Isolation and Some Properties of a Yellow Subgroup Member of Cowpea Mosaic Virus from Illinois

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ABSTRACT

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A twelve-spotted cucumber beetle collected in a soybean field in Edwards County, Illinois, transmitted cowpea mosaic virus (CPMV) to test plants. Infectious CPMV also was recovered when the beetle was homogenized and the homogenate inoculated to test plants. The CPMV isolate from Illinois (CPMV-IL) was identified as a member of the yellow subgroup of CPMV based on host range and serology. Isolate CPMV-IL produced less top component than did another member of the yellow subgroup, CPMV-Sb. Both isolates possessed two electrophoretic forms of essentially equal mobilities; CPMV-IL consistently had a higher proportion of fast to slow form than did CPMV-Sb. This is the first report of natural occurrence of a member of the yellow subgroup of CPMV in the United States.

The beetle-transmitted viruses that cause cowpea mosaic were divided by Agrawal (1) into two subgroups. The yellow subgroup is typified by an isolate first described from Nigeria (3) and includes an isolate reported from Surinam (1), whereas the severe subgroup includes viruses isolated in Trinidad (4, 5) and Arkansas (14). This grouping has proved to be useful and now is generally accepted (17).

Among the leaf-feeding beetles we collected from soybean fields in Illinois in July 1975, was a twelve-spotted cucumber beetle, *Diabrotica undecimpunctata howardi* Barber, that transmitted a virus to cowpeas resulting in symptoms similar to those caused by a yellow subgroup isolate, CPMV-Sb (1). Similar symptoms were obtained when the beetle was homogenized and inoculated to test plants. In this report we describe results of our experiments to determine the relationship between this beetle-transmitted isolate (designated CPMV-IL) and CPMV-Sb. To our knowledge, CPMV-IL is the first member of the yellow subgroup to be reported in the USA. Smith reported a beetle-transmitted cowpea mosaic in the USA in 1924 (15), but it is not known which virus subgroup was involved.

MATERIALS AND METHODS

Virus isolates.—Leaf-feeding beetles collected from soybean fields in southern Illinois were caged individually for one to several days on each of a series of several species of test plants in the greenhouse. The virus isolate thus obtained (CPMV-IL) was given five serial single-lesion passages in *Phaseolus vulgaris* L. 'Scotia'. Virus isolates were maintained in cowpeas, *Vigna unguiculata* (L.) Walp. 'Early Ramshorn'. CPMV-Sb and CPMV-Sb antiserum and CPMV-Ark and CPMV-Ark antiserum were obtained from H. A. Scott and J. P. Fulton, respectively, of the University of Arkansas.

Host range.—Plants were mechanically inoculated 8-10 days after seeding with inoculum prepared from infected leaf tissue homogenized in 0.01 potassium phosphate buffer, pH 7.0. Plants were maintained in the greenhouse and symptoms were recorded 10-15 and 24 days after inoculation.

Purification and sedimentation studies.—Isolates CPMV-IL and CPMV-Sb were purified concurrently from greenhouse-grown Early Ramshorn cowpeas by a slightly modified published procedure (16). After two cycles of differential centrifugation, partially purified virus was subjected to sucrose density gradient centrifugation (4 hr, Beckman SW 27.1 rotor, 25,000 rpm). Gradient fractions were collected with an ISCO Model D density gradient fractionator and those containing virus were pooled and concentrated by ultracentrifugation. Nucleoprotein concentrations were determined spectrophotometrically using an extinction coefficient $E^{0.01\%}_{260nm}$ of 8.1 (16).

Component proportions of virus isolates were determined in a Beckman Model E analytical ultracentrifuge. Equal amounts of virus isolates were adjusted to 0.01 M potassium phosphate, pH 7.0, and centrifuged in an An-D rotor at 35,600 rpm at 22 C. Sedimentation coefficients were determined according to Markham (11).

Electrophoretic analysis.—Electrophoresis of intact virus was performed at pH 7.8 in 2.5% polyacrylamide gels with 5% cross-linking prepared in 6 mm (i.d.) × 120-mm Plexiglas tubes (10). Prerun gels (4 mA per gel for 1 hr) were loaded with 20-40 μg of purified virus, run at 6 mA per gel for 5-6 hr at room temperature, and scanned at 260 nm using a GCA/McPherson Model 700 spectrophotometer.

Electrophoresis of viral proteins was in 8% polyacrylamide gels with 5% cross-linking in 0.1 M sodium phosphate, pH 7.8, containing 0.1% sodium dodecyl sulfate (SDS). Protein samples (15-20 μg) were layered onto 8-cm gels in 6 mm (i.d.) × 100 mm glass tubes and electrophoresed at 6 mA per gel for 16.5 hr. Protein bands were stained with Coomassie brilliant blue, destained in methanol:water:glacial acetic acid (5:5:1, v/v), and their relative electrophoretic mobilities were determined by the method of Weber and Osborn (18).

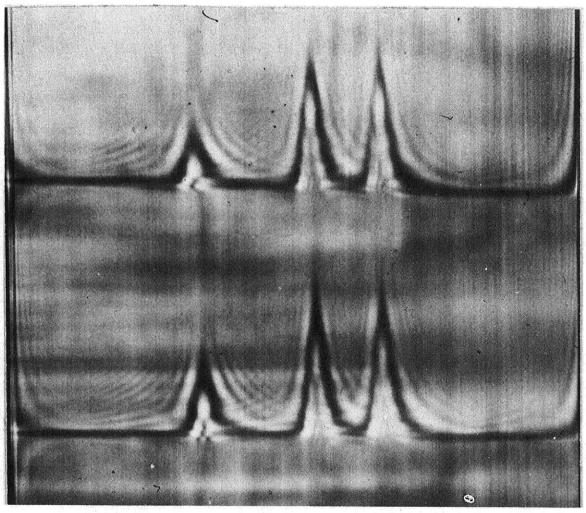

Fig. 1. Schlieren patterns of cowpea mosaic virus (CPMV) isolates, CPMV-IL (upper) and CPMV-Sb (lower) obtained in a Beckman Model E analytical ultracentrifuge. Virus was suspended in 0.01 M KPO_4, pH 7.0 and run at 35,600 rpm at 22 C. This picture was taken 8 min after speed was reached. An apparent difference in proportion of top component between isolates is indicated by the relative heights of the respective peaks. Peaks represent (from left to right) top, middle, and bottom sedimentation components of 58, 91, and 108 S, respectively.

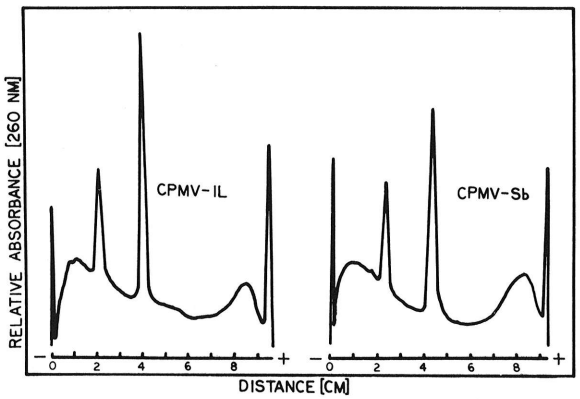

Fig. 2. Ultraviolet scans at 260 nm of 2.5% polyacrylamide gels comparing cowpea mosaic virus (CPMV) isolates, CPMV-IL and CPMV-Sb which were propagated and purified simultaneously, showing different proportions of electrophoretic components between isolates. Electrophoresis was from left (top of gel = 0 cm) to right, 5 hr at 6 mA per gel.

Serology.—Specific antiserum to CPMV-IL was produced in a rabbit following intramuscular injections of 1 mg virus emulsified with an equal volume of Freund's adjuvant administered weekly for 4 wk. Complete adjuvant was used in the first injection and incomplete adjuvant thereafter. Bleedings were made from marginal ear veins before the first injection for pre-immune serum, and before and 1 wk after the final injection for immune serum. Antiserum titer was determined using a microprecipitin test in small capillary tubes. Serological comparisons of virus isolates were made with Ouchterlony double diffusion tests (13).

RESULTS

Isolation of cowpea mosaic virus from a beetle.—Systemic mosaic symptoms developed in *P. vulgaris* 'Tendergreen' and Early Ramshorn cowpea following test feeding by a twelve-spotted cucumber beetle, *D. undecimpunctata howardi*. Thirty days after capture, the beetle died and was homogenized in 0.5 ml 0.01 M potassium phosphate buffer, pH 7.0. Serological tests of the homoginized beetle against CPMV-Sb antiserum were negative, but cowpeas inoculated with the homogenate developed mosaic symptoms. The virus originally transmitted by the beetle was easily sap-transmitted from infected cowpeas to other hosts. Similarity in symptoms on cowpeas and preliminary serological tests (13) indicated a close relationship between the beetle-transmitted virus isolate from Illinois (CPMV-IL) and CPMV-Sb.

Host range.—Both isolates produced chlorotic local lesions on inoculated leaves of Early Ramshorn cowpeas and bright yellow systemic mosaic symptoms in newly emerged leaves. In all soybean cultivars tested (Amsoy 71, Bansei, Beeson, Bragg, Corsoy, Curtis, Dare, Hark, Harosoy, Hill, Kanrich, Marshall, Rampage, Wayne, Wells, and Williams), both isolates produced chlorotic local lesions which later became necrotic. Some cultivars also showed systemic mottling of young trifoliolate leaves and systemic necrosis. *Phaseolus vulgaris* cultivars (Bountiful, Early Golden Cluster, Golden Cluster, Great Northern, Kentucky Wonder, Pinto, Rattlesnake, Red Kidney, Resistant Cherokee Wax, Scotia, Striped Creaseback, Tendergreen, and Topcrop) developed local lesions on primary leaves and occasional systemic symptoms.

Purification and sedimentation analysis.—Plants infected with CPMV-IL and CPMV-Sb yielded 50-60 mg purified virus per 100 g of infected tissue. Both produced similar sedimentation profiles in sucrose density gradients and in analytical ultracentrifugation (Fig. 1), with three sedimentation components of 58, 91, and 108 S. Amounts of middle (91 S) and bottom (108 S) components produced by both isolates appeared to be the

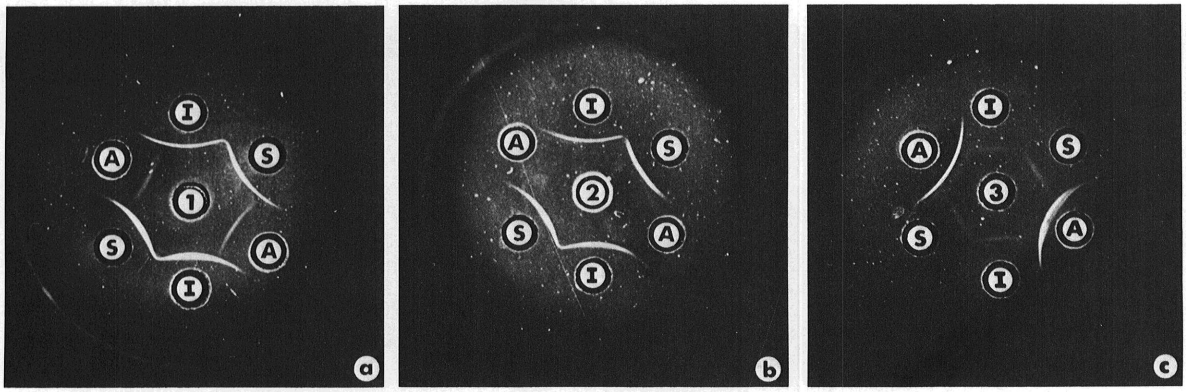

Fig. 3. Serological relationships among cowpea mosaic virus (CPMV) isolates CPMV-IL (I), CPMV-Sb (S), and CPMV-Ark (A) in Ouchterlony double-immunodiffusion tests. Antisera placed in the center wells was against CPMV-IL (a-1), CPMV-Sb (b-2), and CPMV-Ark (c-3). Antiserum to CPMV-Sb (b-2) also reacted slightly with CPMV-Ark, but the precipitation bands were too faint to reproduce photographically. Note the reactions of serological identity between CPMV-IL and CPMV-Sb (a, b).

same. Isolate CPMV-IL, however, consistently produced less top component (58 S) than CPMV-Sb.

Electrophoretic analysis.—Both isolates exhibited two electrophoretic forms (fast and slow) with common electrophoretic mobilities and relatively greater amounts of the fast form (Fig. 2); CPMV-IL had a greater proportion of fast to slow form than CPMV-Sb.

Sodium dodecyl sulfate (SDS) -polyacrylamide gel electrophoresis revealed three protein components of different apparent molecular weights for both isolates. The relative electrophoretic mobilities (R.E.M.) of the three proteins were the same for both isolates. Older virus preparations stored 3 wk at 4 C showed only two protein components. The R.E.M. of the smaller molecular weight protein in older preparations corresponded to that of the smallest of the three observed in fresh preparations. This is consistent with the previously reported behavior of other CPMV isolates (7, 8).

Serology.—The homologous titers of antisera to CPMV-IL obtained before the final injection and 1 wk after were 512 and 1024, respectively. When CPMV-IL and CPMV-Sb were compared in reciprocal Ouchterlony double diffusion tests they gave reactions of identity (Fig. 3). Both isolates produced strong reaction lines with CPMV-IL and CPMV-Sb antisera (Fig. 3-a, b), but very weak reactions with CPMV-Ark antiserum (Fig. 3-c). Homologous reactants formed spurred precipitin lines with heterologous reactants in reciprocal tests of yellow types vs. severe types (Fig. 3-a, c). In all tests normal serum and healthy plant sap controls were negative.

DISCUSSION

Host range, sedimentation profiles in sucrose density gradients, and ultracentrifugation sedimentation analyses showed that CPMV-IL was similar, but not identical, to CPMV-Sb. Consistent differences were noted between CPMV-IL and CPMV-Sb in the relative amount of top component (Fig. 1) and in the relative amount of fast vs. slow electrophoretic forms (Fig. 2). Top component formation is a property which was shown by previous work to vary characteristically among isolates (2, 6), whereas the time between inoculation and purification is reported to influence the proportion of fast to slow electrophoretic forms (9, 12). Slow electrophoretic forms predominate early in infections and fast forms late in infections. Our results with simultaneously purified virus from plants grown under the same conditions, showed differences in the proportions of the respective electrophoretic forms between the two isolates, suggesting that in this respect, the isolates are not identical.

Continuous precipitation lines without spurs between the two isolates in reciprocal double diffusion tests showed that CPMV-IL and CPMV-Sb were serologically identical (Fig. 3-a, b). In our experience, serological tests between yellow subgroup isolates of CPMV always have been found to give reciprocal reactions of serological identity, but serological tests between severe subgroup isolates often show nonidentical reactions.

Major similarities between CPMV-IL and CPMV-Sb indicate that CPMV-IL is a member of the CPMV yellow subgroup. Minor differences between CPMV-IL and CPMV-Sb do not qualify CPMV-IL as a new virus strain. More extensive comparisons with other isolates of this subgroup must be made before such a distinction can be considered. Nevertheless, it is significant that a member of the CPMV yellow subgroup has been found occurring naturally in the United States.

Host range studies reported here and by Agrawal (1) indicate susceptibility of *Glycine max* (L.) Merr. to members of the CPMV yellow subgroup. Considering that CPMV-IL was isolated from a beetle captured in a soybean field, it is logical to question whether CPMV-IL poses a threat to soybean production. Although this single beetle transmission represents the only reported isolation of CPMV in Illinois, the presence of vectors that feed on soybeans and the susceptibility of soybeans to this virus suggest the potential for widespread distribution of CPMV-IL in soybean production areas. Although it is not known where or how this beetle acquired CPMV-IL, it is not unreasonable to speculate that the virus may have existed undetected in wild host plant species, undergoing limited spread owing to the complex nature of virus-host-

vector relationships, or that it was introduced via infected cowpea seed, inasmuch as seed transmission of other isolates in this subgroup has been observed (Thongmeearkom and Goodman, *unpublished observation*) and cowpeas are sometimes grown as a garden crop in southern Illinois. Present practices in virus detection, germ plasm exchange, and commercial and private seed distribution do not preclude these possibilities.

Note added in proof: in Mid-May 1977 we isolated and identified cowpea mosaic virus from naturally infected volunteer soybean plants collected in Union County in southern Illinois. The plants showed foliar damage indicative of feeding by phytophagous beetles. Research is currently underway to further characterize these new CPMV isolates from soybeans.

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