Natural Infection of Potatoes (*Solanum tuberosum*) by a Legume Strain of Cucumber Mosaic Virus

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ABSTRACT

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During the 1984–1985 season, potato plants with severe chlorosis, mosaic, shortened internodes, deformed leaves, and knobby tubers were observed in three fields in Kern County, California. A virus, designated CMV-Py, was recovered from leaves of these plants and from sprouts of knobby tubers collected in these fields. The virus was tuberborne and produced host reactions typical of cucumber mosaic virus (CMV) in all hosts except legumes and potatoes, which were systemically infected. CMV-Py was identified as an isolate of CMV on the basis of serological reaction with antiserum to a nonlegume strain of CMV and the detection of double-stranded RNAs corresponding to CMV RNAs 1, 2, 3, and 4 in extracts of CMV-Py-infected plants. Furthermore, it was identified as a legume strain of CMV on the basis of systemic infection of legumes.

Cucumber mosaic virus (CMV) has a wide host range, and most of the strains cause local lesions on cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata* (5,8). There are, however, "legume strains" of CMV that infect systemically and are seedborne in cowpeas or other legumes (4,9,11,14,15). Natural, systemic infection of Blackeye 5 and other cowpea cultivars grown in California by a legume strain of CMV and the seedborne nature of this virus in cowpeas have been observed in the southern San Joaquin Valley of California for several years (D. H. Hall, *unpublished*). In addition to legumes, legume strains of CMV have been isolated from hosts as diverse as spinach (6), lettuce (16), and peppers (13).

In the fall of 1984, an apparently new disease was observed on potatoes (cultivars White Rose and Red LaSoda) in three fields in Kern County, California, by the late D. H. Hall and the first author. The three fields were adjacent to fields in which cowpeas had been grown during the summer. Although the cowpeas had been harvested when the diseased potatoes were seen, the two crops had overlapped for several weeks. From 60 to 100% of the potato plants were infected and showed systemic mosaic in leaves, chlorosis, severe stunting, and early maturity. Terminal leaves were rosetted, distorted, and small with occasional necrotic patches. Although tuber production was not significantly reduced in symptomatic plants, 95% of tubers from plants showing foliar symptoms were knobbed and unmarketable (Fig. 1), whereas plants with normal foliage had normal tubers of uniform size. The fields were not harvested. The leaf and tuber symptoms were distinct from any described potato disease. Spherical virus particles, in addition to flexuous rods, were observed in diseased leaf tissue with the electron microscope. In this paper, we report the isolation and identification of the spherical virus as a legume strain of CMV.

This publication is dedicated to the memory of Dennis Hall.

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MATERIALS AND METHODS

Virus isolates. The virus isolate from potatoes used for this study was designated CMV-Py. Separation of CMV-Py from the rod-shaped virus(es), which were probably potato virus X and other common potato viruses, by successive inoculation to cowpea and *Cucurbita pepo* L. 'Small Sugar Pumpkin' was confirmed with electron microscopy and host range studies. CMV-Py was recovered from systemically infected leaves of both hosts. Other virus isolates were: CMV-C isolated from a pepper (*Capsicum annuum* L.) in Davis, CA; CMV-Cal isolated from cowpeas in Kern County, California; and peanut stunt virus (PSV) isolate PSV-74-23. The last two isolates were furnished by T. J. Morris (University of California, Berkeley). Isolates of CMV were maintained in *Nicotiana glutinosa* L. and Small Sugar Pumpkin, whereas PSV was propagated in Wisconsin Blackeye cowpeas.

Host range and symptomatology. Host range plants were sown or transplanted into 12-cm-diameter pots containing a pasteurized potting mix. Potato stem cuttings free of potato viruses X, S, Y, and leafroll were made from sprouts that developed after planting whole tubers of virus-indexed cultivars White Rose, Atlantic, Norgold Russet, and Russet Burbank.

Inoculations were made by rubbing Carborundum-dusted cotyledons and expanding leaves with sap from triturated leaf tissue of infected plants. Inoculum was prepared in 0.05 M phosphate buffer (pH 7.5) containing 0.1% sodium diethyldithiocarbamate (DIECA). Infection by CMV was tested serologically.

Antiserum and serology. An antiserum previously prepared against CMV-C had a titer of 1:256 to homologous virus in agar double-diffusion tests and no reaction to sap of healthy *N. tabacum* L. 'Havana 425' from which the virus had been purified. The antiserum was used at a dilution of 1:16 (in 50% glycerol) in these tests. Agar double-diffusion tests were done in a medium of Ionagar no. 2 (Consolidated Laboratories, Chicago Heights, IL) (0.85%), NaCl (0.85%), and NaN_3 (0.02%). A medium containing 0.005 M borate buffer (pH 9.0) and 0.005M EDTA was used for several tests

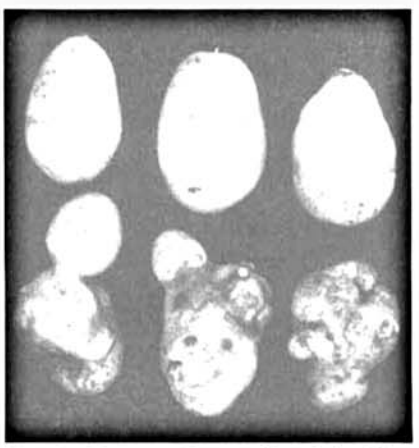

Fig. 1. Potato tubers collected in Kern County, California, from (upper row) plants free of foliar symptoms or (lower row) plants with pronounced foliar mosaic.

(1). Wells were 4 mm in diameter, and the edges of the peripheral wells were 4 mm from the edge of the center well. Crude sap obtained by squeezing leaves wrapped in a layer of cheesecloth was used in the antigen wells. The cheesecloth had been soaked in 1% ascorbic acid and squeezed to remove excess liquid before use. Wells containing sap from healthy, uninoculated plants served as controls.

Electrophoresis. Double-stranded RNAs (dsRNA) were extracted from plants infected with CMV-Py, CMV-Cal, and CMV-C and analyzed by polyacrylamide gel electrophoresis (12; T. J. Morris, *personal communication*).

RESULTS

Symptoms induced by CMV-Py and CMV-C were compared on a range of plants. Many hosts produced the same or similar symptoms after inoculation with either isolate, and these symptoms were in the range of those described for other isolates of CMV (5). Chlorotic and/or necrotic local lesions only developed on the inoculated leaves of *Chenopodium quinoa* Willd. and *C. amaranticolor* Coste & Reyn. Sap from symptomatic leaves of these hosts did not react with CMV-antiserum. Both viruses caused systemic infection of *N. tabacum* 'Havana 425'; *N. glutinosa*; *C. pepo* 'Small Sugar Pumpkin,' 'Cracker,' 'Table Ace,' and 'Ambassador'; *C. moschata* (Duchesne) Poir. 'Early Butternut'; and *Cucumis sativus* L. 'National Pickling.' The cucurbit hosts developed chlorotic local lesions on the cotyledons and epinasty on the young true leaves. CMV infection was confirmed serologically in symptomatic plants. In all experiments, the uninoculated control plants grew normally and their sap did not react with CMV-antiserum.

Symptoms induced by the CMV isolates differed greatly on other hosts. CMV-C produced only necrotic local lesions on cowpea and no symptoms on *Phaseolus vulgaris* L. 'Sutter Pink,' 'Black Turtle,' 'Pinto,' and 'Red Kidney'; *Lycopersicon esculentum* Mill. 'Earlypak 7'; or potato cultivars White rose, Atlantic, Norgold, and Russet Burbank. Sap from these plants did not react with antiserum to CMV. In contrast, CMV-Py caused chlorotic local lesions on primary leaves of cowpea and severe epinasty followed by systemic mottle on trifoliate leaflets. On the four cultivars of beans, CMV-Py caused epinasty and mosaic of systemically infected trifoliate leaflets. Sap from both symptomatic primary leaves and trifoliate leaflets reacted with antiserum to CMV-C. Tomato plants inoculated with CMV-Py showed a mild systemic mottle.

CMV-Py produced leaf symptoms, like those described from the field, on inoculated shoots of four potato cultivars. Symptoms developed on 15 of 20 inoculated potato shoots 3–4 wk after inoculation. CMV infection was confirmed serologically for symptomatic shoots but not for any of the symptomless shoots. A few small tubers produced by these infected plants, and also by uninoculated plants, were stored at 3 C for 5 wk to break dormancy and planted. Of the 11 shoots produced from five tubers from infected plants, one shoot had mottle and assayed positive with CMV-antiserum. The tuber that gave rise to the infected shoot also produced two symptomless shoots. Symptomless shoots failed to react with antiserum to CMV in serological tests.

Eleven knobby tubers collected from field-grown plants with obvious foliar symptoms of virus infection were planted in the glasshouse. Five tubers produced a total of five symptomless and eight symptomatic shoots. Five other tubers produced only symptomless shoots. Seven of eight symptomatic shoots, but none of the symptomless, gave a positive reaction in serological assays. One tuber did not sprout. Tubers collected from field-grown plants showing no foliar symptoms were not distorted and were more uniform in size. In the glasshouse, such tubers gave rise to symptomless shoots only.

Serology. The CMV-Py and CMV-C isolates, but not PSV, reacted with the antiserum prepared against CMV-C (Fig. 2). No spurs or crossed precipitin lines developed between CMV-C and CMV-Py. The same reaction pattern was produced when the test was done in a borate-EDTA medium.

Electrophoresis. There were five dsRNAs in CMV-C and CMV-Cal (Fig. 3). The dsRNA of the satellite RNA (RNA-5) and its presumed dimer, which showed as a distinct band between RNA-4 and RNA-5, were not present in CMV-Py. The dsRNAs 1–4 of the three strains were not distinguishable electrophoretically. Additional minor bands of dsRNAs shown with all three viruses may be disregarded.

DISCUSSION

CMV-Py was identified as a strain of CMV on the basis of serological tests, dsRNA similarities, host range, and host reactions. The systemic infection of cowpea has been designated a key host reaction that characterizes PSV and distinguishes it from CMV in the cucumovirus group (8). Although CMV-Py infected cowpeas systemically, it reacted with antiserum to CMV-C while PSV did not. Our results support the contention that legume strains of CMV are more closely related to CMV than to PSV (3). CMV-Py had four dsRNAs corresponding to those known of CMV (8) but lacked the satellite RNA that was present in CMV-C and CMV-Cal. Thus, satellite RNA was not necessary for pathogenicity to cowpeas, beans, or potatoes. The satellite RNA components of CMV have been associated with symptom potentiation or attenuation in some hosts (3,7,8). The addition of satellite RNA to CMV-Py may result in symptom amelioration in potatoes, but this possibility has not been examined.

Symptoms in potatoes infected with CMV-Py differed from those described previously for CMV (2,10). Bode (2) described leaf rolling as the main symptom associated with infection by a tuberborne strain but acknowledged the weak serological relationship of his strain with antiserum prepared against a known CMV isolate. MacArthur (10) observed one plant with foliar symptoms similar to those resulting from CMV-Py infection. He made no mention, however, of severe stunting or tuber deformation, and he found that the virus was not tuberborne

Fig. 2. Gel double-diffusion test showing reactions of two cucumber mosaic virus strains (CMV-C from peppers and CMV-Py from potato) and peanut stunt virus (PSV) with CMV-C antiserum. Central well contains antiserum (diluted 1:16) prepared against CMV-C. Peripheral wells contain crude sap from cotyledons or primary leaves of plants: A and B = National Pickling cucumber infected with CMV-C, C and D = Wisconsin Blackeye cowpea infected with CMV-Py, E and F = cowpea infected with PSV, G = healthy cucumber, and H = healthy cowpea.

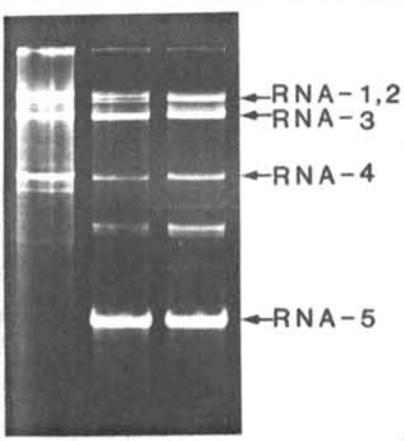

Fig. 3. Polyacrylamide-gel electrophoretic separation of dsRNAs of three isolates of cucumber mosaic virus: (left) CMV-Py, (center) CMV-Cal, and (right) CMV-C.

in the 22 cultivars tested. The present report is the first of a legume strain of CMV in potato and of severe foliar mosaic and tuber distortion caused by it.

The epidemiology of this potato disease in California probably is involved with the cropping pattern for cowpeas and other crops and weeds affected by CMV. In Kern County, cowpeas are raised in the summer and are infected frequently by CMV. Potatoes, grown as a fall crop, emerge before the cowpeas are harvested. The CMV-Py strain caused losses only in potato fields planted close to cowpea fields. Potato plants nearest the cowpea fields probably were infected first because 100% of these plants had foliar and tuber symptoms by October. We thus believe that cowpeas, in which CMV may be seedborne, serve as a source of primary inoculum for the potatoes. Continued in-field spread during the potato season is probably responsible for the widespread losses observed. CMV-Py was tuberborne in potatoes, but infected tubers were probably not an important source of primary inoculum because "seed" potatoes from an outside source are planted annually. In 1985, a strain of CMV was isolated again from potatoes and also from peas (*Pisum sativum* L.) and snap beans grown in fields adjacent to cowpeas in Kern County.

Cultivars of red and white potatoes were infected by CMV-Py in the field. These potatoes also were infected by one or more rod-shaped viruses common to potatoes. Thus, the ability of CMV-Py alone to cause the symptoms observed in the field was tested by inoculating virus-free plants in the glasshouse. The range of foliar symptoms observed in the field was reproduced by CMV-Py. We have not attempted to reproduce the tuber symptoms in the glasshouse, because even the tubers produced by virus-free plants in pots are small and often irregularly shaped. Thus, it has not been established unequivocally that CMV- Py alone causes the tuber distortion. Nevertheless, we believe that the close association between tuber distortion and foliar symptoms in the field, as well as the severity of foliar symptoms caused by CMV-Py, indicate a primary role for CMV-Py in causing tuber deformation.

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