Research Note

SURVEY OF VIRUSES AFFECTING PEPPER (CAPSICUM ANNUUM L.) IN SOUTHERN PUERTO RICO[1]

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Pepper (*Capsicum annuum* L.) is a vegetable crop of major importance in tropical and subtropical regions. In 1983, the farm value of pepper in Puerto Rico peaked at $4.4 million (Departamento de Agricultura, 1984). However, by 1990 it declined to $3.9 million (Departamento de Agricultura, 1991). Among other factors, this decline can be attributed to viral diseases.

In Puerto Rico, viral diseases reduce pepper yield and quality in some cases up to 90 percent (Pérez et al., 1974; Roque and Adsuar, 1941). The common viruses reported infecting peppers in Puerto Rico are potato virus Y (PVY), tobacco etch virus (TEV), tobacco mosaic virus (TMV), and potato virus X (PVX) (Arrollo, 1981; Escudero et al., 1987; Roque and Adsuar, 1941). PVY and TEV have been classified in the potyvirus group (Edwardson, 1974; Mathews, 1981). These two viruses are not seedborne and are transmitted efficiently by a number of aphid species in a non-persistent manner (Delgado-Sánchez, 1970; Sheperd, 1971). TMV is classified in the tobamovirus group (Gibbs, 1977). It is seedborne in several plant species and is not transmitted efficiently by insects. The virus is spread by workers handling infected and healthy plants indiscriminately. PVX belongs to the potexvirus group. It is transmitted by the grasshopper *Melanoplus differencialis* and *Tettigonia viridissima*, and by the fungus *Synchytrium endobioticum*. It is also transmitted through infected seed tubers and by contact between adjacent plants, hands and tools (Bercks, 1970). Virus diseases are also a major problem of peppers in other countries (Edwardson and Christie, 1979).

The major objective of this study was to isolate and identify viruses affecting commercial pepper fields in the semiarid southern coastal plains of Puerto Rico and determine their serological relationships to viral isolates from Florida.

Commercial pepper plantations in the Santa Isabel area were surveyed for virus symptoms at various times from 1980 to 1990. Samples of pepper leaves, cv. Cubanelle, with virus symptoms were collected at random, placed in plastic bags, labelled and refrigerated at 4°C up to one week.

Diseased pepper leaves from plants with different symptoms were separately crushed in 0.01 M Na_2HPO_4 buffer (1:2 w/v, pH 7.2) by using a mortar and pestle previously sterilized, and the sap was squeezed through two layers of cheesecloth. The sap was lightly rubbed on carborundum (400 mesh)-dusted leaves of virus-free seedlings of *Chenopodium quinoa* Willd., *Capsicum annuum* L. cv. Cubanelle, *Nicotiana tabacum* L. cvs. V_{12} and NN, *Datura stramonium* L. and *Lycopersicon lycopersicum* (L.) Karst et Farw. Ten plants of each test species were inoculated separately with each virus isolate for studies.

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Inoculated seedlings and an equal number of healthy control plants were kept in separate cages in a greenhouse at 28° ± 5°C for 30 days.

Sodium dodecyl sulfate (SDS) immunodiffusion tests were carried out as described by Purcifull and Batchelor (1977). Crude extracts obtained from pepper leaves with virus symptoms were crushed in 1.5% SDS and compared with antigens and antisera of PVY, TEV, pepper mottle virus (PeMV), TMV and cucumber mosaic virus (CMV). Antisera and their homologous antigens were provided by D. E. Purcifull, University of Florida. Normal sera and healthy antigen controls were included.

Epidermal leaf strips of affected pepper plants were stained with calcomine orange and Luxol brilliant green as described by Christie and Edwardson (1986) and Edwardson and Christie (1979). The epidermal strips were examined by light microscopy to detect viral inclusion bodies.

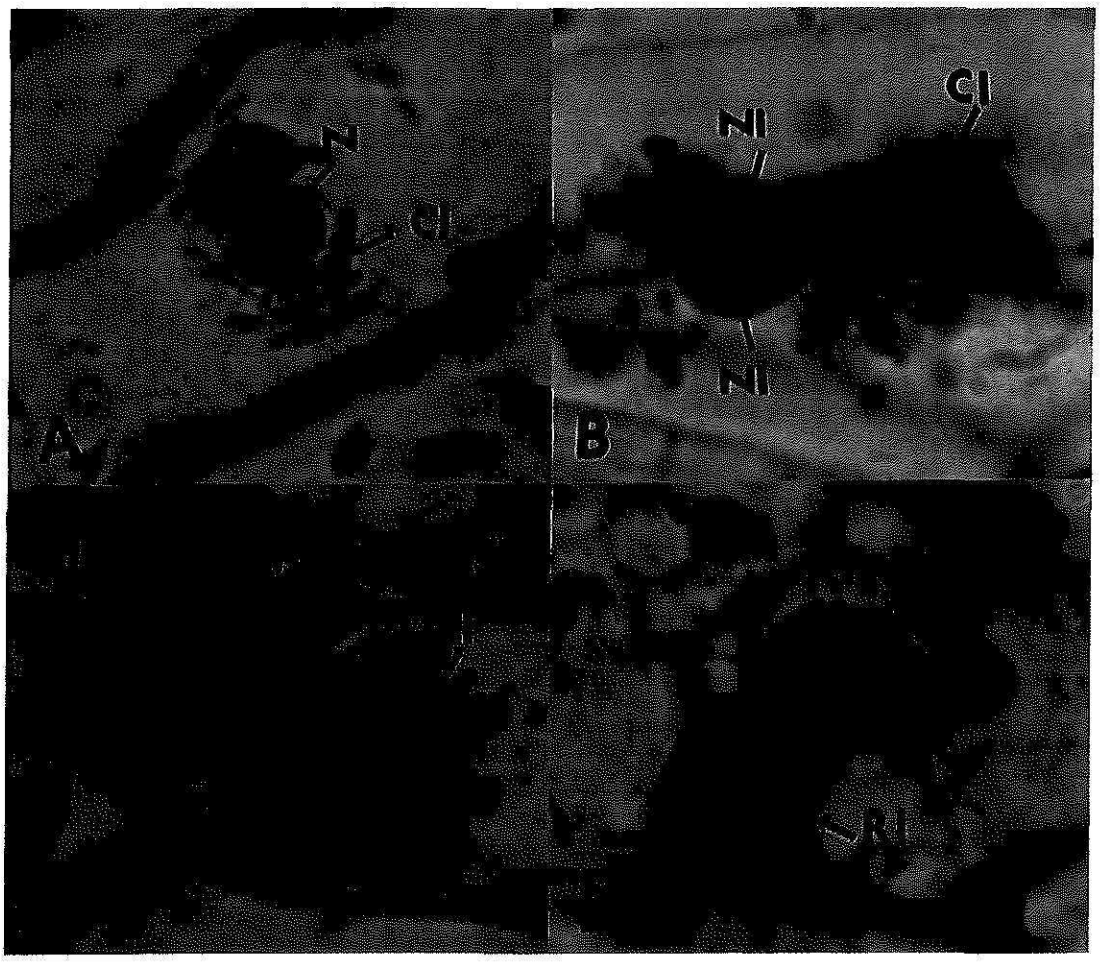

FIGURE 1. Light micrographs of epidermal leaf tissues stained with calcomine orange and "Luxol" brilliant green combination, showing inclusions associated with three potyviruses (A, B, C) and one tobamovirus (D). A) *Capsicum annuum* cell infected with potato virus Y containing nucleus (N) and a group of cytoplasmic cylindrical inclusions (CI). B) *C. annuum* cell infected with tobacco etch virus showing masses of cylindrical inclusions and nuclear inclusions (NI). C) *C. annuum* cell infected with pepper mottle virus containing cylindrical inclusions and irregular cytoplasmic inclusion (II). D) *C. annuum* cell infected with tobacco mosaic virus showing cytoplasmic rectangular crystal inclusion (RI).

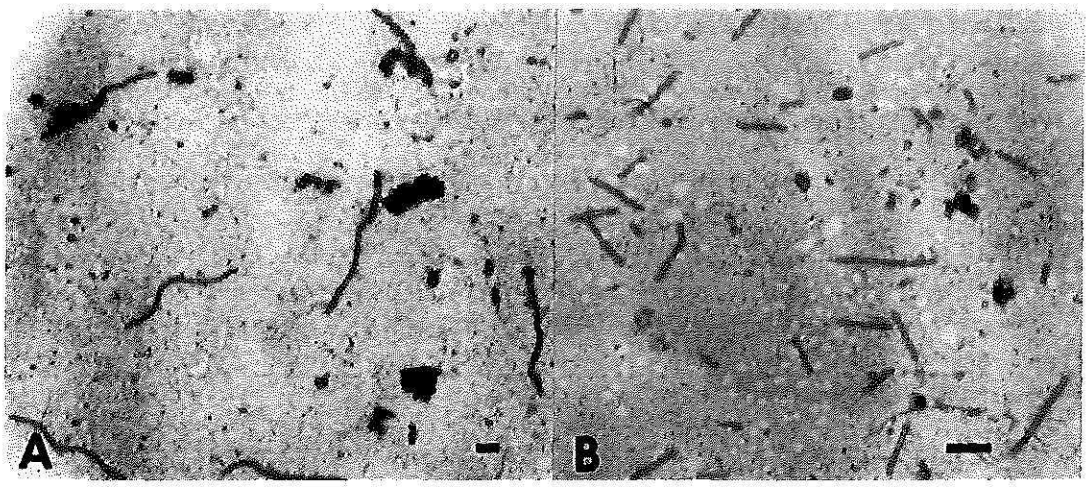

FIGURE 2. Transmission electron micrograph of the two morphologically different virus particle detected in concentrated extract of infected plants. Electron micrographs depict negatively stained: A) potyviruslike flexuous rods from the pepper virus mixture; B) Tobamoviruslike rigid rods of the pepper virus. Bars in A and B, represent 100 nm.

Carbon coated parlodium-films (300-mesh grids) were used for electron microscopy. Crude leaf-dip preparations were stained with 1% phosphotungstic acid. The specimen grids were examined with a Siemens I-A[3] electron microscope for virus particles.

Local necrotic lesions similar to those incited by TMV became evident on inoculated leaves of *C. quinoa*, *N. tabacum* cv. NN and *D. stramonium*. Systemic mosaic was observed in *C. annuum*, *L. lycopersicum*, and *N. tabacum* cv V_{12}.

A systemic mosaic similar to the one induced by PVY isolates was observed on inoculated plants of *C. annuum*, *L. lycopersicum*, and *N. tabacum* cv V_{12}. No symptoms were observed on inoculated *D. stramonium* plants. Plants of *D. stramonium* inoculated with an isolate resembling TEV developed leaf distortion with some mottling and vein banding. Also a systemic mosaic was observed on TEV inoculated *C. annuum* and *L. lycopersicum*. Infected *N. tabacum* cv V_{12} developed clear veins with some etching on their leaves.

Precipitin positive reactions occurred when antigens from local isolates were tested against antisera of PVY, PeMV, TEV and TMV from Florida. No reaction was observed in the controls with healthy antigen and normal serum.

Examination by light microscopy of infected pepper leaf tissues revealed the presence of cylindrical, amorphous, and two types of crystalline inclusions in the cytoplasm and nuclei of epidermal cells. The inclusions observed were similar to those previously described (Edwardson and Christie, 1979; Mejía et al., 1985) for PeMV (Figure 1A), PVY (Figure 1B), TEV (Figure 1C), and TMV (Figure 1D).

Flexuous particles approximately 750-800 nm in length (Figure 2A) and rigid rods about 300 nm long (Figure 2B) were observed in leaf dip preparations of infected peppers. The two types of particles studied were similar in length and morphology to the viruses previously described in the potyvirus (Mathews, 1981) and tobamovirus (Gibbs, 1977) groups.

[3]Trade names in this publication are used only to provide specific information. Mention of a trade name does not constitute a warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

This is the first detection of natural infection by PeMV in Puerto Rico. The virus has been reported to cause about 50% yield reduction of pepper in California (Villalon, 1981). Three other viruses (PVY, TEV and TMV) were also identified in this study. Single and mixed infections of pepper plants by these viruses were commonly observed during the survey.

Differences in virus incidence (sometimes 20 to 90%) were also observed on the various pepper plantations surveyed. Probably these were related to variations of aphid population, varietal differences and the control measures employed. These measures included the use of reflective plastic mulch, oil spray, and other insecticide treatments.

Further work is recommended on the relative incidence of viruses in peppers and other crops in the southern coastal plains of Puerto Rico, as well as evaluation of new pepper hybrids for resistance to our local viruses.

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