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Longidorus breviannulatus as a Vector for Brome Mosaic Virus[1]

DONNA E. HUFF, ROBERT F. DAVIS, AND RONALD F. MYERS[2]

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Longidorus breviannulatus Norton & Hoffmann, 1975 was described from corn in Iowa. Brome mosaic virus (BMV) was initially isolated in the Great Plains area of the United States from perennial brome grass, *Bromus inermis.* Infrequent localized outbreaks of BMV in barley were believed to result from mechanical inoculation by farm machinery moving across infected brome grass and into barley fields (3). *L. breviannulatus* and BMV have several host plants in common, such as barley (*Hordeum vulgare*), corn (*Zea mays*), and Kentucky blue grass (*Poa pratensis*). The habitats of these two pathogens seem to coincide geographically. If *L. breviannulatus* is a vector for BMV, then the potential for serious outbreaks of BMV is present. Our objective was to investigate *L. breviannulatus* as a vector for brome mosaic virus.

Since *L. breviannulatus* was to be placed on mechanically infected barley to acquire the virus, the relative concentration of BMV in barley root tissue was determined 1–10 days after mechanical inoculation of the leaves. Sixty 1-week-old barley seedlings, germinated in vermiculite, were transplanted, one plant per pot, into 10-cm-d plastic pots containing pasteurized soil. Thirty-five plants were inoculated with BMV as follows: BMV-infected barley leaf tissue was ground in 0.02 M sodium phosphate buffer, pH 6, and rub-inoculated onto plants dusted with 500 mesh carborundum. Each day for 10 days, three inoculated barley plants and one control plant were harvested and weighed. Root samples were ground and extracted with phosphate buffer (1:10, w:v) and the extract inoculated onto leaves of four plants of *Chenopodium amaranticolor.* Each test plant received all samples randomized for leaf position, using a Latin square design. Local lesions were recorded after 1 week.

Acquisition of BMV by nematodes from infected barley roots was determined in the following manner: Barley seeds were germinated and plants were grown in 30 cm^3 of autoclaved soil in 17.5 × 2-cm glass test tubes. The test tubes were placed in the greenhouse and maintained at 26 C in a temperature tank. Five treatments were each replicated four times: 1) noninoculated barley, 2) barley rub-inoculated with BMV, 3) soil inoculated with 75 nematodes, 4) noninoculated barley planted in soil inoculated with 75 nematodes, and 5) barley inoculated with BMV and soil inoculated with 75 nematodes. After a 3-week acquisition feeding time, nematodes were extracted from the soil, counted, and assayed for BMV directly using the double sandwich, enzyme-linked immunosorbent assay (ELISA) method (1). Each well contained 10–30 nematodes. Absorbance was read at $A_{405\ nm}$ using a Gilford PR-50 EIA system. The reaction was considered positive if the absorbance of the sample was greater than twice the absorbance of the corresponding healthy control, which in

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[2] Former Graduate Research Assistant, Assistant Professor, and Professor, Department of Plant Pathology, Cook College, Rutgers University, New Brunswick, NJ 08903. Present address of first author: Route 3, Box 323, Pittsboro, NC 27312.
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TABLE 1. Enzyme-linked immunosorbent assay (ELISA)* tests for brome mosaic virus (BMV) on barley plants and *Longidorus breviannulatus* following an acquisition period of 3 weeks.

Treatment group	Barley†	Nematodes‡
Healthy barley	0.004	
BMV barley	> 2.000	
Healthy barley + nematodes	0.005	0.03 (41)§
BMV barley + nematodes	1.600	> 2.00 (45)
Pasteurized soil + nematodes		0.15 (18)

* ELISA reaction ($A_{405\ nm}$) is considered positive if it exceeds twice the value of the noninoculated controls.

† Barley tissue was diluted 1:10⁶ (wt : vol) with buffer. Each value represents the mean of three replicated readings on four plants.

‡ Initial inoculum was 75 nematodes per tube. Each value was a mean of four tubes. Absorbance values obtained were independent of the number of nematodes used per well.

§ Numbers in parentheses are numbers of nematodes recovered.

TABLE 2. Acquisition and transmission of brome mosaic virus (BMV) by *Longidorus breviannulatus* to barley roots using the enzyme-linked immunosorbent (ELISA)* test.

	Acquisition		Transmission	
	Roots†	Nematodes‡	Roots§	Nematodes‖
Noninoculated barley	0.27	0.11	0.28	0.08
BMV-infected barley	1.30	1.90	1.10	> 2.00

* ELISA reaction ($A_{405\ nm}$) is considered positive if it exceeds twice the value of the noninoculated controls.

† Mean of two plants for noninoculated and three BMV-infected plants. Root tissue was diluted 1:100 (wt : vol) with buffer.

‡ Absorbance reading on 8 and 7 nematodes from the noninoculated and BMV-infected treatments, respectively.

§ Root tissue from one plant per treatment diluted 1:10 (wt : vol) in buffer.

‖ Total of 20 nematodes per treatment.

this case consisted of nematodes that had fed on virus-free barley. The tops of the barley plants were ground and tested for the presence of BMV using both the ELISA technique and the formation of local lesions on *Chenopodium* indicator plants.

After determining that *L. breviannulatus* could acquire BMV, a complete acquisition feeding and transmission experiment was undertaken. Because of the limited numbers of nematodes available, only five barley plants were used for virus acquisition. Three plants were mechanically inoculated with BMV while two served as healthy controls. Each plant was infected with 75 nematodes. After 3 weeks, the nematodes were extracted from each plant and the roots of the barley plants were ground in phosphate buffer (1:100, w:v) and tested for BMV by ELISA to confirm the presence of virus. Five to ten nematodes recovered from each plant were also tested for BMV by ELISA to determine if they had acquired virus. The nematodes remaining from the BMV-inoculated barley were combined and placed on a healthy barley plant for transmission feeding. Nematodes remaining from the control barley plants were also pooled and placed on a barley plant. Three weeks after inoculation, the nematodes were extracted and assayed by ELISA, as were the roots of the barley plants, to determine if virus transmission had occurred.

BMV was detected by bioassay in barley roots 3 days after inoculation of leaves. Virus titer increased gradually, as measured by the mean number of local lesions on *C. amaranticolor,* until day 8 when the mean number of lesions was 11 ± 3; this level was maintained through day 10.

Barley plants and nematodes were harvested 3 weeks after the acquisition feeding experiment and assayed for BMV (Table 1). Results from ELISA indicated that BMV was present in leaves of both groups of BMV-inoculated plants but not in leaves of noninoculated plants. This result was confirmed by bioassay on *Chenopodium* plants. Virus was readily detected by ELISA in nematodes that had fed on barley plants inoculated with BMV, but not in nematodes fed on noninoculated plants or in nematodes that had been inoculated into pasteurized soil. These data indicated successful acquisition of virus by nematodes.

The results of the complete virus acquisition and transmission feeding experiment are presented in Table 2. Roots of the barley plants inoculated with BMV contained virus, and nematodes that fed upon those roots acquired virus, as deter-

mined by the ELISA test. Roots and nematodes from the noninoculated barley controls did not contain virus. Nematodes from the noninoculated control and BMV-inoculated barley were placed into soil containing a healthy barley plant for transmission feeding. ELISA absorbance values of roots and nematodes harvested 3 weeks later from these plants indicated that viruliferous nematodes still contained BMV and that BMV was transmitted by nematodes to the healthy barley plant. Again, control plants fed upon by nonviruliferous nematodes were negative for BMV.

ELISA was a practical and useful method for detecting BMV in plant roots and nematodes, producing results in 2 days. The direct assay method of Sanger (4) and procedures for direct observation of the virus in thin sections of the nematodes using the electron microscope (5) are two other methods that have been reported for detection of virus. In this study, virus could not be detected in infected barley plants or nematodes by either of these two methods.

The data presented indicate that *L. breviannulatus* can acquire BMV from mechanically inoculated barley and later transmit the virus to healthy barley. It is interesting to note that BMV was still present within the nematode in high concentrations even though transmission had occurred. Fritzsche (2) reported that BMV persisted in *L. macrosoma* for 21 days, whereas *L. elongatus* retained raspberry ringspot virus for up to 8 weeks (6). Some *L. breviannulatus* survived in pasteurized soil without feeding for 3 weeks.

Since the life cycle of *L. breviannulatus* is longer than the length of these experiments, nematode numbers diminished; this limited the number of nematodes available for the final transmission experiment. Although *L. breviannulatus* acquired and transmitted BMV under restricted experimental conditions, a more extensive confirmatory test is necessary. More important is whether the nematode can transmit this virus to barley and corn in the field, and whether such transmission could result in significant yield losses.

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