Antigenic Relationships Between Isolates of Mild Dark-Green Tobacco Mosaic Virus, and the Problem of Host-Induced Mutation

C. Wetter

Professor, Department of Botany, University of Saarland, D 66-Saarbrücken, West Germany.
The author gratefully acknowledges the competent technical assistance of Mrs. M. Bernard. He is very thankful to R. W. Fulton for valuable information and to R. G. Milne and M. H. V. van Regenmortel for revising the manuscript.
Accepted for publication 30 May 1984.

ABSTRACT

Wetter, C. 1984. Antigenic relationships between isolates of mild dark-green tobacco mosaic virus, and the problem of host-induced mutation. Phytopathology 74:1308-1312.

With the exception of one isolate from pepper, many isolates of mild dark-green tobacco mosaic virus (MDGTMV) from different host plants at widely scattered locations of the world could not be distinguished in immunodiffusion tests using eight antisera against different isolates. *Eryngium planum* was found to be a systemic host of MDGTMV but immune to TMV. By contrast, tomato was found to be a systemic host of TMV but immune to MDGTMV. Experimental results did not support the hypothesis of host-induced mutation from the common strain of TMV into a strain very similar to the U2 strain of MDGTMV.

In 1927, McKinney collected a strain of tobacco mosaic virus (TMV) from *Nicotiana glauca* R. C. Graham in Gran Canaria, Canary Islands, and named it mild dark-green mosaic strain of TMV (MDGTMV) because of the symptoms caused in Havana tobacco (15). MDGTMV differed from the ordinary light green strain of TMV as it did not infect tomato and did not develop yellow spots due to mutation (16).

Similar mild strains of TMV were detected on several occasions independently in Germany (14) and in the United States (11,13,17,21). The relationships among these strains with MDGTMV were not investigated. Even McKinney was not aware that a virus in pungent pepper that he called South Carolina mottling strain of TMV was in fact MDGTMV (17,28). Since no common name for the virus is generally accepted, the designation mild dark-green tobacco mosaic virus (15) is used in this paper and proposed for all strains and isolates that cannot be distinguished by immunodiffusion tests from the type.

The amino acid composition and the sequence (1,20,31) of strain U2 (21) are nearly identical with those of para-tobacco mosaic virus (PTMV) from Germany (14) and with the green mottling strain of tomato atypical mosaic virus (G-TAMV) from the United States (13). These mild viruses belonging to MDGTMV were long neglected, but recently the wide distribution and the economic importance of strains of MDGTMV have been appreciated (7,27–29,35).

The problem of host-induced mutation (33) has been repeatedly raised with respect to MDGTMV (3,11,13). The possibility was supported by Bald et al (4), who claimed to have converted one virus (U1) into M5 (≈U2) by heat treatment of infected tomato plants.

The purpose of this study was to investigate the serological relationships of MDGTMV strains and isolates coming from widely scattered locations of the world. A further objective was to test the hypothesis of the host-induced mutation.

MATERIALS AND METHODS

Virus propagation and purification. Virus isolates were maintained in *Eryngium planum* L. The seed was incubated on wet paper at −20 C to promote germination. Sap was extracted from infected leaves in 0.1 M phosphate buffer (pH 7.0) and mechanically inoculated to Celite-dusted leaves of test plants followed by a tap water rinse. For mass propagation, virus was multiplied in *Nicotiana tabacum* 'Samsun.' It was purified by the polyethylene glycol precipitation method followed by two to three cycles of differential centrifugation. Virus concentrations were determined by using a specific extinction coefficient of 3.16.

Serology. Rabbits were immunized by giving two intramuscular injections (1–10 mg/ml) followed by one subcutaneous injection 2 days later with 1–10 mg of virus per milliliter emulsified in Freund's incomplete adjuvant. Bleedings were taken beginning 4 wk after the last injection. Antiserum titers were determined with 0.1 and 0.05 mg/ml of purified virus by precipitin drop tests on slides (26). Immunodiffusion tests were conducted on slides as described previously (30). The optimal proportions of reactants were determined in preliminary tests with dilution rows of antigen and antisera. The final tests were equilibrated to an antigen concentration of ~10 mg/ml.

Temperature treatment. The effect of temperature on the resistance of tomato to infection with PTMV was studied in growth chambers. In a first experiment, PTMV-inoculated tomato plants were kept at a temperature of 30–35 C and at 80% relative humidity (RH) with continuous illumination for 14–21 days. Control plants were grown in the greenhouse (18–24 C) for up to 32 days. In a second experiment, six tomato and three tobacco plants were kept for 21 days in a phytotron at 40/15 C day/night temperature with a 12-hr light/dark photoperiod and 80% RH. At intervals samples of equal weight from the inoculated leaves were collected, mixed, and ground. The sap was inoculated to *Datura stramonium* and Xanthi tobacco as local lesion hosts. After the treatment the plants were transferred to the greenhouse and newly formed shoots were tested for systemic spread of virus.

RESULTS

Virus sources, host range, and symptomatology. The symptoms induced by MDGTMV and its strains/isolates (Table 1) in Samsun tobacco were a very mild green mottling or mosaic in the systemically infected leaves, and were sometimes hardly detectable on older leaves. Inoculated leaves showed large local patches when they became old and yellow. Burley type tobaccos in the field developed more severe symptoms (27). Some of the symptoms observed with MDGTMV and TMV in various test plants are summarized in Table 2. In addition to the strains listed in Table 1,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

the virus was isolated from naturally infected *N. glauca* plants collected in the following places: Canary Islands—Gran Canaria, Teneriffe, La Gomera, and La Palma; and also from Madeira. Further isolates came from Tunisia, North Africa, and Corsica, France. The virus could be isolated from Brazilian, but not Chinese, cigarettes.

Separation of MDGTMV from TMV using *Eryngium planum* L. Yarwood (33) suggested studying host passage effects on TMV by using *Eryngium aquaticum* L. (sea-holly or button-snakeroot). The problem of host induced "attenuation" (mutation) versus separation of preexisting strains, however, was already solved by Johnson (11) who came to the conclusion that severe and mild strains of TMV (later called U1 and U2 [21]) "occurred prior to the separation or attenuation by the host plant." In other words, sea-holly is a systemic host for MDGTMV but is not susceptible to TMV. In the present study, *E. planum* was used instead of *E. aquaticum*, but both species react similarly. Almost every German cigarette made with blended tobacco contained both TMV and PTMV (29). Samples of cigarettes were inoculated to *E. planum* and in all of the 84 inoculations TMV could be eliminated from the mixture by passage through this host. Including the strains in Table 1, a total of 101 different isolates of MDGTMV were propagated in *E. planum* and all were subsequently free of TMV.

Tomato as a differential host. Whether passage in tomato could induce mutation was considered by Bald et al (4). According to them, periods at 42/5 C day/night temperature combined with 12-hr light/dark photoperiods should favor host induced mutation. We have not repeated these trials because the very rare event of transition from U1 strain to M5 (≈U2) strain reported by Bald et al (4) seems to be below the level of statistical proof. Instead, the survival of virus in leaves inoculated with PTMV under different environmental conditions was investigated.

From inoculated leaves of tomato plants kept in the greenhouse, PTMV could be recovered up to 14 days after inoculation (Table 3). New shoots on these plants were virus-free. On leaves of plants kept at a temperature between 30 and 35 C, the originally inoculated virus survived up to 17 days after inoculation but not up to 32 days. New shoots of these plants were also virus-free. The results indicate that the resistance of tomato to PTMV infection cannot be broken by high temperatures. In the experiments made in a phytotron the courses of infectivity after inoculation of Samsun tobacco as host and tomato as non-host were compared. The infectivity of tomato leaf samples decreased and reached zero after 17 days (Fig. 1A). After the tomato plants were returned to the greenhouse they formed new shoots that were found to be virus-free. These experiments indicate that tomato is immune to infection in contrast to Samsun tobacco, which supports replication after an eclipse period (Fig. 1B). New shoots of Samsun were systemically infected.

The 5-day period of temperature regimes applied by Bald et al (4) to obtain a transition lies within the time of survival of the virus inoculum in our experiments. Supposing that the U1 inoculum contained U2 as a contaminant, this virus could have been recovered after transfer to a local lesion host. Inoculations of tomatoes with strains of MDGTMV were included in every transfer of the virus for many years and in no case was infection observed. All commercial tomato cultivars tested so far were immune to MDGTMV. If the virus is not replicated in the host cells, mutation induced by the host seems unlikely.

Serology. Serological tests were conducted with eight antisera to the following strains of MDGTMV: original strain ATCC PV 226 (2), one antiserum; PTMV, four antisera; G-TAMV, one antiserum; U2, two antisera. The titers in precipitin tests ranged from 1/1,024 to 1/16,400. The serological differentiation index (SDI) between MDGTMV and TMV in reciprocal tests was 2.5. For the pair MDGTMV-ToMV, SDI = 3; and for the pair TMV-

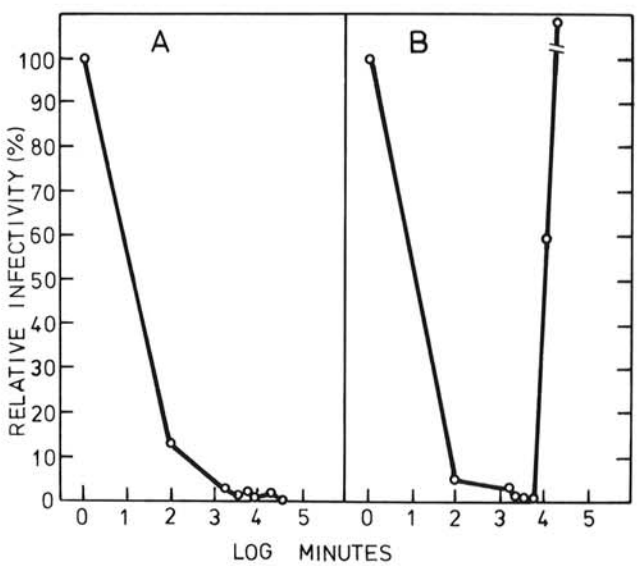

Fig. 1. Comparison of levels of para-tobacco mosaic virus (PTMV) infectivity present on inoculated leaves of immune tomato plants and susceptible tobacco. Virus infectivity was measured in pooled leaf samples collected at different times after inoculation with the PTMV isolate of mild dark-green tobacco mosaic virus (MDGTMV). Plants were kept in a phytotron at 40/15 C day/night with a 12-hr light/dark photoperiod. **A,** Tomato (nonhost); **B,** Samsun tobacco (host). Inoculum was sap of infected *Eryngium planum* which induced >3,000 local lesions per leaf in Xanthi tobacco and *Datura stramonium*. After inoculation to six tomato plants with six leaves each and three tobacco plants with five leaves each and a tap water rinse (zero time) infectivity present in extracts of inoculated leaves was >100 local lesions (= 100% relative infectivity).

TABLE 1. Sources of some strains/isolates of mild dark-green tobacco mosaic virus (MDGTMV)[a]

Strain/isolate	Source	Location	References
MDGTMV (ATCC PV 226)	*N. glauca*	Gran Canaria, Canary Islands	(2,15)
Para-tobacco mosaic virus (PTMV)	*N. tabacum*	West Germany	(14)
Mild mosaic strain of TMV	*E. aquaticum* or field tobacco (?)	Madison, WI, USA	(11)
South Carolina mild mottling strain of TMV (ATCC PV 228)	*C. frutescens*	South Carolina and Georgia, USA	(17,28)
Strain U2	*E. aquaticum* or field tobacco (?)	Madison, WI, USA	(21)
Green-tomato atypical mosaic virus (G-TAMV)	*C. annuum* (?)	Illinois, USA	(13,18)
PTMV (many isolates)	Tobacco from cigarettes	Europe	(29)
PTMV (many isolates)	Field tobacco	West Germany	(27)
Erudina V81-2	*N. glauca*	Australia	cited in (19)
Isolates from pepper	*C. annuum*	Piedmont, Umbria, Italy	(7)
Isolates from gesneriads	Species of Gesneriaceae	USA	(35)

[a]The author is indebted to the following for virus cultures or for collecting leaf samples from infected plants: J. G. Bald; M. Conti; R. W. Fulton; H. Hammer; H. Kaldewey; E. W. Kitajima; C. A. Knight; E. Köhler; M. Marte; K. Müller; J. W. Randles; M. H. V. van Regenmortel; F. W. Zettler.

ToMV, SDI = 2. Similar values were reported earlier (24,30). The serological relationships of these three viruses to odontoglossum ringspot virus was in all combinations nearly equal with SDIs of ~ 3. In immunodiffusion tests, the strains in Table 1 and many other isolates could not be distinguished with the eight antisera (Fig. 2A). Only one strain named Italian III (It III) from pepper reacted differently. In comparative tests It III reacted with a fusion of bands when tested next to U2, G-TAMV, and PTMV with It III antiserum. In contrast, tests with U2, G-TAMV, and PTMV antisera showed that the homologous antigens formed a spur over It III. This strain from pepper was kindly supplied by M. Marte, Perugia, Italy. Results on It III will be published separately.

In immunodiffusion tests with the homologous antiserum MDGTMV formed a spur over TMV, ToMV (Fig. 2C and D) and the other heterologous antigens of Fig. 2B. When an antiserum of PTMV was absorbed with seven heterologous wild strains of TMV (30) it still reacted strongly with the homologous antigen (Fig. 2B).

DISCUSSION

With the exception of isolate It III, which was distinct, no serological differences between MDGTMV isolates from different host plants and locations could be found in immunodiffusion tests. The isolates differed, however, in symptom expression in tobacco and other host plants. The results suggest that the group of MDGTMV isolates is uniform with respect to antigenic properties, like ToMV (25) but in contrast to the antigenic variability of strains of ribgrass mosaic virus (6,12).

MDGTMV has been regarded as a distinct virus of the tobamovirus group (9,13,21,23). The interrelationships between the wild strains TMV, ToMV, and MDGTMV have been characterized by determining the amino acid sequence homology of the coat proteins (9). These values correlate well with the SDIs reported recently (30) (Fig. 3). Though the coat protein of tobamoviruses is coded for by only 10% of the RNA genome, protein homology and SDIs seem to be valuable criteria for the quantitative estimation of natural relationships between these viruses. When the total RNA genomes of TMV (U1) and MDGTMV (U2) in hybridization experiments with complementary DNA were compared under stringent conditions, no sequence homology was found (19). Under less stringent conditions only a small amount of hybridization was found, suggesting a distant relationship. These latter findings strongly support the view that MDGTMV and TMV should be regarded as separate viruses, but they give no additional information on the degree of relationship.

According to Bald (3) 'forms' of viruses are variants that are adapted to special hosts. The U1 strain of TMV would be the form adapted to tobacco and the U2 strain would be adapted to *N. glauca*. This definition of 'forms' seems to be questionable since mild strains like U2 were for a long time reported from field tobacco (3,10,11). PTMV, but not type TMV, is prevalent in Burley tobacco in West Germany. Johnson's mild strain originally came from *E. aquaticum* or from tobacco. Furthermore, strains of MDGTMV are common in pepper (17,28), and gesneriads (35). *N. glauca*, on the other hand, is infected frequently with TMV and MDGTMV (3,8,15), and the strains U2 and G-TAMV were originally isolated from mixtures.

TABLE 2. Comparison of symptoms induced by mild dark-green tobacco mosaic virus (MDGTMV) and tobacco mosaic virus (TMV) in some test plants

Test plant	Virus [a]	
	MDGTMV	TMV
Capsicum annuum L.		
'Sperling's Merit'	M/S	LL
Datura stramonium L.	LL	LL
Eryngium planum L.	M/S	0
Lycopersicon esculentum L.	0	M/S
Nicotiana glauca R. C. Graham	M/S	M/S
N. glutinosa L.	LL	LL
N. sylvestris Speg & Gomes	LL	M/S
N. tabacum L.		
'Samsun'	M/S	M/S
'White Burley'	LL	M/S

[a] Abbreviation for symptoms: LL = local lesions, not systemic. M = mosaic or mottle. S = systemic. 0 = no infection.

TABLE 3. Survival of para-tobacco mosaic virus (PTMV) on inoculated leaves of tomato after different times and under different environmental conditions

Experiment	Number of plants	Treatment	Time of local lesion test after inoculation (days)	Local lesions induced by extracts from inoculated leaves[a]	Local lesions induced by extracts from new shoots
1	4	Greenhouse[b]	32	0	0
2	3	30 C	21	0	0
3	3	32 C	14	22	0
4	4	32 C	14	1	0
5	3	Greenhouse	14	0	0
6	4	35 C	14	5	0
7	3	Greenhouse	14	0	0
8	4	35 C	17	1	0
9	3	Greenhouse	17	0	0

[a] Highest number of local lesions obtained either on *Datura stramonium* or on Xanthi tobacco. The original inoculum induced an average of >100 local lesions per leaf on these hosts.

[b] Mean greenhouse temperatures were 20–24 C.

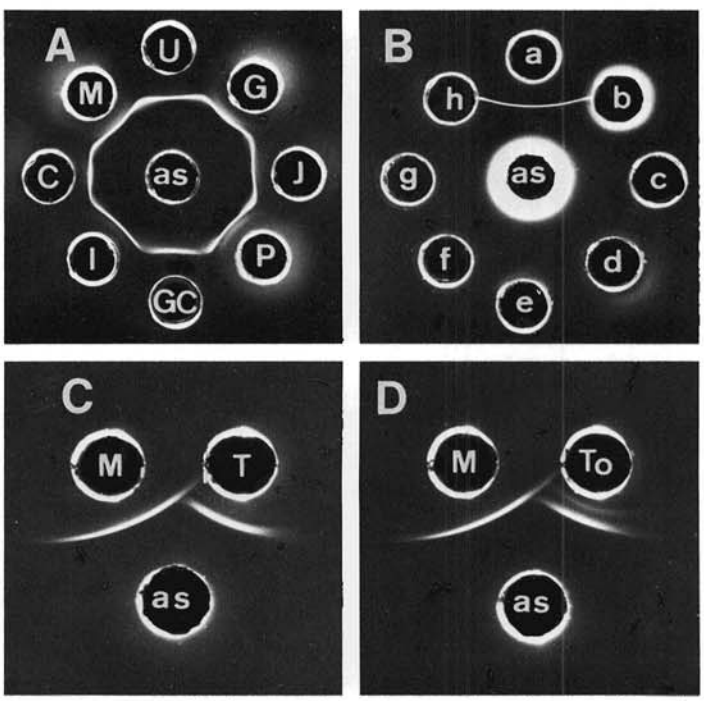

Fig. 2. Immunodiffusion and intragel cross absorption reactions of homologous and heterologous antigens with para-tobacco mosaic virus (PTMV) and mild dark-green tobacco mosaic virus (MDGTMV) antisera. **A,** Antigens are abbreviated as follows: U = U2 strain; G = G-TAMV; J = Johnson's mild strain; P = PTMV from field tobacco, Germany; GC = isolate from *Nicotiana glauca*, Gran Canaria; I = Italian isolate from pepper; C = isolate from *N. glauca*, Corsica; M = MDGTMV (ATCC PV 226); PTMV antiserum (as) was in the central well. **B,** Intragel cross absorption test with PTMV antiserum. Central well was charged initially with antigens b to h and 2 hr later with PTMV antiserum (as). Peripheral wells were charged with the following antigens (30): a = PTMV; b = tomato mosaic virus; c = sunn hemp mosaic virus; d = ribgrass mosaic virus; e = odontoglossum ringspot virus; f = Ohio III virus; g = cucumber mosaic virus 4; h = TMV. **C and D,** Immunodiffusion tests with antiserum to MDGTMV (as). **C,** Test against MDGTMV (M) and TMV (T) antigens. **D,** Test against MDGTMV (M) and ToMV (To) antigens.

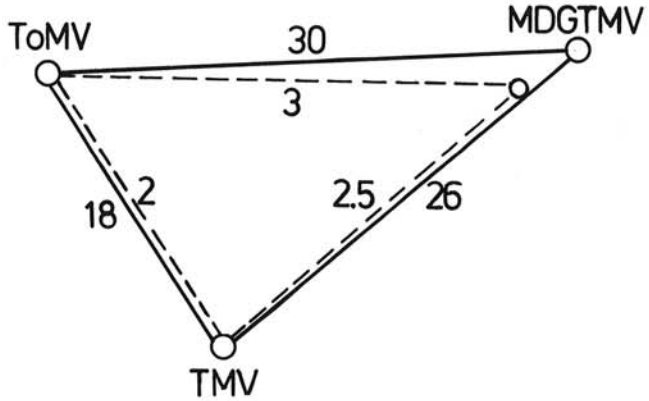

Fig. 3. Interrelationships between tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), and mild dark-green tobacco mosaic virus (MDGTMV). Amino acid sequences of the coat proteins are compared with serological differentiation indices (SDI). Values outside the triangle (solid line) refer to percentages of amino acid exchanges (9). Values inside the triangle (dashed line) refer to SDIs in reciprocal tests (30). The relationship is expressed in length units and TMV-ToMV was chosen as the baseline.

What makes the designation 'form' of a virus still more questionable is the meaning that was given it by Bawden (5). Bawden claimed that reversible changes between a tobacco form and a bean form of the sunn hemp mosaic virus were possible, but these results were not confirmed (33,34). My own experiments with several hundred tobacco and bean plants failed to obtain a reversible 'transformation' (*unpublished*). Very probably, the changes observed by Bawden (5) resulted from contamination. Our experimental results also do not support the hypothesis of a host induced 'transition' of the U1 to the M5 (≈U2) strain as suggested by Bald et al (4).

Other arguments against this type of mutation come from the work on induced and spontaneous mutants of TMV. U2 differs in 34 amino acids from U1, that is 21% of the gross composition and 26% of the sequence (9). In their work on about 200 TMV mutants, Wittmann and Wittmann-Liebold (32) did not find more than three exchanges per mutant. Similar results were obtained by Tsugita (22) who studied more than 80 naturally occurring strains and induced mutants. His results are of special interest since he investigated five mutants of G-TAMV induced by three mutagenic agents and found that only two differed in one amino exchange from type G-TAMV. Since our own results failed to demonstrate any multiplication of MDGTMV in tomato we conclude that TMV and MDGTMV are naturally occurring strains that evolved over a long time by mutation and selection in other host plants.

In light of these results, an explanation for the obscure origin of G-TAMV (13,31) may be offered. Probably, the infected leaf sample supplied to Knight by Thornberry contained a mixture of two viruses, because Miller and Thornberry (18) originally worked with two isolates. One came from field pepper (possibly G-TAMV) and the other came from field tomato (possibly Y-TAMV ≈ ToMV). Both of these isolates were considered to be identical and were propagated in tobacco (18), a host that easily permits contamination. Although the isolates are difficult to distinguish by host reactions, Knight et al (13) were able to separate them. This explanation on the origin of G-TAMV seems to be plausible because G-TAMV and U2, also studied at the same time, differed by one amino acid exchange (23). The alternative explanation on the origin of G-TAMV mentioned by Knight et al (13) would be a host-induced mutation occurring during repeated transfers. This hypothesis has been refuted in the present study.

The name green tomato atypical mosaic virus (G-TAMV) would thus seem particularly unsuitable for a virus that was not isolated from tomato. It seems preferable to use the name of mild dark-green tobacco mosaic virus originally proposed by McKinney (15) for the group of serologically closely related entities labeled para-tobacco mosaic virus by Köhler and Panjan (14) and U2 by Siegel and Wildman (21).

LITERATURE CITED

1. Altschuh, D., Reinbolt, J., and van Regenmortel, M. H. V. 1981. Sequence and antigenic activity of the region 93-113 of the coat protein of strain U2 of tobacco mosaic virus. J. Gen. Virol. 52:363-366.
2. American Type Culture Collection. 1981. Catalogue of strains II. Third ed. Rockville, MD. 413 pp.
3. Bald, J. G. 1960. Forms of tobacco mosaic virus. Nature 188:645-647.
4. Bald, J. G., Gumpf, D. J., and Heick, J. 1974. Transition from common tobacco mosaic virus to the *Nicotiana glauca* form. Virology 59:467-476.
5. Bawden, F. C. 1958. Reversible changes in strains of tobacco mosaic virus from leguminous plants. J. Gen. Microbiol. 18:751-766.
6. Chessin, M., Juretić, N., Miličić, D., Perryman, J., and Giri, L. 1980. A distinctive strain of ribgrass mosaic virus from Montana, USA. Phytopathol. Z. 97:295-301.
7. Conti, M., and Marte, M. 1983. Virus, virosi e micoplasmosi del peperone. Ital. Agric. 120:132-152.
8. Faccioli, G., and Paul, H.-L. 1967. Vergleichende Untersuchung von vier Isolaten des Tabakmosaik-Virus aus dem mediterranen Raum. Phytopathol. Mediterr. 6:41-47.
9. Hennig, B., and Wittmann, H. G. 1972. Tobacco mosaic virus: Mutants and strains. Pages 546-672 in: Principles and Techniques in Plant Virology. C. J. Kado and H. O. Agrawal, eds. Van Nostrand Reinhold Co., New York. 688 pp.
10. Johnson, E. M., and Valleau, W. D. 1935. Mosaic from tobacco one to fifty-two years old. Pages 264-271 in: Kentucky Agric. Exp. Stn. Bull. 361. Lexington.
11. Johnson, J. 1947. Virus attenuation and the separation of strains by specific hosts. Phytopathology 37:822-837.
12. Juretić, N., and Wetter, C. 1973. Serological relationship among some necrotic strains of the Holmes' ribgrass mosaic virus. Plant Virology (Pages 369-375 in: Proc. 7th Conf. Czech. Plant Virol., High Tatras, 1971), Slovak Acad. Sci., Bratislava. 573 pp.
13. Knight, C. A., Silva, D. M., Dahl, D., and Tsugita, A. 1962. Two distinctive strains of tobacco mosaic virus. Virology 16:236-243.
14. Köhler, E., and Panjan, M. 1943. Das Paramosaikvirus der Tabakpflanze. Ber. Dtsch. Bot. Ges. 61:175-180.
15. McKinney, H. H. 1929. Mosaic diseases in the Canary Islands, West Africa, and Gibraltar. J. Agric. Res. 39:557-578.
16. McKinney, H. H. 1935. Evidence of virus mutation in the common mosaic of tobacco. J. Agric. Res. 51:951-981.
17. McKinney, H. H. 1952. Two strains of tobacco mosaic virus, one of which is seed-borne in an etch-immune pungent pepper. Plant Dis. Rep. 36:184-187.
18. Miller, P. M., and Thornberry, H. H. 1958. A new viral disease of tomato and pepper. Phytopathology 48:665-669.
19. Palukaitis, P., and Symons, R. H. 1980. Nucleotide sequence homology of thirteen tobamovirus RNAs as determined by hybridization analysis with complementary DNA. Virology 107:354-361.
20. Rentschler, L. 1967. Aminosäuresequenzen und physikochemisches Verhalten des Hüllproteins eines Wildstammes des Tabakmosaikvirus. I. Analyse der Primärstruktur (Pos. 62-134) des Hüllproteins vom Wildstamm U2. Molec. Gen. Genet. 100:84-95.
21. Siegel, A., and Wildman, S. G. 1954. Some natural relationships among strains of tobacco mosaic virus. Phytopathology 44:277-282.
22. Tsugita, A. 1962. The proteins of mutants of TMV: Classification of spontaneous and chemically evoked strains. J. Mol. Biol. 5:293-300.
23. Van Regenmortel, M. H. V. 1967. Serological studies on naturally occurring strains and chemically induced mutants of tobacco mosaic virus. Virology 31:467-480.
24. Van Regenmortel, M. H. V. 1975. Antigenic relationships between strains of tobacco mosaic virus. Virology 64:415-420.
25. Wang, A. L., and Knight, C. A. 1967. Analysis of protein components of tomato strains of tobacco mosaic virus. Virology 31:101-106.
26. Wetter, C. 1965. Serology in virus-disease diagnosis. Annu. Rev. Phytopathol. 3:19-42.
27. Wetter, C. 1980. Occurrence of para-tobacco mosaic virus in field tobacco in South-West Germany. Z. Pflkrankh. Pflanzenschutz 87:150-154.
28. Wetter, C. 1984. Serological identification of four tobamoviruses infecting pepper. Plant Dis. 68:597-599.
29. Wetter, C., and Bernard, M. 1977. Identifizierung, Reinigung und serologischer Nachweis von Tabakmosaikvirus und Para-Tabakmosaikvirus aus Zigaretten. Phytopathol. Z. 90:257-267.
30. Wetter, C., Conti, M., Altschuh, D., Tabillion, R., and van Regenmortel, M. H. V. 1984. Pepper mild mottle virus, a tobamovirus infecting pepper cultivars in Sicily. Phytopathology 74:405-410.

31. Wittmann, H. G. 1965. Die primäre Proteinstruktur von Stämmen des Tabakmosaikvirus. Teil IV. Aminosäuresequenzen (Pos. 1-61 und 135-158) des Proteins des Tabakmosaikvirus-Stammes U2. Z. Naturforsch. 20b:1213-1223.
32. Wittmann, H. G., and Wittmann-Liebold, B. 1966. Protein chemical studies of two RNA viruses and their mutants. Cold Spring Harbor Symposia on Quantitative Biology 31:163-172.
33. Yarwood, C. E. 1979. Host passage effects with plant viruses. Adv. Virus Res. 25:169-190.
34. Zaitlin, M., Beachy, R. N., and Bruening, G. 1977. Lack of molecular hybridization between RNAs of two strains of TMV. A reconsideration of the criteria for strain relationships. Virology 82:237-241.
35. Zettler, F. W., and Nagel, J. 1983. Infection of cultivated gesneriads by two strains of tobacco mosaic virus. Plant Dis. 67:1123-1125.