A MOSAIC OR NONPERSISTENT VIRUS ISOLATED FROM PUMPKIN IN SOUTH DAKOTA [1]

W. N. Stoner
Entomologist, Northern Grain Insects Research Laboratory
Agricultural Research Service, U. S. Department of Agriculture
Brookings, South Dakota 57006

ABSTRACT

A nonpersistent-type virus was isolated from pumpkin plants showing mosaic symptoms in a field near Brookings, South Dakota. Physical properties of the virus were as follows: thermal inactivation at 80°C for 10 min; dilution endpoint 1:80,000 in sterile distilled water; longevity *in vitro* 30+ days at 20°C. Fifty-two species of 40 genera in 15 plant families were tested for susceptibility to the Pumpkin Mosaic Virus (PMV) isolate. Transmission of the virus was affected in seven species of three genera of the Cucurbitaceae and in one species each of four genera in the Leguminosae. Comparison of the physical properties, symptom syndromes, and host ranges of Squash Mosaic Virus (SMV) and PMV indicates a close relationship. Nevertheless, the PMV isolate can be distinguished from SMV isolates by its ability to infect definitive host plants in the Leguminosae and its failure to infect definitive host plants in the Umbelliferae, Solanaceae, and Cucurbitacea.

In a continuing plant virus survey in South Dakota, each year a few abnormal corn, *Zea mays*[2], plants are found with symptoms resembling those induced by infections of Cucumber Mosaic Virus (CMV). Repeated attempts to recover CMV from these plants have failed. In one instance where such corn plants were seen near Brookings, South Dakota, an adjacent field was planted to 'Young's Beauty' pumpkin. Several pumpkin plants along the border of the field were showing mosaic symptoms similar to those induced by CMV infections. Portions of both the corn and pumpkin plants were taken for a study in the laboratory and greenhouse.

Damp chamber tests and microscopic examination of the field specimens proved them to be free of pathogenic fungi and bacteria.

[1]Mention of a trademark or propietary product does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

[2]Botanical nomenclature taken from L. H. Bailey — The Standard Cyclopedia of Horticulture. 3 Vols., 3639 pp., 4056 Figs., Eighteenth Printing, 1960. The Macmillan Company, New York.

Mechanical inoculations were made to an assay host range of plants using whole freshly extracted sap from the individual corn and pumpkin field plants. No transmissions occurred from the corn plants, but in each case pumpkin was infected from every pumpkin specimen. None of the assay host range plants definitive for CMV was infected. This proved that the virus transmitted from the pumpkins was *not* CMV. Additional studies were necessary to determine the identity of the pumpkin virus. A portion of these studies is herein presented.

MATERIALS AND METHODS

The summer squash variety Early Yellow Prolific Straight-Neck (EYPSN), *Cucurbita Pepo* Var. *condensa*, was used as a control, test, and recovery plant throughout the experiments.

Virus sources were whole sap extractions from young, new growth portions of the test plants taken from above the point of inoculation.

The test plants were isolated in a greenhouse that was routinely fumigated with nicotine or Tepp®. No insects were noted and all control plants remained healthy throughout the investigations.

All transmissions in the experiments were made by the carborundum or leaf rubbing method. Test plants were immediately rinsed with tap water after inoculation.

Whenever test plants developed symptoms, a virus recovery trial to 10 EYPSN squash was made. Symptomless test series were all reassayed by bulk recovery trials to 10 EYPSN squash. Recoveries, when obtained, were almost always 100% and in no case less than 70%. Susceptibility ratings were based on virus recovery trials and, where observed, on symptom expression readings.

The method used in determining the temperature at which the PMV isolate was inactivated consisted of placing 5 cc aliquots of whole freshly extracted infective sap in test tubes and plunging these into a constant temperature water bath for 5- and 10-min intervals. The 5-min trials were agitated once for 1 min after being in the bath 2 min. In the 10-min trials, the tubes were agitated three times for 30 sec at about 3-min intervals. The treated sap was cooled in a 55-mm stender dish. Then the heat-treated sap was inoculated to the cotyledons of healthy EYPSN squash seedlings.

Dilution endpoint of infection was determined by taking whole infective sap of the PMV isolate and diluting it with sterile distilled water. Solutions of the various dilutions were immediately inoculated to the cotyledons of healthy EYPSN squash seedlings.

Longevity *in vitro* of the PMV isolates was determined by making daily inoculations to 7-10 day old EYPSN squash plants from a large stock volume of infective whole sap stored at 20 C in a B.O.D. bacteriological incubator. Each succeeding day, 10 EYPSN squash seedlings were inoculated with the stored sap.

RESULTS

Thermal Inactivation: Three experiments were conducted to determine the thermal inactivation temperature of the PMV isolate. The experiments included temperatures ranging from 50 to 90 C at 5- and 10-min intervals. In Experiment 1, there was 100% infection of the test plants except for the last increment at 70 C and 10 min of treatment. In this case, there was a 60% drop in infectivity. Experiment 2, conducted from 66 to 75 C, indicated strongly that the 10-min interval at 75° was approaching the endpoint of thermal inactivation of the virus. Experiment 3 was conducted through a 20° temperature range from 70 to 90 C. The results of Experiment 3 strongly indicated that the thermal inactivation temperature of the PMV isolate was very close to 80 C because the last infection of the virus was obtained from infective sap treated at 80 C at 5 min, whereas no infection occurred from 80 at 10 min onward in the tests. A final test series of 100 EYPSN squash seedlings was inoculated with infective sap heated to 80 C at the 10-min interval. No visible symptoms developed in any of these plants, nor was it possible to recover the virus from them. This final test confirmed that thermal inactivation of PMV isolate occurs between a 5- and 10-min heat treatment of whole infective sap at 80 C.

Dilution Endpoint: Three experiments were conducted to determine the dilution endpoint of the PMV isolate. In Experiment 1, a 1:10,000 dilution produced a 50% reduction in infectivity of whole, infective sap. Experiment 2 was conducted from a 1:1,000 to a 1:40,000 dilution. At 1:40,000, 100% infectivity was still obtained. In Experiment 3, dilutions up to 1 to 90,000 were chosen to extend the dilution range. Experiment 3 with one infection in 20 inoculations at the 1:80,000 dilution indicated an approach to the dilution endpoint. As a final test, 100 squash seedlings were inoculated with a 1:90,000 dilution. None of these plants was infected, indicating that the dilution endpoint of the PMV isolate is very close to 1:80,000.

Longevity in vitro: Three experiments were conducted in an attempt to determine the longevity *in vitro* of the PMV isolate. In Experiment 1, the daily inoculations were discontinued at 23 days. Because the last infection with the virus had occurred at 9 days, this test was carried to the 23-day termination in order to cover the maximum period for definitive symptom development. No inoculations were made in the 1st 4 days of Experiment 2 because test plants were not available. On the 5th day, plants became available and inoculations were made for 10 succeeding days. A 100% infection was obtained in all 10 days. It is believed that the sap in Experiment 1 was only infective for the 1st 9 days because there was an acidic fermentation. In Experiment 2, the sap putrified, indicating a bacterial fermentation. For this reason, a third experiment was conducted. In Experiment 3, the sap remained infective through 30 days and on the 30th day, 5 of the 10 test plants were infected. The experiment was terminated at 30 days because the original volume of sap was exhausted. In the third experiment, the sap again putrified instead of developing an acidic fermentation. The results of these three experiments do not determine the longevity of the PMV isolate *in vitro* precisely, but they do indicate that it is fairly stable and can persist for at least 30 days *in vitro*.

Symptomology: Foliar symptoms described and illustrated in Early White Bush Scallop squash (5) and those given for both foliar and fruit symptoms in Yellow Crookneck squash (7) for plants infected with SMV are similar to, if not indentical with, those induced by PMV isolate infecting EYPSN squash.

To the author's knowledge, however, nothing has been published on the symptoms of SMV in the flowers. EYPSN plants infected with PMV in the early seedlings stage rarely flower. Plants that are infected with PMV after they are half grown, or more, develop leaf symptoms in the new growth and will flower. The blossoms, however, are smaller than normal, but not distorted, and light yellow rather than the normal orange color. Fruits developed from flowers showing decided symptoms of PMV infection frequently produce only a few seeds.

Host Range: Fifty-two species and varieties of 40 genera in 15 plant families were tested for susceptibility to the PMV isolate. In these host range studies, the PMV isolate proved to be infective in all of the Cucurbitaceae tested except two genera *Citrullus* and *Luffa*. The virus was also found to infect seven varieties in four species of four genera of the Leguminosae. Two of these, Lentil and Fenugreek, were symptomless carriers of the virus. In the other legumes, the only discernible symptom of infection was a transitory diffused mottling that continued to appear in the young foliar growth and then disappeared as the leaves matured.

Plants Susceptible to PMV: CUCURBITACEAE — West Indian Gherkin, *Cucumis Anguria;* Cantaloupe, *C. Melo,* vars. Emerald Gem, Golden Champlain, Granite State, Hale's Best, Honeydew, Netted Gem, and Pride of Wisconsin; Cucumber, *C. sativus,* vars. Boston Pickling, Burpee's Hybrid, Improved White Spine, Marketer, Ohio MR17, Smoothie, and Straight 8; Squash, *Cucurbita maxima,* vars. Blue Hubbard, Buttercup, Red Hubbard, Pink Banana, and Warren Turban; Squash, *C. moschata,* var. Butternut 23; Cushaw, *C. mos-*

chata, var. Large Cheese; Pumpkin, *C. Pepo,* vars. Connecticut Field, Jack O'Lantern, Small Sugar, and Young's Beauty; Squash, *C. Pepo,* vars. Boston Marrow, Royal Acorn Bush, Table Queen Bush, and Table Queen Vine; Summer Squash, *C. Pepo* Var. *condensa,* vars. Black Zucchini, Early Yellow Prolific Straight-Neck, and Mammoth White Bush Scallop; Gourd, *C. Pepo* Var. *ovifera,* vars. Apple-shaped, Crown of Thorns, Nest Egg, Flat Striped, Miniature Bottle, Orange, Pear-shaped, Pear-shaped Green and White, Pear-shaped Yellow and Green Striped, Small Spoon, Turks Turban, and Warted; Gourd, *Lagenaria leucantha,* vars. Bottle Large, Dipper Large Fruited, and Hercules' Club; LEGUMINOSAE — Sweet Pea, *Lathyrus odoratus,* var. Spencer; Lentil, *Lens esculenta;* Garden Pea, *Pisum sativum,* vars. Blue Bantam and Freezer 37; Field Pea, *P. sativum* Var. *arvense,* var. Canadian Field Pea; Pod Pea, *P. sativum* Var. *saccharatum,* var. Dwarf Gray Sugar Pod; Fenugreek, *Trigonella Foenum-Graecum.*

Plants Not Susceptible to PMV: AMARANTACEAE — Amaranth, *Gomphrena globosa,* var. Globe Amaranth Purple; APOCYNACEAE — Madagascar Periwinkle, *Vinca rosea,* var. Little Pinkie Dwarf; BALSAMINACEAE — Sultana, *Impatiens Sultani,* var. Scarlet Baby Dwarf; CHENOPODIACEAE — Swiss Chard, *Beta vulgaris* Var. *Cicla,* var. Fordhook Giant; COMPOSITAE — China Aster, *Callistephus chinensis,* var. Burpee's Pompom; Zinnia, *Zinnia elegans,* var. Golden Gem Lilliput Pompom; CRUCIFERAE — Cabbage, *Brassica oleracea* Var. *capitata,* var. Copenhagen; Stock or Gilliflower, *Matthiola incana* Var. *annua,* var. Trysomic 7 weeks; CUCURBITACEAE — Watermelon, *Citrullus vulgaris,* vars. Blue Ribbon, Charleston Gray, Rhode Island Red, and Sugar Baby; Vegetable Sponge, *Luffa cylindrica;* EUPHORBIACEAE — Castor Bean, *Ricinus communis,* var. Red Spire; GRAMINEAE — Sweet Corn, *Zea saccharata,* var. Golden Bantam; LABIATAE — Coleus, *Coleus Blumei* Var. *Verschaffeltii,* var. Vaughn's Rainbow; LEGUMINOSAE — Soybean, *Glycine Soja,* vars. Bansei and Chippewa; Lima Bean, *Phaseolus lunatus Var. macrocarpus,* vars. Fordhook Concentrated and Fordhook 242; Greenbean, *P. vulgaris,* vars. Blue Lake Pole, Blue Lake Stringless, Taylor's Horticultural, Bountiful, Resistant Asgrow Valentine, Sure Crop Wax, and Resistant Tendergreen; Field Bean, *P. vulgaris,* var. Soldier Bean; Pod Pea, *Pisum sativum* Var. *saccharatum,* var. Mammoth Melting Sugar Pod; Clover, *Trifolium repens,* var. Ladino; Horse Bean, *Vicia Faba,* var. Fava; Cowpea, *Vigna sinensis,* var. Early Ramshorn; SOLANACEAE — Pepper, *Capsicum annum* Var. *abbreviatum,* var. Christmas Candle; Sweet Pepper, *C. annuum* Var. *grossum,* var. Yolo Wonder; Jimson Weed, *Datura Stramonium;* Henbane, *Hyoscyamus niger;* Tomato, *Lycopersicum esculentum,* var. Earliana; Evening Star, *Nicotiana alata* Var. *grandiflora,* var. Crimson Bedder; Tobacco, *N. Tabacum,* var. Connecticut Broad Leaf; Tobacco, *N. glauca;* Tobacco, *N. glutinosa;* Tobacco, *N. rustica;* Petunia, *Petunia hybrida,* var. Fire Chief; Solanum, *Solanum Capsicastrum,* var. Felger's Orange Cherry; Husk Tomato, *Physalis Franchetti,* var. Chinese Lantern; TROPAEOLACEAE — Nasturtium, *Tropaeolum majus* Var. *nanum,* var. Globe of Fire; UMBELLIFERAE — Salad Chervil, *Anthriscus Cerefolium;* Celery, *Apium graveolens,* var. Golden Self Blanching; Coriander, *Coriandrum sativum;* VIOLACEAE — Pansy, *Viola tricolor,* var. Swiss Giant.

DISCUSSION

There are several viruses of Cucurbitaceous and Leguminous plants that can closely simulate the symptoms of the PMV isolate in summer squash and garden peas. These are Cucumber Mosaic Virus (1), Watermelon Mosaic Virus (2), Muskmelon Mosaic Virus (3), Pea Mosaic Virus (4), and Bean Yellow Mosaic Virus (8). All of these, however, can be separated from the PMV isolate and the various strains of SMV by differences in physical properties and divergent host ranges. Pea Mosaic Virus and Bean Yellow Mosaic Virus produce mosaic foliage symptoms in garden pea very similar to those induced by the PMV isolate. Neither of these viruses, however, can be transmitted to Cucurbitaceous hosts. An inoculation to two host plants, garden pea and summer squash, will serve to separate these two viruses from PMV. Cucumber Mosaic Virus has an extremely wide host range but can be definitively separated from all other viruses under discussion by inoculation to either Turkish tobacco or *N. glutinosa* which are both susceptible to CMV but not to the others. The remaining cucurbit viruses under discussion are divided into two groups: the melon mosaics and the squash mosaics. The melon mosaics can be separated from the squash mosaics in that they are infectious in watermelon. The squash mosaic group will not infect watermelon with the exception of one called Wild Cucumber Mosaic which does infect watermelon and is serologically distinct from other members of the squash mosaic group (6).

The PMV isolate discussed in this paper is very similar to SMV in its various strains. It can be separated from the other squash mosaic strains or variants, however, in that three species in three genera in the Leguminoseae, Lentil, *Lens esculenta;* Garden Pea, *Pisum sativum;* and Fenugreek, *Trigonella Foenum-Graecum,* are susceptible to the PMV isolate only. Two species in two genera of the Umbelliferae, Salar Chervil, *Anthriscus Cerefolium,* and Coriander, *Coriandrum sativum,* are susceptible to SMV only.

Separation and identification of the PMV isolate can be affected in two ways — 1 inoculation with untreated infective sap to a host range consisting of EYPSN, garden pea, a tobacco, watermelon,

and Salad Chervil or Coriander. If a virus complex is involved, such an inoculation should also serve to separate the complex; (2) separation and identification of the PMV isolate can also be accomplished by the heat treatment of infective sap for 10 min at 70°C and then inoculating with the heat-treated sap to Garden Pea and Salad Chervil or Coriander. Such manipulation should also produce pure cultures of PMV isolate.

The symptomology, physical properties, and host range of the PMV isolate are very similar to those of SMV. It is useful as a working hypothesis to assume that PMV may be a strain or variant of SMV.

LITERATURE CITED

1. Ainsworth, G. C. 1935. Mosaic diseases of the cucumber. Ann. Appl. Biol. 22:55-67.

2. Anderson, C. W. 1954. Two watermelon mosaic virus strains from central Florida. Phytopathology 44:198-202.

3. Anderson, C. W. 1954. Two muskmelon mosaic virus strains from central Florida. Phytopathology 44:371-374.

4. Doolittle, S. P., and F. R. Jones. 1925. The mosaic disease in the garden pea and other legumes. Phytopathology 15:763-772.

5. Freitag, J. H. 1956. Beetle transmission, host range, and properties of Squash Mosaic Virus. Phytopathology 46:73-81.

6. Linderg, G. D., D. H. Hall, and J. C. Walker. 1956. A study of melon and squash mosaic viruses. Phytopathology 46:489-495.

7. Middleton, J. T. 1944. Seed transmission of squash-mosaic virus. Phytopathology 34:405-410.

8. Pierce, W. H. 1934. Viruses of the bean. Phytopathology 24:87-115.