b]	SMo	SMo	SMo
C. communis	···	SMo	SMo	SMo
Datura stramonium	···	LLn	RSn	···
Vigna unguiculata (L.) Walp. subsp. *unguiculata* 'Monarch'	···	LLn	···	···

[a] All inoculated plants were tested by serology and back-inoculated to *Chenopodium quinoa*.
[b] S = symptomless host, SMo = systemic mosaic, LLn = necrotic local lesions, RSn = necrotic ring spots, ··· = no infection.

Virus was further purified by one high-speed (80,000 *g* for 90 min) and one low-speed (5,000 *g* for 10 min) centrifugation. Final pellets were resuspended in 50 mM of sodium acetate and 1 mM of magnesium acetate, pH 5.0. The RNAs of the isolates and the BMV-type were analyzed by 2.9% polyacrylamide gel electrophoresis using Lane's method (3). Antisera to BMV-1, BMV-2, and BMV-3 (titer 1:16) were produced in rabbits by four weekly subcutaneous injections of 0.5 ml (5 mg/ml) suspensions of virus emulsified with 0.5 ml of Freund's incomplete adjuvant. Antiserum to BMV-type was supplied by H. A. Scott. Serological comparisons among the three isolates and BMV-type were made with purified virus in Ouchterlony double-diffusion tests using 1% agarose in 0.01 M phosphate buffer, pH 6.0 (8).

To evaluate the relative occurrence of the isolates in the area of original isolation, leaf samples from *C. communis, C. diffusa, Poa pratensis* L., *Digitaria sanguinalis* (L.) Scop., *D. ischaemum* (Schreb.) Muhl., *Muhlenbergia schreberi* Gmel., and *Paspalum pubiflorum* Rupr. showing mosaic symptoms were collected from the lawn. Samples were tested by serology for BMV, and the isolate type was identified by mechanical inoculation to *Datura stramonium* L. and *C. communis*.

Lema sexpunctata Oliv., a chrysomelid beetle that feeds on *Commelina* species, was tested for ability to transmit BMV-1 to *C. communis*, using procedures previously reported (10).

RESULTS

The reactions of differential hosts to mechanical inoculations with the three isolates and BMV-type are shown in Table 1. The lawn isolates were clearly differentiated on *D. stramonium*: BMV-1 induced necrotic local lesions (Fig. 1A), BMV-2 induced necrotic ring spots (Fig. 1B), and BMV-3 did not induce symptoms. All three lawn isolates induced mosaic symptoms on *C. communis* and *C. diffusa* (Fig. 1C), but BMV-type was latent in *C. diffusa* and did not infect *C. communis*. Other susceptible hosts that reacted similarly to the three isolates and to BMV-type were: *H. vulgare* 'Pike,' *Triticum aestivum* L. 'Rosen,' 'Cocker,' and 'McNair,' *Zea mays* L. 'Golden Cross Bantam,' *C. quinoa* Willd., and *C. capitatum* (L.) Aschers, with chlorotic local lesions and systemic mosaic; *C. album* and *C. amaranticolor*, with necrotic local lesions; and *Cucumis sativus* L. 'Model,' with chlorotic local lesions.

Each lawn isolate contained the typical four RNA electrophoretic components of BMV and did not differ from BMV-type RNA. Coalescing bands in Ouchterlony double-diffusion tests indicated that all three lawn isolates were serologically identical to each other and to BMV-type.

All leaf samples showing symptoms collected from the lawn were infected with BMV. Isolate BMV-3 was the most common and the most widely distributed among the natural hosts, occurring in all hosts sampled (Table 2). Isolate BMV-2 was obtained only from *C. diffusa* and BMV-1, only from *C. communis*.

In beetle transmission trials, three of the 66 *L. sexpunctata* tested transmitted BMV-1 from *C. communis* to *C. communis*.

Table 2. Relative natural occurrence of three isolates of brome mosaic virus (BMV) in a lawn

Natural host	Total samples collected[a]	BMV isolate[b]		
		BMV-1	BMV-2	BMV-3
Commelina diffusa	25	0	1	24
C. communis	20	2	0	18
Poa pratensis	7	0	0	7
Digitaria sanguinalis	5	0	0	5
D. ischaemum	10	0	0	10
Muhlenbergia schreberi	4	0	0	4
Paspalum pubiflorum	4	0	0	4

[a] Leaf sample from single plants showing mosaic symptoms.
[b] Virus was identified by serology and type of isolate was determined by reaction of *Datura stramonium* and *C. communis*.

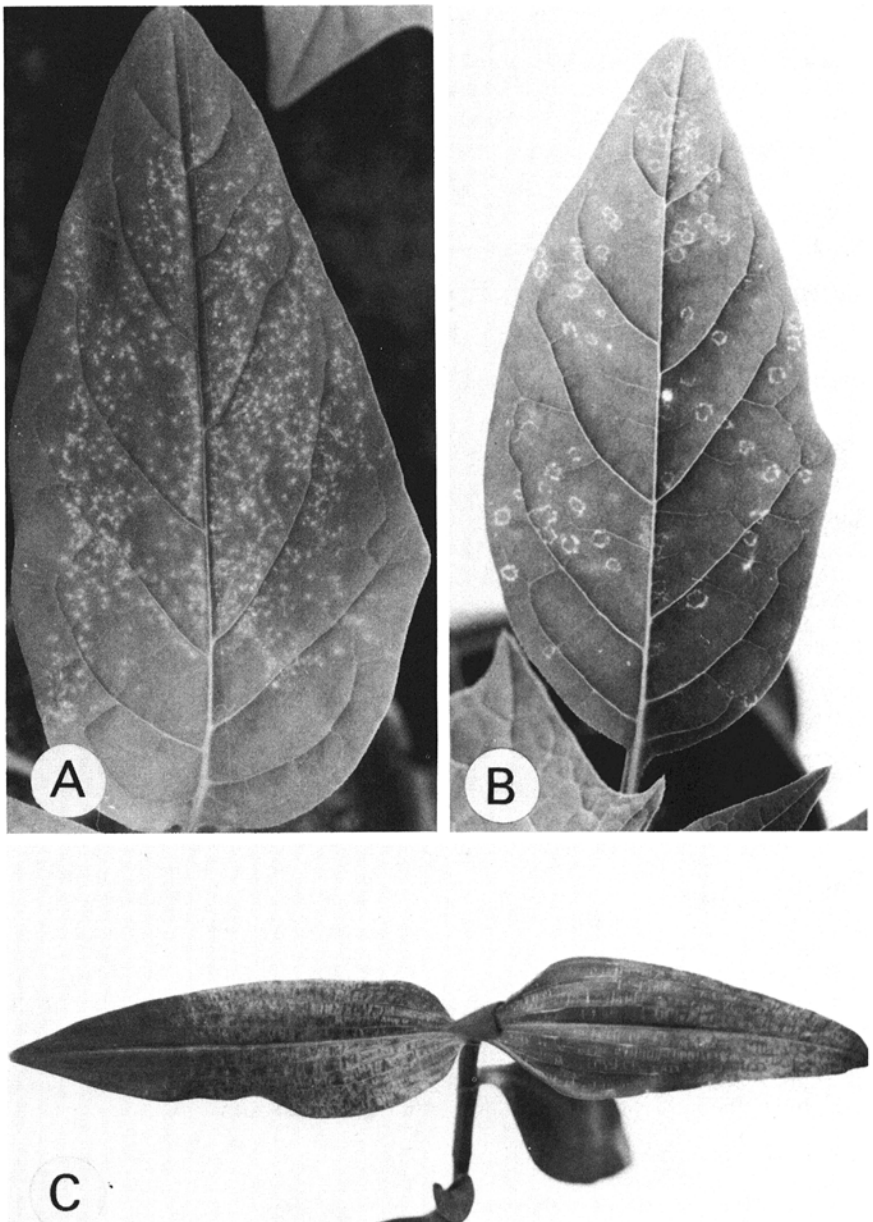

Fig. 1A,B. Symptoms on *Datura stramonium* after mechanical inoculation with brome mosaic virus (BMV) isolates **(A)** BMV-1 and **(B)** BMV-2. **(C)** Symptoms on *Commelina diffusa* induced by BMV-3; similar symptoms were obtained with BMV-1 and BMV-2.

DISCUSSION

Brome mosaic virus isolates from different areas tend to be indistinguishable (5). All contain four similar RNA components and are serologically identical. Differences in symptom severity on *D. stramonium* among isolates from the United States, Yugoslavia, and Germany have been reported (7). The ring spots induced by BMV-2 on *D. stramonium* are similar to those described for BMV by Chiu and Webster (1). Ford et al (2) and Stoner et al (9) reported both local lesions and ring spots when their BMV isolates were inoculated to *D. stramonium*. In this study, local lesions were produced by BMV-1 and ring spots, by BMV-2. Both lesions and ring spots were observed only when inoculum consisted of a mixture of these two isolates.

The occurrence of three isolates with distinguishable host reactions in a lawn of approximately 75 m^2 is an interesting phenomenon, particularly since BMV-3 was present in most samples collected and BMV-1 and BMV-2 were present in only three samples. Isolate BMV-3 may be better adapted for survival than the other isolates; BMV-3 was found in perennial grasses, whereas BMV-1 and BMV-2 were found only in annual *Commelina* species. It is possible also that BMV-1 and BMV-2 are mutants of BMV-3.

Brome mosaic virus has been transmitted by beetles and nematodes in European laboratories (5). Because bromoviruses in general seem to be inefficiently transmitted by beetles (5), the low level of transmission obtained here with *L. sexpunctata* is not unexpected. McKinney (6) demonstrated mechanical transmission of BMV in pastures. Mechanical transmission of the three BMV isolates during mowing may be an important factor in disseminating the virus in the lawn.

The occurrence of BMV in *C. communis* and *C. diffusa* expands the natural host range of this virus.

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