Differences in Virus-Replicating Capacity Among Plant Species Inoculated with Tobacco Mosaic Virus

P. C. Cheo and John S. Gerard

Chief and Research Assistant, respectively, Research Division, Los Angeles State and County Arboretum, Arcadia, California 91006.

Supported in part by Research Grant GB-8543 from the National Science Foundation.

Accepted for Publication 22 March 1971.

ABSTRACT

Fifty-eight plant species were artificially inoculated with tobacco mosaic virus (TMV). Their virus content was determined by means of spectrophotometric measurement at 260 nm and/or by biological assays on cucumber (*Cucumis sativus* L. 'Chicago Pickling') cotyledons. The results can be classified into 5 categories: I) very susceptible, having a virus content above 1,000 μg TMV/g fresh wt plant tissue, 11 species; II) susceptible, having a virus content from 10 μg to 1,000 μg TMV/g fresh wt plant tissue, 12 species; III) slightly resistant, having a virus content from 0.1 μg to 10 μg TMV/g fresh wt plant tissue, 12 species; IV) subliminally infected, resistant, having a virus content of less than 0.1 μg TMV/g fresh wt plant tissue, 20 species; and V) very resistant, probably immune with no recovery of TMV, 3 species. Phytopathology 61:1010-1012.

Comparative resistance to tobacco mosaic virus (TMV) infection among plant species is well established (5). The local lesion reaction in plants is generally considered to represent the resistant type. However, hypersensitivity in the local lesion reaction does not represent genuine physiological resistance. Rather, it is a functional resistance which minimizes further spread of virus and reinfection. Cheo (1) recently reported the subliminal infection of cotton by TMV, and indicated that in cotton a high degree of physiological resistance exists.

Further attempts have been made to investigate the extent of subliminal infection among plant species and to define a quantitative range of subliminal infection. Among 58 plant species studied, a wide range of recoverable virus content is demonstrated. The purpose of this paper is to demonstrate that virus-replicating capacity (VRC) may justifiably be used for classifying relative physiological resistance against virus infection.

MATERIALS AND METHODS.—Seeds of each species were germinated in 4-inch plastic pots containing two parts perlite and one part peat moss. The emerging young seedlings were transplanted to 3-inch clay pots containing one part peat moss and one part silt. The greenhouse, which was fitted with carbon filters for smog control, operated within a diurnal temperature range of 21-29 C. When plants reached the 4-6 leaf stage, they were inoculated with a standard inoculum (0.25 μg/ml of purified TMV solution) by means of the airbrush spray method (6). At weekly intervals for 3 weeks after TMV inoculation, 15 to 25 g fresh wt of inoculated tissue were collected and frozen. The sample was later purified, and the virus content was calculated in μg virus/g fresh wt of tissue. The highest virus content of the weekly collection is used to represent its VRC.

The virus content in plants was measured either spectrophotometrically when virus content was high or biologically when virus content was low. Frozen plant tissue was homogenized (Virtis "45" homogenizer) with three volumes of 0.01 M neutral phosphate buffer containing 0.01 M cysteine HCl. The homogenate was filtered through one layer of cheesecloth, clarified at 60 C for 10-20 min, and given one cycle of low-speed (3,200 *g* for 30 min), high-speed (54,000 *g* for 2 hr), and low-speed (3,200 *g* for 30 min) centrifugation (Beckman Model L ultracentrifuge). The final TMV pellet was resuspended in a known amount of distilled water (8 ml for biological assay and 5 ml for spectrophotometric determination) for virus content determination.

Final preparations having a virus content above 100 μg/ml were measured spectrophotometrically using the Cary 15 spectrophotometer. On the basis of optical density readings of purified TMV solutions (0.27 at 260 nm for an 0.01% solution), approximate virus content was determined. The biological assay was used to determine virus content of samples containing less than 100 μg/ml of virus. A standard curve of purified TMV was prepared by plotting the spectrophotometrically determined concentration of TMV against the average number of starch lesions produced on cucumber cotyledons (1). Final determination of virus content was based on the average number of starch lesions per sample dilution and its proportion to the lesion count of a standard inoculation (0.1 μg/ml TMV) on the same day under the same conditions. By this method, the lesion count was then converted to the value of μg/ml of TMV according to the standard curve. Several dilutions were usually made, so that the logarithmic zone of lesion increase could be used for determination. *Cucumis sativus* L. 'Chicago Pickling' was the starch lesion host used for biological assay. About 7 days after inoculation, the cucumber cotyledons were harvested and stored 24 hr in a dark moist chamber to remove excess starch. Subsequently, the chlorophyll was removed by heating in 70% ethanol. Starch lesions were developed by placing the bleached cotyledons in an IKI-lactic acid stain mixture. The stock IKI mixture was 2% iodine and 6% KI, with further dilution containing 50% lactic acid.

RESULTS.—Fifty-eight plant species were assayed and classified according to their virus-replicating capacity into 5 categories (Table 1). Since seasonal varia-

TABLE 1. Classification of plant species assayed for tobacco mosaic virus (TMV) virus content

Plant species	µg TMV/g fresh wt tissue
I. High virus-replicating capacity[a]	
+*Solanum sarrachoides* Sendt. ex Mart	9,525
+*Saracha umbellata* G. Don	8,422
+*S. edulis* Thellung	5,197
+*Salpiglossis sinuata* Ruiz et Pav.	5,130
+*Physalis ixocarpa* Brot.	2,977
+**Trachymene caerulea* R. Grah.	2,700
+*Marrubium vulgare* L.	2,325
+*Solanum ovigerum* Dun.	1,769
Antirrhinum majus L. 'Floral Carpet Rose'	1,672
+**Beta vulgaris* L.	1,445
**Lactuca sativa* L.	1,185
II. Moderate virus-replicating capacity[b]	
+*Nicotiana arentsii* Goodspeed	735
Tagetes erecta L. 'Golden Jubilee'	503
**Erigeron canadensis* (L.) Cronq.	426
Eucalyptus lansdowneana F. Muell. & J.E. Br.	394
Coleus blumei Benth. (green-yellow pattern)	258
Abutilon indicum S.W.	250
**Nepeta cataria* L.	186
**Atropa belladonna* L.	117
+*Solanum aviculare* Forst.	86
**Cirsium afrum* DC.	32
Scopolia tangutica	30
**Chrysothamnus nauseosus* (Pall.) Britton	24
III. Low virus-replicating capacity[c]	
+*Amaranthus spinosus* L.	2.3
**Clarkia elegans* Dougl.	1.0
Polypodium aureum L.	0.8
Prezewalskia tanjutica	0.56
**Impatiens balsamina* L.	0.46
Amaranthus lividus L.	0.43
Physochlaina orientalis	0.40
**Althaea rosae* (L.) DC.	0.24
Centranthus ruber (L.) DC.	0.22
Rumex patientia L.	0.13
Cassia corymbosa Lam.	0.11
Sida napaea Cav.	0.11
IV. Subliminal infection[d]	
**Zea mays* L. 'Midway'	0.10
**Cucurbita maxima* Duch.	0.085
**Raphanus sativus* L.	0.078
Cestrum aurantiacum Lindl.	0.075
**Zea mays* L. 'Duel'	0.075
Anoda cristata Schlecht.	0.063
**Citrullus vulgaris* Schrad.	0.062
**Cyphomandra betacea* Sendt.	0.060
**Polygonum hydropiper* L.	0.060
**Momordica charantia* L.	0.040
Mandragora officinarum L.	0.040
Lavatera thuringiaca L.	0.031
**Gossypium hirsutum* L. 'Acala 4-42'	0.030
Galega officinalis L.	0.013
**Campanula latifolia* L.	0.0096
Foeniculum vulgare Mill.	0.0096
Tropaeolum vulgaris	0.005
Sophora secundiflora Lag.	0.005
Andropogon condensatus H.B. & K.	0.001
**Pisum sativum* L. 'Freezer 69'	0.001
V. Probably immune to TMV infection	
Cistus villosus L.	
Begonia acuminata Dryand. hybrid	
Saint paulia ionantha H. Wendl. 'Wild White'	

[a] Considered very susceptible, containing more than 1,000 µg TMV/g fresh wt plant tissue. * = Plant species that are reported to be insusceptible to TMV infection listed by Thornberry (9). + = Plants showed visible symptoms, mottling, and chlorosis.

[b] Considered susceptible, containing 10 µg-1,000 µg TMV/g fresh wt tissue.

[c] Considered slightly resistant, containing 0.1 µg TMV/g fresh wt tissue.

[d] Considered resistant, containing less than 0.1 g TMV/g fresh wt tissue.

tions in light and temperature occurred in our greenhouses, some environmentally induced fluctuations in the total virus content were to be expected. The results presented here are not exact values; they represent only a comparative range of the VRC.

Plant species listed under Class I showed systemic symptoms of mottling and yellowing, except *Antirrhinum majus* L. and *Lactuca sativa* L., which were symptomless. All other plant species tested, except *Solanum aviculare* L. and *Amaranthus spinosus* L., were symptomless.

Polypodium aureum L., a species of fern, was infected when artificially inoculated with TMV. No symptoms, including growth inhibition, were observed on infected plants. The VRC in *Polypodium* was not affected by continuous light or continuous dark treatments in excised culture study. This is the first report that a member of the ferns can be infected by TMV. Two monocotyledonous species, *Zea mays* L. and *Andropogon condensatus* H.B. & K., were subliminally infected with TMV.

DISCUSSION.—This paper presents evidence that virus-replicating capacity (VRC) can be used as a means of classifying degrees of plant resistance to TMV infection. Studies with cotton (1) have established that the VRC in cotton is a constant feature, irrespective of the concentration of inoculum used. Similarly in a systemically susceptible host, such as *Physalis floridana* Rybd., the concentration of inoculum can only affect the rate of virus increase during the early stage of infection, not the eventual virus content (*unpublished data*). The environmental conditions under which plants are grown before inoculation, and the conditions at the time of inoculation and during the development of disease, can have profound effects on the course of infection. A plant that is highly susceptible to a given virus under one set of conditions may be completely resistant under another. However, within a standard set of environmental conditions, the VRC could be a species characteristic indicating a degree of resistance or acceptance to TMV infection (Table 1). The physiological condition or resistance in the plant cell, which has not been understood, could be a determining factor in regulating the course of virus infection.

The investigation described in this paper is part of a project concerned with the studies of subliminal infection and immunity of plants when artificially inoculated with TMV. No systematic approach to a taxonomic relationship was intended. The selection of plant species for testing was random, depending on the availability of seed source and with some guide lines from the list of insusceptible species to TMV infection compiled by Thornberry (9). Therefore, our findings have no taxonomic significance. However, we concur with the conclusion made by Price (7) in his host range studies of six plant viruses that "the ability of a plant to support increase of a virus in its tissues is a characteristic of the species itself, and has little or no relationship to taxonomic position of the family".

The lowest limit of our present biological assay appeared to be 0.001 μg TMV/g plant tissue. This concentration of TMV is calculated to contain about 1.5×10^7 TMV molecules. The sensitivity of the cucumber assay is limited to the least level of 1.5×10^8 TMV particles/ml. Therefore, 10 g of plant tissue would be sufficient to detect virus replication for plants having 0.001 μg TMV/g plant tissue. Since a portion of virus is lost during the purification process, it is possible that less than 0.001 μg TMV/g plant tissue, or 1.5×10^7 TMV molecules/g plant tissue, could occur in the immune plant species and not be detected by the present method. Suss et al. (8) reported an increase of infective TMV ribonucleic acid in Chlorella, an alga; TMV replication is reported here on *Polypodium aureum*, a fern. Hamilton & Dodds (4) recently reported subliminal infection of TMV in barley, a monocotyledon. In this report, two cultivars of corn and *Andropogon condensatus* developed subliminal infection by TMV. Evidence is accumulating that TMV could be an independent infective entity, equipped to carry on replication when building materials and energy supply are provided. This is further supported by works of Cochran et al. (3) indicating cell-free biosynthesis of TMV and TMV replication in chick embryo (2). Therefore, plants listed as probably immune (V) should be investigated further. In other words, this immunity should be understood as either a quantitative difference that escapes our present method of detection, or a true immunity in which no infection takes place. African violet (*Saintpaulia ionantha*), for example, is not immune to TMV infection and can be induced by special treatment (*unpublished data*). These studies will be considered in a later report.

LITERATURE CITED

1. Cheo, P. C. 1970. Subliminal infection of cotton by tobacco mosaic virus. Phytopathology 60:41-46.
2. Cochran, G. W., G. M. Fisher, A. Shigematsu, R. A. Smart, L. Shube, & J. L. Chidester. 1969. Replication of an infectious form of tobacco mosaic virus in chicken embryos. Nature 224:1313-1314.
3. Cochran, G. W., A. S. Shaliwal, J. L. Chidester, J. H. Venekamp, C. R. Lamborn, H. L. S. Wang, & R. D. Wilcoxson. 1964. Cell-free biosynthesis of complete tobacco mosaic virus. 6th Int. Congr. Biochem. 226 (Abstr.).
4. Hamilton, R. I., & J. A. Dodds. 1970. Infection of barley by tobacco mosaic virus in single and mixed infection. Virology 42:226-268.
5. Holmes, F. O. 1965. Genetics of pathogenicity in viruses and of resistance in host plants. Advances Virus Res. 11:139-161.
6. Lindner, R. C., & H. C. Kirkpatrick. 1959. The airbrush as a tool in virus inoculation. Phytopathology 49:507-509.
7. Price, W. C. 1940. Comparative host range of six plant viruses. Amer. J. Bot. 27:530-541.
8. Suss, R., E. Sander, B. Roltger, & H. Singer. 1965. An increase on infective ribonucleic acid from tobacco mosaic virus in Chlorella. Biochim. Biophys. Acta 95:388-397.
9. Thornberry, H. H. 1966. Index of plant virus diseases. USDA Handbook No. 307. p. 393-395.