

Host Range, Cytology, and Transmission of an Alyce-clover Isolate of Blackeye Cowpea Mosaic Virus

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ABSTRACT

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A blackeye cowpea mosaic virus (BICMV) isolated from alyce-clover, designated as BICMV-AC, was partially characterized. Cylindrical inclusions containing scrolls induced by BICMV-AC were detected in infected plants by light, electron, and immunofluorescence microscopy. Isolate BICMV-AC infected *Alysicarpus vaginalis*, *Crotalaria spectabilis*, *Indigofera hirsuta*, *Lupinus albus*, *Macroptilium lathyroides*, *Nicotiana benthamiana*, *Phaseolus vulgaris* 'Black Turtle 2,' *Vigna unguiculata* subsp. *unguiculata* 'California Blackeye,' *Chenopodium amaranticolor*, and *C. quinoa*. No seed transmission of BICMV-AC was detected in alyce-clover, cowpea, or hairy indigo. Isolate BICMV-AC was transmitted in a nonpersistent manner by *Myzus persicae*. Infection with BICMV-AC, peanut stripe virus, or both significantly reduced plant height, dry weight of tops, and seed yield of both alyce-clover and hairy indigo but had no effect on nitrogen concentration of tops or roots. Genotypes of alyce-clover and hairy indigo reacted differently to infection by the two viruses, but all were altered in some growth parameters by viral infections.

Alyce-clover (*Alysicarpus vaginalis* (L.) DC.) and hairy indigo (*Indigofera hirsuta* L.) are summer annual legumes used extensively in Florida for soil improvement (2). Both can be grazed or harvested for hay or silage (10). When properly managed, hairy indigo is a desirable livestock feed because of its high protein content and digestibility (10).

At least 34 viruses have been reported to infect forage legumes in the United States (1,7,13). Six of these viruses are widely distributed in the Southeast and often cause severe damage (3,12). Recently, viruses associated with alyce-clover and hairy indigo have been considered to pose potentially serious problems (13,19).

Peanut stripe virus (PStV) was first observed in 1982 in Georgia in peanut (*Arachis hypogaea* L.) plants raised from seeds imported from the People's Republic of China (6). PStV is seed-transmitted in peanut and is serologically related to blackeye cowpea mosaic virus (BICMV) (6). Recently, PStV was reported to infect mechanically inoculated plants of alyce-clover and hairy indigo (13, this paper). The effects of the virus on these crops have not been reported.

A virus isolated from alyce-clover plants caused severe mosaic, stunting,

and leaf distortion of both alyce-clover and hairy indigo (19). The virus was identified as a potyvirus based on the presence of cylindrical inclusions (CIs) in infected plants and its serological relationship to BICMV (19). The alyce-clover isolate has been identified as a strain of BICMV, designated as BICMV-AC. In this paper we report some biological properties of BICMV-AC and the effects of this virus and of PStV on the growth of alyce-clover and hairy indigo.

MATERIALS AND METHODS

Virus sources. BICMV-AC used in this study was isolated from naturally infected alyce-clover plants collected in a research field at Gainesville, FL (19). The virus was maintained in white lupine (*Lupinus albus* L.). PStV used in this study was isolated from a peanut seedling grown from seed produced in an experimental planting in Florida (D. Purcifull and E. Hiebert, unpublished) and was maintained in peanut cultivar Virginia Jumbo.

Host range of BICMV-AC. All test plants (five to 20 plants per test) were mechanically inoculated with sap from cowpea or white lupine plants infected with BICMV-AC, and virus infections were confirmed by sodium dodecyl sulfate (SDS) immunodiffusion tests (17). These experiments were repeated at least once for each test plant studied.

Light, electron, and immunofluorescence microscopy of CIs induced by BICMV-AC. Epidermal strips from plants of alyce-clover, cowpea, hairy indigo, *Nicotiana benthamiana*, or white lupine infected with BICMV-AC were

stained with calamine orange and Luxol brilliant green (4) and examined by light microscopy. Partially purified preparations of CIs (20) were negatively stained with 1% uranyl acetate (UA) (5) and examined with a Hitachi H-600 electron microscope.

Immunofluorescence microscopy was performed as described by Hiebert and co-workers (9). Epidermal strips from *N. benthamiana* infected with BICMV-AC were incubated first with antiserum against the CI protein of BICMV-AC (19) and then with protein A-conjugated rhodamine. The CIs were revealed by immunofluorescence microscopy.

Determination of BICMV-AC particle size. Crude leaf extract from infected cowpea plants was negatively stained with 1% UA (5). Viral particles were examined and photographed, and the projected image of a diffraction grating (2,160 lines per millimeter) was used to measure particle images in projected micrographs.

Seed and aphid transmission tests. Seeds collected from alyce-clover, hairy indigo, and cowpea that had been mechanically inoculated with BICMV-AC were germinated, and the seedlings were maintained in a greenhouse and tested for virus infection by SDS immunodiffusion (17) and light microscopy (4). Tests for aphid transmission of BICMV-AC were performed as described by Noordam (16) for viruses transmitted in a nonpersistent manner.

Effects of BICMV-AC and PStV on alyce-clover and hairy indigo. Four genotypes of alyce-clover (AC-1, -3, -4, and -100) and three of hairy indigo (HI-24, -44, and -101) were used. Seedlings were transplanted into super-cell Cone-Tainers (Ray Leach Cone-Tainer Nursery, Canby, OR) in a greenhouse. Each Cone-Tainer held 150 cm³ of methyl bromide-fumigated arredo fine sand topsoil containing cowpea-type *Rhizobium* inoculant. Temperatures in the greenhouse were maintained at 31 C during the day and 24 C during the night. Light intensity and day length were similar to natural conditions because the greenhouse was not shielded.

The experiment was conducted as a 2×7 factorial; a split-plot design was used, with four combinations of virus infection as the main plots, seven genotypes of alyce-clover and hairy indigo as subplots, and 20 replicates.

Twenty plants from each genotype were mechanically inoculated 30 days after transplantation with BICMV-AC alone, PSTv alone, both BICMV-AC and PSTv, or buffered extracts from virus-free plants. Plant heights from the soil surface were measured at the time of inoculation.

Sixty days after inoculation, 10 plants from each treatment were randomly selected, and plant height, plant width, and number of branches were recorded. Roots from these plants were removed from the cones and gently washed. The tops or roots of these plants were dried

at 50 C for 72 hr to determine dry weights. The nitrogen concentration of tops and roots was analyzed separately in the Evaluation Support Laboratory of the University of Florida as described by Moore and Mott (14).

The remaining 10 plants in each treatment were grown to seed maturity, except for AC-4 plants, which did not produce mature seed during the study. Seeds were hand-harvested as they matured.

Virus infections were confirmed by SDS immunodiffusion tests (17), light microscopy (4), and visual observation of the symptoms of inoculated plants.

RESULTS

Host range and symptomatology. Alyce-clover and hairy indigo plants mechanically inoculated with BICMV-AC showed mosaic and distortion in systemically infected leaves 6–10 days after inoculation. Later, stunting developed.

Numerous chlorotic ringspots developed on inoculated primary leaves of *Vigna unguiculata* subsp. *unguiculata* 'California Blackeye.' Necrosis started from the center of chlorotic areas, and then reddish, necrotic ringspots were formed. In systemically infected leaves of cowpea, vein-clearing was followed by mosaic symptoms. Mild mottle symptoms were also observed in white lupine and *N. benthamiana*.

Isolate BICMV-AC caused chlorotic lesions in *Chenopodium amaranticolor* and *C. quinoa*. Yellow stripe and mosaic symptoms were observed in young leaves of *Crotalaria spectabilis*. Epinasty and yellowing were observed in *Phaseolus vulgaris* 'Black Turtle 2,' and mosaic and leaf distortion were observed in *Macropodium lathyroides*.

Based on the absence of symptoms and negative serological results, we concluded that BICMV-AC did not infect *Arachis glabrata* 'Arblik' and 'Florigraze,' *Arachis hypogaea* 'Florunner' and 'Virginia Jumbo,' *Cucurbita pepo* 'Small Sugar,' *Glycine max* 'Bragg,' 'Braxton,' 'Centennial,' 'Cobb,' 'Foster,' and 'Kirby,' *L. angustifolius* 'Bitter Blue,' *Lycopersicon esculentum*, *Medicago sativa* 'FL-77,' *N. tabacum*, *P. vulgaris* 'Black Turtle 1' and 'Topcrop,' *Pisum sativum* 'Alaska' and 'Little Marvel,' *Stylosanthes guianensis*, *S. scabra*, *Trifolium incarnatum*, *T. pratense*, *T. repens*, *T. subterraneum*, *T. vesiculosum*, *Vicia faba*, or *V. u.* subsp. *unguiculata* 'Knuckle Purple Hull.'

Light, electron, and immunofluorescence microscopy of CIs induced by BICMV-AC. Cytoplasmic CIs containing scrolls (Fig. 1) were present in epidermal leaf strips from BICMV-AC-infected alyce-clover, cowpea, hairy indigo, *N. benthamiana*, and white lupine but not in epidermal strips from virus-free control plants. The CIs were

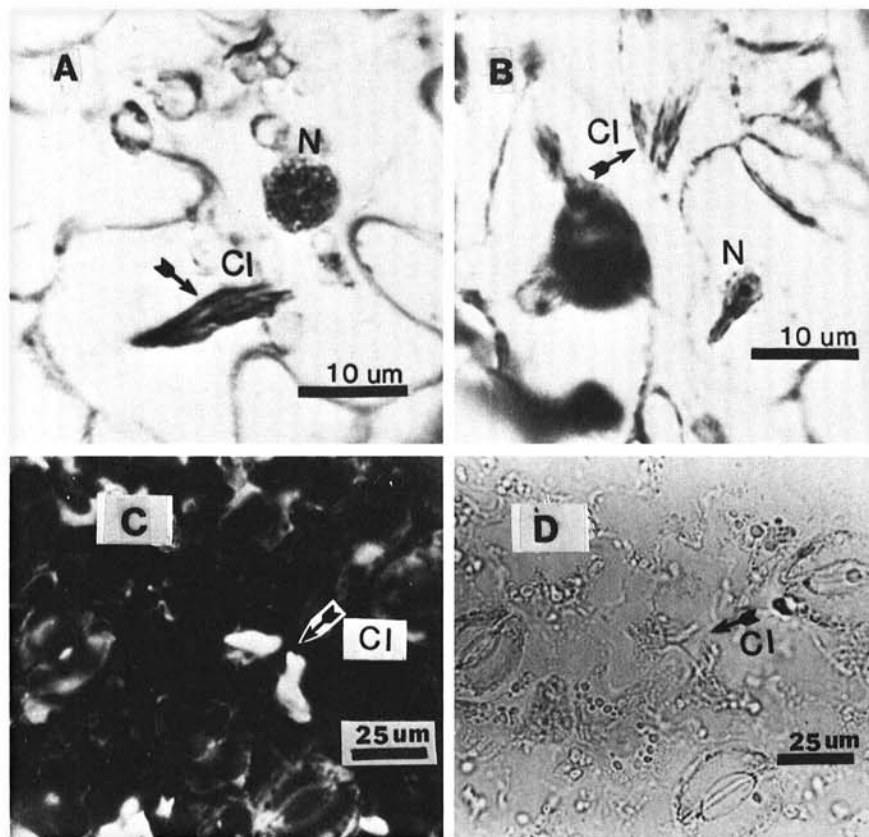


Fig. 1. Light and immunofluorescence microscopy of cylindrical inclusions (CIs) (arrows) induced by an alyce-clover strain of blackeye cowpea mosaic virus (BICMV-AC). **A and B,** CIs in epidermal strips of BICMV-AC-infected hairy indigo (**A**) and *Nicotiana benthamiana* (**B**) plants stained with calomine orange and Luxol brilliant green and viewed by light microscopy. **N** = nucleus. **C and D,** CIs in epidermal strips of infected *N. benthamiana* plants incubated with antiserum to BICMV-AC CI protein and labeled with protein A-conjugated rhodamine, as revealed by immunofluorescence microscopy ($\times 400$) (**C**) and viewed by light microscopy without fluorescence (**D**).

Table 1. Dry weight of tops and seed yield of four genotypes of alyce-clover and three genotypes of hairy indigo mechanically inoculated with an alyce-clover strain of blackeye cowpea mosaic virus (BICMV-AC), peanut stripe virus (PStV), both viruses (BICMV-AC + PStV), or buffered extracts from virus-free plants (control)^y

Treatment	Alyce-clover genotype				Hairy indigo genotype		
	AC-1	AC-3	AC-4 ^z	AC-100	HI-24	HI-44	HI-101
Dry weight of tops (g)							
Control	8.12 aB	10.01 aAB	8.06 aB	10.31 aA	12.32 aA	10.48 aB	8.38 aC
BICMV-AC	7.66 aA	2.85 bB	7.71 aA	5.87 bA	7.71 bA	7.29 bA	4.29 bB
BICMV-AC + PStV	4.99 bA	3.41 bA	4.68 bA	3.91 bcA	4.46 cA	3.45 cAB	2.14 cB
PStV	3.98 bA	4.48 bA	1.60 cB	2.98 cA	4.30 cA	3.40 cAB	2.78 cB
Seed yield (g)							
Control	0.23 aB	0.99 aA	0.00 aC	0.80 aA	0.20 aC	1.86 aA	0.73 aB
BICMV-AC	0.00 bB	0.40 bA	0.02 aB	0.00 bB	0.00 bA	0.00 bA	0.04 bA
BICMV-AC + PStV	0.00 bA	0.16 cA	0.00 aA	0.00 bA	0.00 bA	0.00 bA	0.00 bA
PStV	0.00 bA	0.00 cA	0.00 aA	0.00 bA	0.00 bA	0.00 bA	0.00 bA

^y Means for alyce-clover and hairy indigo followed by the same letter (lowercase for columns and uppercase for rows) do not differ significantly at $P = 0.05$ according to Duncan's multiple range test.

^z AC-4 was not mature during the time of harvesting.

also revealed by electron and immunofluorescence microscopy in partially purified preparations and infected tissues, respectively.

Particle size of BICMV-AC. We examined 120 rod-shaped particles of BICMV-AC from crude leaf extract, and 70% of the particles ranged from 656 to 960 nm in length. The modal length was 733 nm.

Seed and aphid transmission. We found no evidence of seed transmission of BICMV-AC in alyce-clover (200 seedlings), hairy indigo (200 seedlings), or cowpea (350 seedlings): no symptoms were expressed in seedlings grown from seeds harvested from infected plants, and light microscopy and immunodiffusion assays of about 45 plants from the 350 cowpea seedlings were negative.

Myzus persicae (Sulzer) transmitted isolate BICMV-AC in a nonpersistent manner to healthy cowpea test plants. Six of 10 plants infested with viruliferous aphids (five aphids per plant) developed mosaic and vein-clearing symptoms 10 days after inoculation.

Effects of BICMV-AC and PStV on alyce-clover and hairy indigo. Virus infection significantly reduced dry weights of tops and seed yields of alyce-clover and hairy indigo (Table 1) and also reduced plant heights and dry weights of roots (*data not shown*). The viruses had no effect on the nitrogen concentrations of the two genera (*data not shown*). PStV was usually the more severe of the two viruses; it reduced dry weights of tops significantly more than BICMV-AC in three of the four alyce-clover genotypes and with all three hairy indigo genotypes (Table 1).

Because there was a significant interaction between virus infection and genotype, the response of each genotype to virus infection was analyzed separately. For example, BICMV-AC-infected AC-3 produced significantly more seeds than other AC genotypes (Table 1).

DISCUSSION

Four potyviruses—clover yellow vein virus (CYVV), peanut mottle virus (PMoV), PStV, and watermelon mosaic virus-2 (WMV-2)—are reported to infect alyce-clover (1,13). None of these potyviruses induce the formation of scrolls alone (4,7). BICMV-AC induces CIs containing only scrolls. The host range of isolate BICMV-AC also differed from

the published host ranges of CYVV, PMoV, PStV, and WMV-2 (7).

Originally isolated from field-grown cowpea in Florida (BICMV-FL), BICMV induces CIs containing scrolls in infected plants (8,11); thus, BICMV-AC was similar to BICMV-FL in this respect. Isolate BICMV-FL infects mechanically inoculated plants of alyce-clover (G. S. Zhao, *unpublished*), and BICMV-AC and BICMV-FL also have similar host ranges (11,15). Both isolates cause mosaic symptoms on trifoliolate leaves of California Blackeye cowpea (11), whereas BICMV-AC also caused necrotic ringspots on inoculated primary leaves. The South Carolina isolate of BICMV (BICMV-NR) also causes necrotic ringspots on inoculated primary leaves of cowpea, but BICMV-AC and BICMV-NR differ in host range (15). Isolate BICMV-NR infects *Arachis hypogaea* 'Florunner,' *G. max* 'Bragg,' *Vicia faba*, and *V. u. subsp. unguiculata* 'Knuckle Purple Hull' (15), which were not infected by BICMV-AC in this study. Furthermore, BICMV-FL is seed-transmitted in cowpea (18), but seed transmission of BICMV-AC in alyce-clover, cowpea, or hairy indigo was not detected.

Infection with BICMV-AC, PStV, or both resulted in severe damage to alyce-clover and hairy indigo under greenhouse conditions, as measured by large reductions in plant height, dry weight, and seed yield.

None of the genotypes we studied was resistant to the two viruses, but two (AC-1 and HI-24) appeared to be more tolerant than the others. Plant improvement through selection for increased tolerance may be possible if the variation in symptom expression measured in this study is genetic in nature.

Because the effects of double infection seemed to be less severe than those of PStV alone and more severe than those of BICMV-AC alone, no additive or synergistic effect appeared to be involved.

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