

## STUDIES ON VIRUS DISEASES OF PAPAYA (CARICA PAPAYA) IN PUERTO RICO

### III. Property Studies of Papaya Mosaic Virus

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During the last two years a couple of virus diseases (Adsuar, 1, 2) have been found attacking papaya *Carica papaya* in Puerto Rico. One of them, the so called "bunchy top" disease, is of general occurrence through the island and resembles in general appearance the papaya viroses reported from Trinidad, Cuba and Santo Domingo. "Bunchy top" is extremely difficult, if not impossible, to transmit either mechanically or by grafting, and it has been only recently that we have been able to obtain evidence of its transmission by means of a leafhopper vector (3). The other disease, a typical *mosaic*, differs from "bunchy top" not only symptomatologically, but also in the fact that it is easily transmissible by mechanical means as well as by grafting. Its insect vector is an aphid (2).

Due to its stability *in vitro* and to the ease of mechanical transmission, experiments were undertaken to determine the physical properties, filterability, etc., of the papaya mosaic virus.

#### MATERIALS AND METHODS

The virus was obtained from artificially infected papaya plants kept inside wire-screened shelters. The plants were inoculated with the aid of carborundum following the method described by Adsuar (1). All experiments were conducted in sets of four plants each. After treatment the plants were transplanted into 12 inch pots and transferred to the shelters. The aphids used in the retention experiment were taken from papaya trees, starved, and then fed on infected leaves for the required periods of time, inside Petri dishes. After feeding was completed, they were removed and placed on healthy plants for the retention studies.

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## EXPERIMENTAL STUDIES ON THE PAPAYA MOSAIC VIRUS

*Resistance to Heat:*

Leaves from recent cases of *mosaic* were pounded in a mortar with sterile sand and 1 cc. of distilled water gradually added. The juice was expressed through heavy muslin, and about 2 cc. of juice obtained. To these more water was added, making a final dilution of approximately 1:12. Samples were then heated in corked tubes holding about 1¼ cc. each, to temperatures of 50°C, 55°C and 60°C for 3, 10 and 30 minutes periods at each temperature. (Table I).

TABLE NO. 1  
THERMAL INACTIVATION OF PAPAYA MOSAIC

Temperature °C	Minutes heated	Number of Plants Inoc	Infected
50.....	3	4	4
50.....	10	4	4
50.....	30	4	1
55.....	3	4	4
55.....	10	4	1
55.....	30	4	0
60.....	3	4	0
60.....	10	4	0
60.....	30	4	0

The results indicated that papaya mosaic virus has a thermal inactivation range between 55°C to 60°C for 10 min. Exposures to 55°C for 10 min. show a decrease of infectivity, with complete inactivation at 60°C for the same period of time.

*Tolerance to Dilution:*

Infected leaves showing early symptoms were crushed in a mortar with a little sterile sand and distilled water, the pulp squeezed through muslin, and the resulting green fluid diluted as follows: 1:4; 1:16; 1:64; 1:256 and 1:1024.

The virus remained infective up to and including 1:256. No infections were obtained with the 1:1024 dilution.

*Longevity in vitro at room temperature:*

Samples obtained as above and diluted approximately 1:12 were stored at laboratory temperature (29°C) and tested at 24, 48 and 72 hrs. No fermentation or putrefaction was noted. Chlorophyll settled leaving a clear fluid above. Part of the same juice was put in the refrigerator at 10°C and tested at different intervals of time.

The results indicated that the virus is completely inactivated in 48 hrs. at room temperature. At refrigerator temperature, the virus was still active at the end of 15 days.

*Filterability:*

Leaves of plants showing the disease were ground with approximately three times their weight of distilled water. Juice was extracted through muslin and centrifuged for 15 minutes in a clinical centrifuge. To the supernatant fluid was added  $\frac{1}{4}$  cc. of a 19 hr. culture of *Sarcina lutea* in broth (Difco). The mixture was then filtered through a Seitz disc No. 5114-C10.

The filtrate proved to be free from bacteria as determined by inoculation in sterile nutrient broth and on nutrient agar slants. The virus passed the filter, but apparently did so with much difficulty. Of our plants inoculated, only one came down with symptoms. Controls inoculated with the unfiltered mixture all showed the disease.

*Inactivation by Drying:*

Recently affected young leaves (showing vein clearing and chlorotic spotting) from previously inoculated plants were picked, tied together and suspended in a shady but breezy part of the laboratory for drying. Some of the leaves were macerated immediately after collection with the addition of 2 cc. of water and the juice tested for infectivity. The rest of the leaves were allowed to dry for 48 hours and 72 hours periods. Samples extracted as explained above were obtained at the required time and inoculated on healthy plants. Plants inoculated with juice from leaves extracted immediately after picking all came down with the disease. None of the plants inoculated with juice extracted from leaves air dried from 48 to 72 hours became infected.

*Retention of Virus by Aphids:*

Winged adults of *Aphis spiraeicola* Patch were collected and starved for about 1 hour, transferred to infected leaves, and allowed to feed for 15 minutes. Aphids in lots of five were then taken to individual papaya seedling and allowed to feed for 3 hours. After that interval they were removed to new seedlings. Table 2 shows the result of the experiment.

TABLE No. 2  
RETENTION OF PAPAYA MOSAIC BY APHIS SPIRAECOLA PATCH

Lot	Number Aphids Used	Plants inoculated during 3 hrs. period	Plants infected during 3 hrs. period	Plants inoculated after 3 hrs. period	Plants infected after 3 hrs. period
1	5	1	1	1	0
2	5	1	1	1	0
3	5	1	1	1	0
4	5	1	0	1	0

The results demonstrated that the aphids failed to retain the virus for more than 3 hours.

#### SUMMARY

The physical properties of papaya mosaic virus are reported. The virus is inactivated by a 10-minute exposure at 60°C. No infection was obtained when juice was diluted up to 1:1000. The virus was completely inactivated in 48 hours at room temperature. Filterability was accomplished but with difficulty through a Seitz germicide filter. The virus was found to lose its infectivity in the leaves when air dried for 48 hours.

*Aphis spiraecola* Patch retained the virus the first 3 hours but failed to infect a second lot of plants after that time.

#### RESUMEN

Se ha informado sobre las propiedades físicas del virus del mosaico de la papaya. El virus queda inactivo, mediante una exposición a 60°C durante 10 minutos. No se obtuvo infección alguna cuando el jugo fué diluído hasta 1:1000. El virus queda competamente inactivo en el término de 48 horas a la temperatura del laboratorio. Se efectuó con dificultad la filtración a través de un filtro tipo Seitz No. 5114-C10. Se descubrió que el virus perdió su infectividad en las hojas expuestas al aire durante 48 horas.

El afídido *A. spiraecola* Patch retuvo el virus durante las primeras tres horas; pero, después de ese tiempo, no logró infectar un segundo número de plantas.

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