A New Virus Disease of Peppers in Puerto Rico

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INTRODUCTION

Pepper mosaic virus was reported to occur in Puerto Rico by Cook (*1*)[2] in 1927. A thorough study of a mosaic of peppers, found widespread throughout the Island, was made by Roque and Adsuar (*2*) in 1941. Their studies disclosed that pepper production in Puerto Rico was greatly limited by the disease and they named the causal agent Puerto Rican pepper mosaic virus (PRPMV). Later studies by Pérez and Adsuar (*3*) indicated that potato virus Y and PRPMV were serologically related, although PRPMV had a minor antigenic component that does not occur in potato virus Y.

Another virus has been found recently attacking pepper varieties resistant to PRPMV. This new virus tentatively has been named VPLLT (virus producing local lesions on tobacco) because, unlike PRPMV, it produces localized necrotic lesions on NN type tobacco, *Nicotiana glutinosa*, and *Datura stramonium*. These lesions are different from those produce by the common type of tobacco mosaic virus. Studies were undertaken to characterize this newly found pepper virus.

MATERIALS AND METHODS

HOST RANGE, THERMAL INACTIVATION AND DILUTION ENDPOINT STUDIES

Plants used for host range studies were grown and kept in screened greenhouses. The leaves of young plants were dusted with carborundum (400 mesh) and then rubbed with a cotton swab dipped in sap expressed from leaves of previously inoculated pepper plants showing symptons of PRPMV.

For the thermal inactivation tests, a range of temperatures between 50° and 90° C. at 10° intervals was used. Constant-temperature water baths were calibrated accordingly, except for the last temperature interval in which the difference was only 5° C. One ml. of sap expressed from diseased pepper plants of variety Blanco del País was placed in thin-walled tubes and immersed in each of the corresponding baths for a period of 10 minutes. The tubes were then quickly removed and placed in iced water and plants of *N. glutinosa* were inoculated with the heat-treated extracts.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 410.

For the dilution endpoint tests, tenfold dilutions of the infective sap were made in distilled water. *Nicotiana glutinosa* was also used as a test plant to determine the dilution endpoint. To study the longevity *in vitro*, undiluted sap was maintained at laboratory temperature (29° C.) and inoculated on *N. glutinosa* plants every day for a period of 14 days.

FILTRATION EXPERIMENTS

Filtration experiments were carried out by treating crude juice extracted from virus-infected pepper leaves with 0.1 M potassium phosphate buffer at pH 4.0. The juice was centrifuged at low speed (3,000 r.p.m.) and the clear supernatant passed through a Millipore, 0.45 μ filter. Presence of the virus in the filtrate was determined by inoculation on *N. glutinosa*.

PARTIAL PURIFICATION AND SEROLOGY

The VPLLT virus was inoculated on about 200 plants of pepper variety Blanco del País. Healthy plants of the same variety were left uninoculated as controls and were processed as described below for infected plants. Eighteen days after inoculation the healthy and virus infected leaves were harvested separately and frozen for 72 hours. The leaves were then homogenized for 5 minutes in a pre-cooled Waring Blendor in a mixture containing 0.5 M phosphate citrate buffer, chloroform, and leaf tissue, in a proportion of 1 ml. buffer and 2 ml. chloroform per g. of leaf tissue. The final pH was 6.7 (initial extract). The homogenate was then filtered through a triple layer of cheesecloth and centrifuged in a pre-cooled #850 angle head rotor in a model UV International Centrifuge at about 3,000 gravities (g) for 30 minutes. The precipitate was discarded and the supernatant centrifuged for 2 hours at 78,000 g in a model L Spinco Ultracentrifuge using a pre-cooled #30 rotor. The pellets were then re-suspended in .005 M Na-citrate pH 7.6 in an amount equivalent to $\frac{1}{25}$ volume of the initial extract, and stirred for 30 minutes. They were then centrifuged in an 813-angle head using a model UV International Centrifuge at approximately 1,300 g for 20 minutes. The cycle of centrifugation was repeated. The final concentration of the virus was 25 times that in the initial extract, disregarding losses. The pellets were dissolved in 0.05 M phosphate-citrate buffer and diluted in tenfold steps with cold distilled water. The purified virus (VPLLT) preparation produced necrotic lesions on both NN-type tobacco and *Nicotiana glutinosa* up to a dilution of 10^{-6}. The preparation obtained from healthy pepper leaves did not produce lesions or other symptoms in the above-mentioned plants.

Two rabbits were inoculated intramuscularly with a suspension of the partially purified virus in Freund's incomplete adjuvant. The rabbits were given two injections 2 weeks apart. The rabbits were exsanguinated 50 days

after the first injection and the serum separated and stored in aliquots in the frozen state until used. The precipitin endpoints of the sera were determined by the tube method and tests were carried out in a 37° C. incubator. Twofold dilutions of the sera were made and tested against the next to the highest dilution of the VPLLT preparation that gave a visible precipitate with its homologous serum. The dilution endpoint was read after 2 hours incubation at 37° C. and overnight refrigeration. The endpoints of both sera were $\frac{1}{256}$. The VPLLT preparation was also tested as described above, against constant concentrations ($\frac{1}{40}$ dilution) of specific antisera to tobacco mosaic virus, PRPMV, tobacco severe etch virus, tobacco ringspot virus, green mottle mosaic virus, squash mosaic virus (*Echinocystis lobata* strain) and melon mosaic virus (MMV) (P.R. squash mosaic isolate), sugar cane mosaic virus, and potato virus X (J1X strain) but did not react with any of the antisera. The preparation obtained from healthy plants did not react with the VPLLT serum and the other antisera used.

TRANSMISSION VIA INSECTS

Limited transmission studies with insects were conducted by feeding adults of the aphid species *Aphis gossypii* and *Myzus persicae* separately on VPLLT-infected, Blanco del País pepper leaves for a period of 3 hours and then transferring them to healthy pepper plants. The insects were fasted for 1½ hours previous to feeding. No transmission was evidenced.

IMMUNOLOGICAL RELATIONSHIPS

Cross-immunity tests between the VPLLT virus and the common tobacco mosaic virus (TMV) were carried out using the method described previously. Plants of pepper variety Blanco del País were inoculated mechanically with VPLLT virus and after a few days inoculated with TMV. As expected, on pepper plants of this variety, VPLLT induced a systemic mottling, while TMV produced only localized necrotic spots. To test for a possible relationship between the PRPMV and VPLLT, *N. glutinosa* plants were first inoculated with PRPMV and then challenged with VPLLT.

RESULTS

HOST RANGE

In all inoculated varieties of pepper, VPLLT produced symptoms after 8 to 12 days. *Capsicum annuum* varieties California Wonder, Blanco del País and P.R. Wonder (resistant to PRPMV) developed vein clearing, followed by mottling, chlorosis, and leaf wrinkleness. Retardation of growth was observed frequently. Pepper variety Madame Genet, in addition, showed necrotic blotches and defoliation of the leaves. *Capsicum frutescens*

variety Cuaresmeño, a hot pepper highly resistant to PRPMV imported from Mexico, was found to be highly susceptible to VPLLT. It exhibited mottling, necrotic spots, and necrotic blotches on the leaves followed by stem necrosis and death. *Capsicum frutescens* variety Large Bell Hot displayed vein clearing, followed by faint systemic mottling, without necrosis of the veins or defoliation.

Other solanaceous hosts susceptible to VPLLT and their reactions were: *Solanum ciliatum*, marked mottling of the leaves; *Datura stramonium*, localized chlorotic spots on inoculated leaves, no systemic symptoms; *S. guanicensis*, localized necrotic lesions on inoculated leaves, no systemic symptoms; and *S. quitoensis*, exhibiting mild systemic mottling only. *Physalis peruviana* displayed a very faint systemic mottling.

No symptoms were obtained when the following plants were inoculated with VPLLT: *Carica papaya* varieties P.R.6-65 and Solo, *Gomphrena globosa*, *Nicotiana tabacum* var. Virginia-12, *N. sylvestris*, *Cucumis sativus* variety Black Diamond, *Cajanus cajan*, *Cucumis melo* var. P.I.180280, *Luffa acutangula*, *Phaseolus vulgaris* var. Criolla, *Vigna sinensis* var. Black, *Chenopodium amaranticolor*, *Solanum indicum*, *S. mamosum*, *S. melongena* var. Rosita, *Cassia tora*, *Althaea rosea*, *Phytolacca decandra*, *Passiflora serrata digitata*, and *Lycopersicum esculentum* var. Rutgers. Recovery tests made from the leaves of these plants on *N. glutinosa* were negative.

THERMAL INACTIVATION, DILUTION END-POINT AND LONGEVITY IN VITRO

The virus withstood a dilution of 1:100,000 and was inactivated between 85° and 90° C. The extracted sap was still infectious after 14 days at room temperature.

FILTRATION

The VPLLT virus filtrate produced the characteristic localized necrotic lesions when inoculated on *N. glutinosa*. No bacterial growth developed after inoculation of the filtrate in sterile nutrient broth.

SEROLOGY

Dilutions of the VPLLT preparation reacted only with VPLLT antiserum and did not react with antisera to tobacco mosaic virus, tobacco severe etch virus, pepper (PRPMV) mosaic virus, tobacco ringspot virus, green mottle mosaic (cucumber #3) virus, squash mosaic virus, sugarcane mosaic virus, or potato virus X.

TRANSMISSION VIA INSECTS

No transmission of VPLLT was obtained by aphids *A. gosypii* and *M. persicae* on pepper variety Blanco del País.

IMMUNOLOGICAL RELATIONSHIPS

VPLLT induced mottling of the leaves after inoculation on pepper variety Blanco del País. When these leaves were re-inoculated with tobacco mosaic virus (TMV) characteristic local necrotic lesions of TMV appeared. Leaves of *N. glutinosa* plants after inoculation with PRPMV exhibited systemic mottling. Local necrotic lesions were observed when these leaves were re-inoculated with VPLLT.

These results demonstrated that VPLLT was not able to protect invaded tissues of pepper against infection by TMV. On the other hand, infection by PRPMV was unable to confer protection against VPLLT on *N. glutinosa*.

DISCUSSION AND CONCLUSIONS

The viruses reported thus far as infecting pepper plants in the field in Puerto Rico are PRPMV, TMV, and TEV (tobacco etch virus). Of these, PRPMV is still the predominant virus. A fourth virus, unrelated to PRPMV, TMV, or TEV, was found recently on mosaic-affected pepper plants at the Station grounds and later at the Isabela Substation.

The virus, named VPLLT, produces discrete, localized, necrotic lesions on *N. glutinosa* and NN-type tobacco and localized, chlorotic spots on *D. stramonium*, has a high dilution endpoint (1–100,000), and resists ageing for more than 14 days at laboratory temperature. Although resembling TMV in its thermal inactivation point and in the symptoms induced on *N. glutinosa*, VPLLT differs from TMV in its immunological as well as serological relationships, as demonstrated in the course of this investigation. VPLLT also differs from both PRPMV and TEV in serological properties.

It is of interest to note that serological tests being conducted presently at the Station as part of a survey on the virus diseases of peppers in the Island have detected the presence of VPLLT almost as frequently as PRPMV (*4*).

A literature survey has not disclosed a virus in peppers displaying the the characteristics of the VPLLT virus, although it is possible that one may have been reported.

Finally, and most important, all pepper varieties tested thus far have been found susceptible to the VPLLT virus, including P.R. Wonder sweet pepper and the Cuaresmeño hot pepper, both of which are highly resistant to PRPMV in Puerto Rico. If VPLLT eventually becomes as widespread as PRPMV, it certainly will pose a serious menace to pepper growing in the Island unless new sources of resistance to VPLLT are found.

SUMMARY

A previously undescribed virus, (VPLLT) causing mottling, chlorosis, leaf wrinkleness and retarded growth on *Capsicum annuum* varieties California Wonder, Blanco del País and P.R. Wonder, was isolated at the Agricultural Experiment Station 2 years ago.

VPLLT is not inactivated by heating at 85° C., can be diluted to 1–100,000, and is still active after 14 days at room temperature (29° C.).

It is not serologically related to tobacco mosaic virus, tobacco severe etch virus, Puerto Rican pepper mosaic virus, tobacco ringspot virus, green mottle mosaic (cucumber #3) virus, squash mosaic virus, potato virus X, sugarcane mosaic virus, and MMV (Puerto Rico isolate) of squash mosaic virus.

No transmission of VPLLT was obtained using the aphid species *A. gossypii* and *M. persicae* as vectors.

The virus easily passes through a Millipore 0.45 μ filter, and is not immunologically related to TMV or PRPMV.

It was found that P.R. Wonder and Cuaresmeño hot pepper, two varieties highly-resistant to PRPMV, are fully susceptible to the VPLLT virus.

RESUMEN

Se informa la presencia de un nuevo virus del pimiento en Puerto Rico, que ataca las variedades de pimiento California Wonder, Blanco del País y P.R. Wonder. El virus fue hallado por primera vez en los terrenos de la Estación Experimental Agrícola hace alrededor de 2 años. Se le ha adjudicado la sigla VPLLT (virus del pimiento que produce lesiones localizadas en tabaco) debido a los síntomas que produce en el tabaco tipo-NN.

Las propiedades físicas del virus VPLLT son las siguientes: No se inactiva a 85° C., resiste una dilución de 1–100,000 y permanece infeccioso por más de 14 días a la temperatura del laboratorio (29° C.). El virus VPLLT no reacciona serológicamente con antisueros preparados contra los siguientes virus: mosaico común del tabaco, "salpicado" del tabaco, mosaico del pimiento de P.R., virus X de la papa, mosaico de la caña de azúcar, mosaico del calabacín de P.R. y mosaico verde del pepinillo (cucumber virus 3).

No se logró transmitir el virus VPLLT utilizando los afídidos *A. gossypi* y *M. persicae*. Se verificó la filtración del virus a través de un filtro Millipore (0.45 μ). No se observó el fenómeno de interferencia inmunológica al virus VPLLT en plantas previamente inoculadas con los virus TMV y PRPMV.

Se comprobó la susceptibilidad de las variedades de pimiento P.R. Wonder y Cuaresmaño al virus VPLLT.

LITERATURE CITED

1. Cook, M. T., Annual Report Ins., Expt. Sta., Dept. of Agric. and Labor of Puerto Rico, Fiscal Year 1927–28: 65, 1929.
2. Roque, A., and Adsuar, J., Studies on the mosaic of peppers (*Capsicum frutescens*) in Puerto Rico, *J. Agr. Univ. P.R.*, *25* (4): 1-11, 1941.
3. Pérez, J. E., and Adsuar, J., Antigenic relationship between Puerto Rican pepper mosaic virus and a strain of potato virus Y., *J. Agr. Univ. P.R. 39* (3): 165–7, 1955.
4. Pérez, J. E., Personal communication.