Local Epidemic of NL-8 Strain of Bean Common Mosaic Virus in Bean Fields of Western New York

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ABSTRACT

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A viral disease that severely affected bean fields of the cultivar Sanilac in two areas of western New York in 1982 was caused by the NL-8 strain of bean common mosaic virus (BCMV). This strain was identified using differential bean cultivars and enzyme-linked immunosorbent assay with antisera to specific strains of BCMV. It was demonstrated experimentally that NL-8 is seed-transmitted in Sanilac, but attempts to detect its presence in the seed lot used for field plantings were unsuccessful. Because NL-8 is one of the temperature-independent, necrosis-inducing strains of BCMV and is able to overcome resistance in some cultivars with the *I* gene, the necessity to reassess the present approach to breeding for resistance is discussed.

In 1982, six bean fields of the cultivar Sanilac were severely affected by a viral disease that significantly reduced the yield and quality of the seed crop. These fields were located in two areas of western New York but had been planted with seed of the same lot produced in Michigan. Most of the plants were stunted and showed foliar chlorosis, veinal browning, and other symptoms that are usually associated with an infection of bean common mosaic virus (BCMV) (1). In preliminary greenhouse tests, 50 virus isolates recovered from randomly collected specimens incited typical BCMV symptoms in plants of Black Turtle 2 and a lethal necrotic reaction in plants of Black Turtle 1, a bean line usually resistant to local strains of the virus (9). Because the greenhouse temperature had been maintained at 25–27 C, these results suggested that the causal agent was one of the temperature-independent, necrosis-inducing strains of BCMV (3). Electron microscopy of negatively stained leaf dips revealed virus particles similar in size and shape to those of BCMV; however, immunodiffusion tests in sodium dodecyl sulfate (SDS) agar gel with an antiserum to the type strain of BCMV (11) were negative.

Considering the severity of the disease and the hectarage involved (about 50 ha), this study was undertaken to confirm the causal agent as BCMV and identify its strain, establish the primary source of the virus, and determine the reactions of a number of cultivars of *Phaseolus vulgaris* L., particularly those possessing factors for BCMV resistance.

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MATERIALS AND METHODS

Virus isolate NY82-20, a typical field isolate from Sanilac, was used throughout this study. An isolate of the original NL-8 strain of BCMV was obtained from Drijfhout (3). Both virus isolates were maintained and propagated in plants of the BCMV-susceptible line Black Turtle 2. Inoculum was prepared by macerating leaves showing prominent symptoms in 0.01 M phosphate buffer (K^+), pH 7.2. Test plants were mechanically inoculated by rubbing leaves previously dusted with 400-mesh Carborundum. The host range included 80 bean cultivars, 20 of which represented the international standard set of BCMV differentials (5), as well as other leguminous and nonleguminous species. All plants, regardless of their reactions after inoculation, were assayed for local and systemic infection using back-transfers to plants of Black Turtle 1 and Black Turtle 2 or Sutter Pink.

Enzyme-linked immunosorbent assay (ELISA) was also used for viral strain determination and assays. Antisera to BCMV strains US-1, US-2, CR, NL-3, and NL-4 had been prepared for a previous study (13). Direct ELISA was conducted by the methods of Clark and Adams (2) and Voller et al (12). For indirect ELISA, the method of Lommel et al (8) was followed. Additional serological tests were conducted in SDS agar gel (0.85% Ionagar, 1% NaN_3, and 0.5% SDS), using antisera to the following viruses: bean yellow mosaic (BYMV), blackeye cowpea mosaic (BlCMV), clover yellow vein (CYVV), cowpea aphidborne mosaic (CAbMV), and peanut mottle (PMV).

To determine the original source of the virus, seed of Sanilac were secured from the lot used for field plantings. To demonstrate seed transmission of isolate NY82-20 in Sanilac and Black Turtle 2, plants of these lines were mechanically inoculated in the primary leaf stage and allowed to set seed. All plants were maintained in an insect-free greenhouse at 25–27 C, and extreme care was taken to avoid any contamination with other strains of BCMV or any other virus.

RESULTS

Reactions of differential bean cultivars. Isolate NY82-20 caused systemic mosaic in plants of Dubelle Witte, Stringless Green Refugee, Sanilac, Michelite 62, and Red Mexican 34. It also incited local and systemic necrosis followed by death in plants of Widusa and Black Turtle 1. Plants of Redlands Greenleaf B, Redlands Greenleaf C, Puregold Wax, Imuna, Great Northern 31, Great Northern 123, Monroe, Pinto 114, Red Mexican 35, Jubilia, Topcrop, and Amanda reacted with local chlorotic or necrotic spots, but assays indicated that the virus did not invade the plants systemically. These cultivars were considered resistant according to the terminology of Drijfhout et al (5). When these results were compared with those reported by Drijfhout and Bos (4), it was evident that isolate NY82-20 behaved identically to strain NL-8. Additional tests with the same differential bean cultivars demonstrated that all 49 remaining isolates recovered from infected Sanilac plants were identical to NL-8.

Serology. Results of direct ELISA (Table 1) indicated a close serological relationship between isolate NY82-20 and strain NL-3. No reaction was obtained with antisera to US-1, US-2, CR, and NL-4, thus confirming previous work (13) that NL-8 is a member of a unique serogroup of BCMV that also includes NL-3 and NL-5. With indirect

ELISA, however, isolate NY82-20 reacted with US-2 antiserum but NL-3 did not. This distinction can be exploited to serologically differentiate NL-8 from NL-3. In immunodiffusion tests, antigens from bean plants infected with NY82-20 or NL-8 reacted with an antiserum to BYMV, forming precipitin lines of equal intensity that fused with each other. No reaction was visible with antisera to BlCMV, CYVV, CAbMV, and PMV. A serological relationship between NL-8 and BYMV was reported also by Drijfhout and Bos (4).

Reactions of bean cultivars. All cultivars inoculated with isolate NY82-20 or NL-8 were classified in three categories:

Resistant cultivars. Astro, Avalanche, Barbuni, Bush Blue Lake 47, Bush Blue Lake 109, Cacahuate, California Light Red Kidney, Canario 107, Checkmate, Cherokee Wax, Corbett Refugee, Early Gallatin, Earliwax, Eastern Butterwax, Gaelic, Goldrush Wax, Gold Crop Wax, Great Northern 1140, Idagreen, Lake Shasta, Moon Gold Wax, Pencil Pod Wax, Pencil Pod Black Wax, Provider, Redkote, Redcloud, Roma, Seneca BL6, Slimgreen, Spartan Arrow, Sprite, Sunrise Wax, Tenderette, Topnotch Golden Wax, White Kidney, and experimental lines G-1009, G-1575, and G-1913 were classified as resistant. In plants of this group, viral infection was confined to the inoculated leaves, which responded with chlorotic or necrotic lesions or with some veinal chlorosis. No systemic symptoms were visible and assays confirmed that infection was not systemic.

Fig. 1. Reddish brown spots induced by the NL-8 strain of bean common mosaic virus on pods of bean cultivar Sanilac grown in the greenhouse.

Susceptible cultivars. Antigua, Arriaga, Black Turtle 2, Jamapa, Negro Patzicia, Pinto 111, Pioneer, Rabia de Gato, San Martin, and Sutter Pink were classified as susceptible. Plants of these cultivars developed local and systemic symptoms usually associated with BCMV infection. Infected leaves showed light and dark green mosaic, puckering, malformation, downward cupping along the main vein, and green veinbanding. Growth was retarded and pods were small and green mottled; however, unusual reddish brown streaks developed in most of the Sanilac pods (Fig. 1). Under field conditions, these symptoms could easily be attributed to a fungal infection.

Systemic hypersensitive cultivars. Aurora, Black Turtle 1, Bush Blue Lake 94, Jutiapan, Kentucky Wonder Wax, Midnight, Pico, Quetzal, Sataya, Suchitan, Tamazupala, and line G-1822 were classified as systemic hypersensitive. Plants of these cultivars developed distinct necrotic local lesions and local vein browning. Systemic symptoms consisted of apical and stem necrosis, which caused premature death of plants. This condition has been attributed to the ability of the virus to overcome resistance (3). According to Drijfhout (3), cultivars belonging to host resistance group 8 possess the *I* gene without *bc-u* (the strain-unspecific gene) or any of the other strain-specific genes (*bc-1*, *bc-1*^{2}, *bc-2*, *bc-2*^{2}, and *bc-3*).

Reactions of other plant species. The following species and cultivars were neither locally nor systemically infected by isolate NY82-20 and NL-8: *Chenopodium amaranticolor, C. quinoa, Cucumis sativus* 'Marketer,' *C. melo* 'Iroquois,' *Cucurbita pepo* 'Seneca Zucchini,' *C. maxima* 'Emerald,' *C. moschata* 'Butternut,' *Citrullus lanatus* 'Charleston Gray,' *Nicotiana tabacum* 'Havana 423,' *N. rustica, N. glutinosa,* and *Pisum sativum* 'Bonneville,' 'Early Perfection 3040,' 'Perfected Freezer 60,' 'Venus,' 'Wando,' 'Sirod,' and several other cultivars known to be resistant also to BYMV (10). The following were systemically infected: *N. benthamiana, Vicia faba* 'Improved Long Pod,' *Vigna unguiculata* subsp. *unguiculata* 'California Blackeye,' *V. unguiculata* subsp. *cylindrica,* and *P. sativum* 'Alderman,' 'Alaska,' 'Giant Stride,' 'Freezonian,' 'Lincoln,' 'Early Sweet 11,' 'Tall Telephone,' 'World's Record,' and several other cultivars known to be susceptible also to BYMV (10). These pea cultivars were uniformly susceptible, responding with very mild chlorotic mottle or without symptoms. Only an occasional plant of Improved Long Pod fava bean was susceptible and developed moderate mosaic. Viruses recovered from susceptible hosts incited BCMV-like symptoms on Black Turtle 2 and necrotic local lesions and systemic necrosis on Black Turtle 1. NL-8 appears to be unique among strains of BCMV because it is able to infect certain cultivars of *P. sativum* and *V. faba.*

Seed transmission. Of 1,825 Sanilac seeds of the same lot used for field plantings, 1,198 (66%) developed viable plants but none showed symptoms resembling those of BCMV infection. Assays for viral infection made when plants had reached the four- to five-leaf stage were all negative. Transmission of isolate NY82-20 occurred in seed derived from plants inoculated under greenhouse conditions and ranged from 35% (68/192) in Sanilac to 43% (116/270) in Black Turtle 2. All virus isolates recovered from plants infected via seed were identical to NY82-20.

DISCUSSION

Until a few decades ago, BCMV was one of the most prevalent agents affecting the bean crop of New York; however, widespread use of certified seed and the advent of resistant cultivars have almost eliminated this virus as a threat to commercial production. Occasionally, some seed lots contain unusual strains of this virus that may cause unexpected losses.

The virus infecting Sanilac in fields in western New York was identified as the NL-8 strain of BCMV that was first detected in The Netherlands a few years ago (4). NL-8, NL-3, and NL-5 have been categorized as temperature-independent, necrosis-inducing strains, because they induce a lethal systemic reaction in cultivars carrying the *I* gene (3), regardless of the ambient temperature. In particular, NL-8 is able to infect bean plants in which resistance is conditioned by the *I* gene alone, whereas NL-3 infects plants with *I* and *bc-1*, and NL-5, those with *I* and *bc-1*^{2} (3).

Of 80 cultivars tested in this study, 64% were classified as resistant and 36% responded to inoculation with either a foliar mosaic or lethal necrosis. The latter

Table 1. Absorbance values ($A_{405\ nm}$) obtained in direct double-antibody sandwich enzyme-linked immunosorbent assay with antisera to five strains of bean common mosaic virus

	Antiserum against				
Antigen	**US-1**	**US-2**	**CR**	**NL-3**	**NL-4**
NY82-20	0.05	0.0	0.0	2.74	0.12
Healthy sap	0.10	0.04	0.0	0.35	0.20
Homologous strain	0.93	1.80	1.21	2.74	2.74

group included several cultivars known to be resistant to BCMV strains prevalent in the United States. Furthermore, cultivars bearing the same name but different accession numbers differed in resistance. Bush Blue Lake 47 and Bush Blue Lake 109 were resistant, but Bush Blue Lake 94 showed systemic necrosis. A similar situation was reported by Drijfhout with the Great Northern and Redlands Greenleaf lines (3).

It was demonstrated experimentally that NY82-20 is seedborne in Sanilac and Black Turtle 2; however, attempts to detect the virus in the Sanilac seed lot used for field plantings were not successful. This failure can be attributed either to the low germination of the remaining seed or to an exceedingly low rate of virus transmission. It is also possible that the virus originated from other bean fields.

The presence of foreign strains of BCMV in New York (NL-8), in the Northwest (NL-4 and NL-8) (6), and in Michigan (NL-3) (7) demonstrates that they are a constant threat to the bean industry of the United States. Consequently, it seems advisable to reassess our approach to breeding for resistance, which is presently confined to the domestic strains (US-1, NY-15, and Western). Drijfhout (3) demonstrated that all known strains can be controlled successfully by the genes present in line IVT 7233 (*I, bc-u, bc-1*^{2}, and *bc-2*^{2}). This combination and others can be obtained easily by manipulating the available genes (3). Meanwhile, steps must be taken to produce commercial seed lots absolutely free of any strain of BCMV, because infected seed represents the main avenue of infection. Seed certification should be based on zero tolerance, and foreign cultivars preferably should be subjected to a strict quarantine.

Wang et al (13) demonstrated that NL-8, NL-5, and NL-3 constitute a unique serogroup among BCMV strains. Drijfhout and Bos (4) reported that NL-8 was serologically related to BYMV. We have confirmed this relationship and established that pea cultivars resistant or susceptible to BYMV are also resistant or susceptible to NY82-20 and NL-8. Further characterization of NL-8, as well as NL-3 and NL-5, is in progress.

LITERATURE CITED

1. Bos, L. 1971. Bean common mosaic virus. Description of Plant Viruses. No. 73. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England.
2. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
3. Drijfhout, E. 1978. Genetic interaction between *Phaseolus vulgaris* and bean common mosaic virus with implications for strain identification and breeding for resistance. Agric. Res. Rep. 872, ISBN 90 220 0671 9 (vii), Wageningen, Netherlands. 98 pp.
4. Drijfhout, E., and Bos, L. 1977. The identification of two new strains of bean common mosaic virus. Neth. J. Plant Pathol. 83:13-25.
5. Drijfhout, E., Silbernagel, M. J., and Burke, D. W. 1978. Differentiation of strains of bean common mosaic virus. Neth. J. Plant Pathol. 84:13-26.
6. Hampton, R. O., Silbernagel, M. J., and Burke, D. W. 1983. Bean common mosaic virus strains associated with bean mosaic epidemics in the northwestern United States. Plant Dis. 67:658-661.
7. Kelly, J. D., Saettler, A. W., and Morales, M. 1984. New necrotic strain of bean common mosaic virus in Michigan. Bean Improv. Coop. Annu. Rep. 27:38-39.
8. Lommel, S. A., McCain, A. H., and Morris, T. J. 1982. Evaluation of indirect enzyme-linked immunosorbent assay for detection of plant viruses. Phytopathology 72:1018-1022.
9. Provvidenti, R. 1983. Two useful selections of the bean cultivar Black Turtle Soup for viral identification. Bean Improv. Coop. Annu. Rep. 26:71-72.
10. Schroeder, W. T., and Provvidenti, R. 1964. Evaluating *Pisum sativum* for resistance to pea mosaic. N.Y. State Agric. Exp. Stn. Bull. 806. 10 pp.
11. Uyemoto, J. K., Provvidenti, R., and Schroeder, W. T. 1972. Serological relationship and detection of bean common and bean yellow mosaic viruses in agar gel. Ann. Appl. Biol. 71:235-242.
12. Voller, A., Bartlett, A., Bidwell, D. E., Clark, M. F., and Adams, A. W. 1976. The detection of viruses by enzyme-linked immunosorbent assay (ELISA). J. Gen. Virol. 33:165-167.
13. Wang, W.-Y., Mink, G. I., and Silbernagel, M. J. 1982. Comparison of direct and indirect enzyme-linked immunosorbent assay (ELISA) in the detection of bean common mosaic virus. (Abstr.) Phytopathology 72:954.