IDENTIFICATION OF TWO SEROTYPES IN SQUASH MOSAIC VIRUS STRAINS

by

Hjalmar F. K. Knuhtsen

A Thesis Submitted to the Faculty of the

DEPARTMENT OF PLANT PATHOLOGY

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

1 9 6 8

STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for an advanced degree at the University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from, or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Richard F. C. Hunter

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

Merritt R Nelson
M. R. NELSON
PROFESSOR OF PLANT PATHOLOGY

Oct. 19, 1967
Date

ACKNOWLEDGMENTS

The author wishes to express his gratitude for the guidance and counsel of Dr. Merritt R. Nelson, Professor of Plant Pathology, The University of Arizona, under whose leadership this investigation was conducted.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

ABSTRACT

The common and watermelon strains of squash mosaic virus have similar physical and biological properties. While cross reactions of antisera and antigens of each indicate a close serological relationship, cross absorption studies and immunodiffusion tests show that each strain has at least some antigenic sites not common to the other. The watermelon strain appears to produce the greatest number of strain specific antibodies.

Immunoelectrophoretic studies in agar gel show no appreciable differences in the electrophoretic mobility of the virus strains at pH 8.6.

Of four isolates tested, only two serotypes are identified in this study.

INTRODUCTION AND LITERATURE REVIEW

Early reports of squash mosaic virus (SMV), which include those by Dolittle and Gilbert (5), Kendrick (11) and Middleton (13), indicated that this virus was seed borne and beetle but not aphid transmitted.

Freitag's original description (6) characterized the virus as being aphid and beetle transmitted, though he subsequently ascertained that it was only beetle transmitted (8). Physical properties were reported by Freitag (7) as: a dilution end point of 10^5 to 10^6, longevity in vitro for as long as six weeks at 20 C or six months at 0 C, and a temperature of 75 C for 10 minutes would inactivate all of the virus in a sample. Resistance of the virus to elevated temperatures was restudied later by Lindberg, Hall and Walker (12), who found this thermal inactivation point to be between 60 C and 70 C.

A cucurbit ring mosaic virus, also reported by Freitag (6), resembled the squash mosaic virus described above with respect to insect vectors, host range, and physical properties. This virus showed a remarkable similarity to others described below.

Anderson (1, 2) noted chlorotic spots or rings as symptoms on three isolates of cucurbit mosaic viruses

from central Florida. Two of these isolates, the typical muskmelon mosaic and latent muskmelon mosaic virus, were placed in the squash mosaic virus group with the cucurbit ring mosaic virus of Freitag (6) by Lindberg, Hall and Walker (12). These authors found the above three isolates to be identical with respect to symptoms expressed on Cucurbita pepo host range, physical properties, insect transmission, morphology, and serological cross reactions.

The muskmelon mosaic virus described by Rader (16) and Aycock (3) resembles the above viruses with respect to symptoms expressed on C. pepo L. or Cucumis melo L. and should be considered as strains of SMV on the basis of their high inactivation temperatures, and host ranges.

Keener (10) reported a seed borne virus of cucurbits of low incidence in both field and greenhouse trials, along with a description and photographs of an unidentified virus which produced vein banding and chlorotic interveinal areas. The percentages of plants showing these symptoms were also of low incidence when sampled in a 40 acre stand.

Grogan, Hall and Kimble (9) obtained virus infected cantaloupe plants from commercial seed. They noted that all plants, when indexed in a differential host range study, gave reactions typical of the "cucurbit ring mosaic strain" or cucurbit ringspot strains of

SMV (6). This virus was reported to be transmitted by Diabrotica undecimpunctata Mann. beetles, and the physical properties of the virus samples were within the ranges set by Freitag (7) and Lindberg, Hall and Walker (12).

Nelson, Matejka and McDonald (14) isolated a strain of SMV from cantaloupe in Arizona which, according to criteria previously established (12), is similar to the common strain of SMV but differs principally by its ability to infect watermelon (Citrullus vulgaris Schrad.). The Arizona isolate has been found to be similar to SMV (common strain) with respect to thermal inactivation, dilution end point, retention of infectivity when aged in vitro, insect transmission and cross protection studies. Serological studies with both viruses and antiserum to the SMV (common strain) demonstrated that the common and Arizona watermelon strains both reacted strongly, indicating a close relationship (14). In addition, the symptoms produced by these two strains on hosts susceptible to both are different. This difference in the host range and symptomatology prompted this study to determine whether an antigenic heterogeneity exists and the extent of this difference. There are numerous reports of such differences between strains of plant viruses but none previously for SMV.

MATERIALS AND METHODS

Viruses, Hosts, and Purification

The common strain of SMV originated from cucurbits grown in Wisconsin and was increased most satisfactorily in the small sugar pumpkin (Cucurbita pepo L.). The watermelon strain was isolated from cantaloupes, and produced the highest virus concentrations in this host. These isolates were used for preparation of the antisera.

SMV (mild) and SMV (pumpkin) isolates were obtained respectively from W. H. Sill at Kansas State University and W. H. Stoner of the Northern Grain Insects Research Laboratory, ARS, USDA, Brookings, South Dakota. The other watermelon isolate SMV-W (4-25-26) was isolated from Arizona melons by M. R. Nelson. Symptoms induced by the five different isolates, and associated host range data, are shown in Table 1.

Seedlings are inoculated during the cotyledon stage, and leaf tissue was harvested, generally after the true leaves bore evidence of a systemic infection such as mottle or vein banding (7-20 days).

The following procedure was used for purification of both viruses, and was found to be the simplest and

Table 1

Symptoms of the Common and Watermelon Isolates of Squash Mosaic Virus

Strain	Cucurbita pepo L.	Cucumis melo L.	Citrullus vulgaris Sch.
*SMV-C	Severe systemic mottle and distortion	Very mild mosaic	No infection
SMV-P	Severe systemic mottle and distortion	Very mild mosaic	No infection
SMV-M	Mild mosaic, little distortion	Very mild mosaic	No infection
*SMV-W (10-28-4)	Chlorotic rings, later mild mosaic which disappear in 10-15 days	Early, severe vein banding; later, mild mosaic	Severe stunt, necrotic, chlorotic lesions
SMV-W (4-25-26)	Chlorotic rings, later very mild mosaic; symptoms persist	Mild mosaic	Mild mosaic

* Antisera prepared for these isolates.

most productive. Plant sap was extracted by mincing infected tissue in a blender in phosphate buffer (0.1 M at pH 6.8) in a proportion of 1:1 (w/v) tissue to buffer. The resulting extract was expressed through cheesecloth and chilled to less than 10 C in an ice bath. The suspension was then centrifuged for 20 min at 10,000 rpm in a Lourdes model A-2 Beta-Fuge. The pellet was discarded, and the supernatant was heated at 60 C for 10 min in a constant temperature water bath. The heated sap was cooled rapidly to less than 10 C; this green debris was removed by centrifugation at 10,000 rpm for 20 min. The extract was subjected to two to three cycles of differential centrifugation alternating between the Beckman model L-2 preparative ultracentrifuge with the No. 30 rotor at 28,000 rpm and 10,000 rpm in the Lourdes model A-2 Beta-Fuge. Viral concentrations in mg/ml in each preparation were determined using the absorbance values of Bancroft (4) at 260 mμ.

Production and Testing of Antisera

Prior to injection into rabbits, the purified virus preparations were tested for purity and freedom from strain contamination by inoculations of three different susceptible cucurbitaceous hosts. Contamination of the watermelon by the common strain could be detected easily by symptom differences, whereas, contamination of

the common by the watermelon strain was detected by host range differences.

To produce an antiserum specific for each virus strain, 1-2 mg of the respective purified viral preparations were injected intravenously into adult rabbits. These injections were made at weekly intervals over a period of four weeks, after which blood was obtained via non-terminal cardiac puncture. The antisera were then titrated by the tube precipitin test.

Titrations were also carried out by a simple modification of the double diffusion technique of Ouchterlony (15) using 85 x 15 mm disposable plastic petri dishes. Fifteen ml of 1.0% Ion Agar II (Consolidated Laboratories, Chicago Heights, Illinois) dissolved in glass distilled water with 0.85% NaCl and 1:5000 w/v sodium azide added as a weak preservative, were poured and allowed to harden overnight. The test pattern for the above titrations consisted of three rows of wells 5 mm wide, equally spaced 1 cm apart from their centers with the center row offset 5 mm to the right. Uniformity was maintained by the use of an apparatus similar in design to that suggested by Wright and Stace-Smith (19). Titrations obtained by use of this technique were in good agreement with those performed by the tube precipitin method.

Cross Reaction

Cross reaction studies were carried out in agar gels as described, and antisera were titrated using a two-fold dilution scheme beginning with a 1:10 dilution of each antiserum and terminating with a dilution of 1:1280. These diluted antisera were then tested against each antigen, and read to the last barely visible precipitin band after an incubation period of 48-72 hrs.

Cross Absorption Procedure

Cross absorption was performed using antisera with titers of 1:640. Quantities of 0.5 ml of antiserum were allowed to warm to the incubation temperature of 37 C for 30 min prior to the addition of 1.5 ml (0.1 mg/ml) of each hetrologous antigen. This mixture was allowed to incubate for 2 hrs. Then the precipitate was removed by centrifugation at 2,000 RPM for 20 min in an IEC clinical centrifuge. The supernatant was saved, and the procedure repeated until a precipitate no longer formed. The absorbed antisera were adjusted to a dilution of 1:10 with phosphate buffered saline, serial dilutions made to at least 1:1280, and tested against their respective homologous antigens in agar gels.

Immunodiffusion

Immunodiffusion tests were done in agar gel prepared as above. The pattern utilized consisted of a center well 1.0 cm in diam with six peripheral wells spaced 2.0 cm from the center well as measured from the centers of the wells. Antigens were adjusted to the same concentration, and antisera with a titer of 1:640 were used consistently.

Immunoelectrophoresis

Immunoelectrophoretic analysis of the viruses was performed by the microslide method of Scheidegger (18) in 0.75% agar gel (Ion agar II) dissolved in 0.05M pH 8.6 barbitalacetate buffer. Electrophoresis (250 v, 50 ma) was terminated after 2.5 hr, then the center trough was cut between and 4 mm from the antigen wells, 100 μ liters of antiserum were diffused against the virus zones for 12 hr.

RESULTS

Reactions of the Viruses on Three Cucurbitaceous Hosts

The watermelon strain induced symptoms on C. pepo which were described best as a ring spot mottle. The spots varied in size (2-4 mm) and number on the first true leaves to emerge from the plant which had been inoculated during the cotyledon stage (Fig. 1-B, 1-C). The symptoms appeared suddenly about the fifth day after inoculation, and were lost entirely within 14 days, although the presence of virus could still be demonstrated in the near symptomless plants.

Symptoms expressed on the same host by the common strain of SMV also were noted first on the fifth day after inoculation, and began with a solid chlorotic pin point about 1 mm in diameter, later developing into solid spots. Eventually the typical vein banding, cupping, blistering, and epinasty accompanied by severe leaf distortion were observed (Fig. 1-E, 1-F). The symptoms did not fade in a young actively growing plant. Symptom overlap occurred between these isolates as shown in Fig. 1-D. When the density of the chlorotic spots became very great, they coalesced and resembled the symptoms expressed

Fig. 1. Reactions of the common and watermelon strain of SMV on *C. pepo* L.

A. Healthy leaf.

B. Infected leaf SMV (w) showing the large cucurbit ring symptom.

C. Infected leaf showing the small cucurbit ring symptom.

D. Infected leaf showing small cucurbit ring symptoms after coalescence of rings and some necrosis.

E. Infected leaf showing the type of systemic mottle induced by the common strain of SMV.

F. Same as E; the diseased leaf is exhibiting distortion, epinasty, and reduction in interveinal tissue.

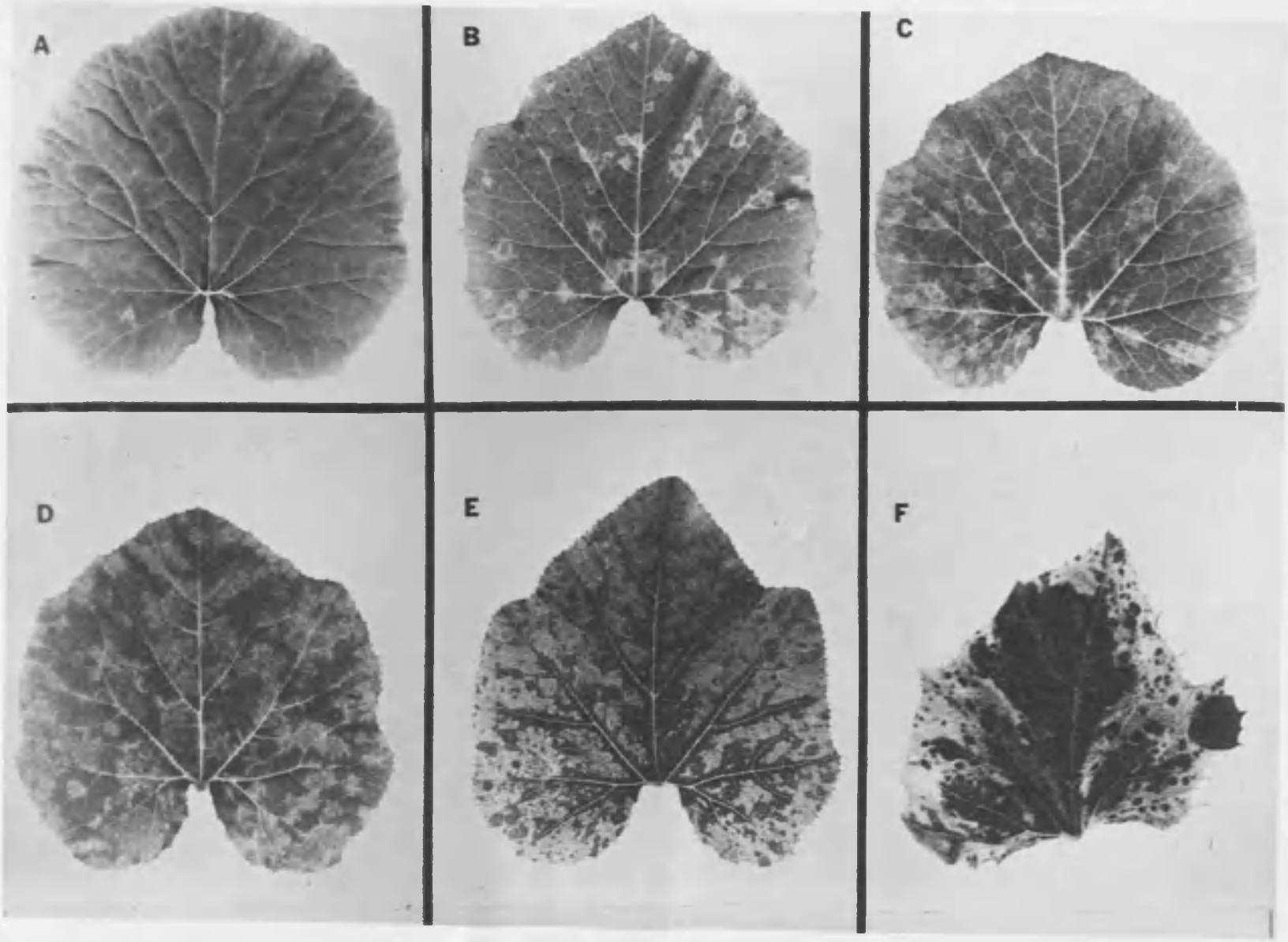

Fig. 1. Reactions of the common and watermelon strain of SMV on C. pepo L.

on this host by the cucurbit virus isolate described by Aycock (3).

C. melo was also susceptible to both isolates, and symptoms induced by the watermelon strain of SMV were marked by a very obvious vein banding on the first true leaf; the veins were bordered by an area of dark green set off by an area of lighter green (Fig. 2-B). As the disease advanced several different symptoms were noted on the plant, but the mottle was rarely as definite as shown (Fig. 2-B) but were marked usually by a mild chlorotic mottle showing little or no reference to the veins (Fig. 2-C, 2-D). Some leaves showed a serrated effect due to reduced interveinal tissue (Fig. 2-E). Leaves, which produced this effect, might or might not show mottle. Leaf distortion was found infrequently and usually took the form of curling along the longitudinal axis of the leaf.

The common strain of SMV induced very mild symptoms on this host beginning with vein clearing, and later caused a very mild mosaic pattern similar to the pattern shown in Fig. 2-C. These symptoms might not be obvious in all infected plants, and infection by this isolate might be easily overlooked on this host.

Citrullus vulgaris Schrad. was found to be susceptible only to the watermelon strain of SMV and showed

Fig. 2. Reactions of the watermelon strain of SMV on *C. melo* L.

A. Healthy leaf.

B. Infected leaf showing dark green vein banding and islands of lighter green between the veins.

C. Infected leaf showing the mild generalized mottle.

D. Infected leaf showing the stronger generalized mottle.

E. Infected leaf showing the serrated edge caused by reduction of interveinal tissue and exhibiting no mottle.

F. Infected plant from the greenhouse showing the typical early vein banding symptom.

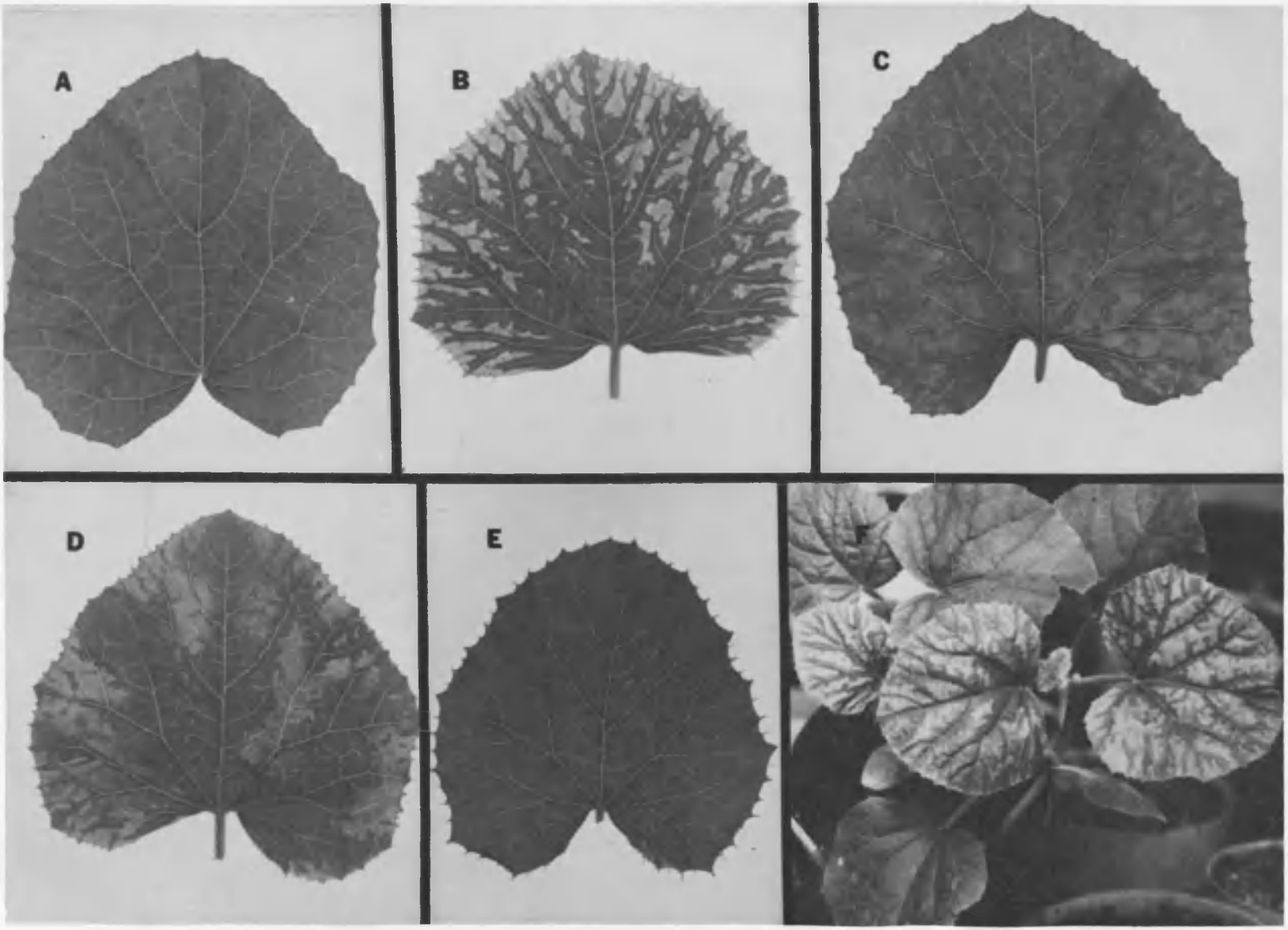

Fig. 2. Reactions of the watermelon strain of SMV on C. melo L.

a hypersensitivity marked by a severe stunt and leaf distortion (Fig. 3-B, 3-C). Chlorotic spots developed on the leaves after 2-3 weeks which later became necrotic giving the appearance of local lesions. The stunting effect was partially overcome but the plant never again attained the growth rate of the healthy control.

Serology

General

Pellets from the final high speed centrifugation of the purified virus preparation yielded 9-10 mg of virus/100 g of infected host tissue. The pellets were clear and exhibited an ultraviolet absorption spectrum which was typical of nucleoproteins with a minimum absorption at 240 mμ and a maximum at 260 mμ; extracts from healthy tissue absorbed ultraviolet light only slightly showing no peak at 260 mμ. These final virus preparations were found to be infectious when inoculated into susceptible test plants.

Cross Reaction Studies

These data confirmed the original work of Nelson, Matejka and McDonald (14) and indicated that antiserum to each strain reacted strongly with the heterologous antigen. These studies indicated that simply reacting diluted antiserum against a constant concentration of either virus

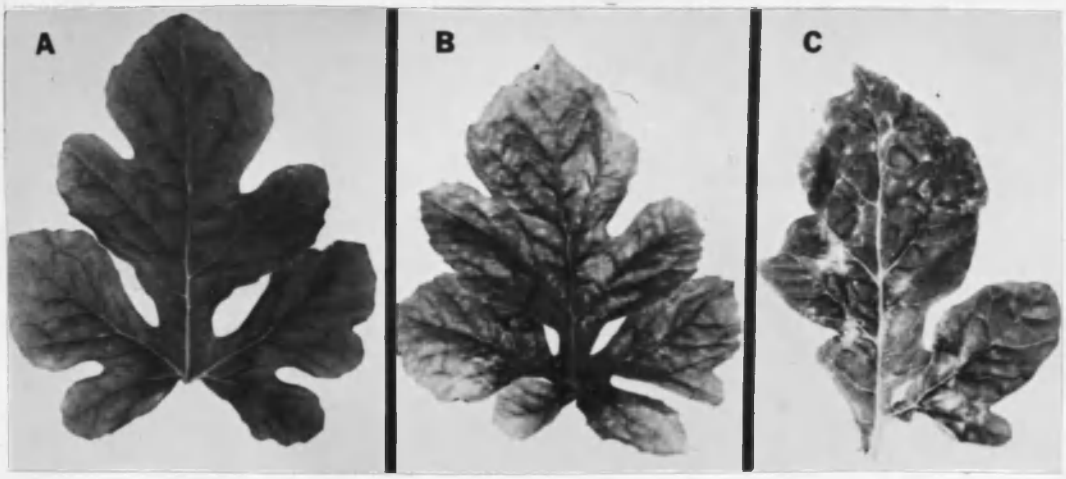

Fig. 3. Reactions of the watermelon strain of SMV on C. vulgaris Schrad.

A. Healthy leaf.

B. Infected leaf showing generalized systemic mottle and chlorosis.

C. Infected leaf showing stunt, distortion, chlorosis and necrosis which has at times been described as "local lesions."

strain in agar gels did not result in an appreciable depression of the titer of either antiserum.

Cross Absorption

In an attempt to demonstrate the antigenic difference between two virus isolates, cross absorption studies were performed. The data thus obtained showed that a considerable antigenic difference did exist between these two SMV strains (Table 2). Both antisera were completely absorbed by the heterologous antigen. In both cases, the absorbed antisera, when combined with their homologous antigen, still reacted strongly to an antiserum dilution of 1:40. It was necessary to use 0.6 mg of the watermelon strain antigen to completely absorb the antiserum to the common strain. Of the common strain antigen, 1.4 mg was needed to completely absorb the antiserum to the watermelon strain.

Immunodiffusion

The results of the cross absorption studies were confirmed in immunodiffusion tests (Fig. 4). A typical reaction of confluence or serological identity was shown (Fig. 4-a) and was produced by reacting a homologous antigen and antiserum. Figures 4-B and 4-C showed the reactions of homologous and heterologous antigens with each antiserum. Note in each case a relatively thin spur

Table 2

Results of Testing Absorbed Antisera of the Common and Watermelon Strains of SMV Against Both Antigens

Antiserum to	Absorbed with	Test antigen[a]	10	20	40	80	160	320	640	1280
SMV	0	SMV	+	+	+	+	+	+	+	-
SMV	0	SMV (W)	+	+	+	+	+	+	+	-
SMV	SMV (W)	SMV	+	+	+	-	-	-	-	-
SMV	SMV (W)	SMV (W)	+	+	+	-	-	-	-	-
SMV	0	Control[b]	-	-	-	-	-	-	-	-
NS	0	SMV	-	-	-	-	-	-	-	-
SMV (W)	0	SMV (W)	+	+	+	+	+	+	+	+
SMV (W)	0	SMV	+	+	+	+	+	+	+	+
SMV (W)	SMV	SMV (W)	+	+	+	-	-	-	-	-
SMV (W)	SMV	SMV	+	+	+	-	-	-	-	-
SMV (W)	0	Control	-	-	-	-	-	-	-	-
NS	0	SMV (W)	-	-	-	-	-	-	-	-

a. test antigen of 0.1 mg/ml.

b. control material was from healthy plants, treated exactly as those containing viruses.

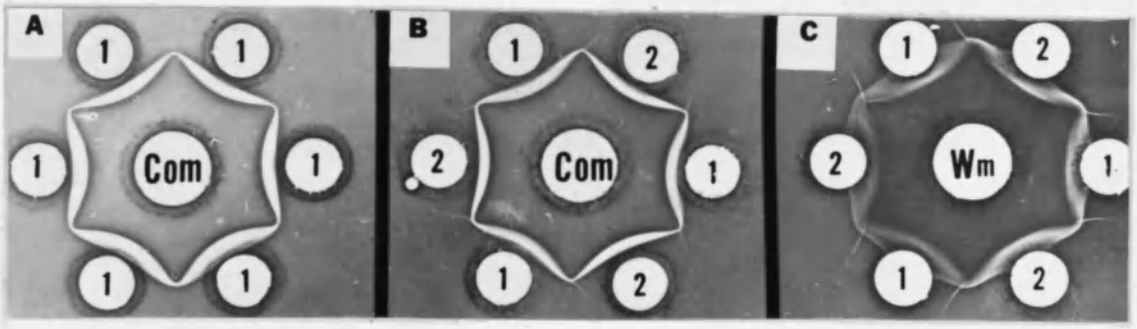

Fig. 4. Comparison of immunodiffusion precipitin patterns of the common and watermelon strains of SMV.

A. Typical pattern of complete serological identity produced by the common strain (com) when reacted against its homologous antiserum at 1:640 dilution.

B. Demonstration of spur formation by the reaction of antiserum prepared against the common strain at 1:640 dilution in the center well with common (1) and watermelon strain (2) antigens alternated in the peripheral wells.

C. Same as B, except with antiserum prepared against the watermelon strain of SMV (W) in the center well.

appeared on the homologous reaction crescent. This spur was more dense, and appeared much sooner when the watermelon strain was reacted with its antiserum (Fig. 4-C) in comparison with the interaction of the common strain and its homologous antiserum (Fig. 4-B).

Immunoelectrophoresis

Both viruses moved about 1 cm toward the anode at approximately the same rate. Precipitin arcs formed when either antiserum was diffused against both virus zones. Only one precipitin arc was formed in the vicinity of either virus zone which indicated freedom from healthy plant contaminants in the virus preparation.

Comparisons of Other SMV Isolates

When compared serologically against antisera to SMV-W (10-28-4), SMV-P, SMV-M and SMV-W 4-25-26 produced a homologous reaction with one serotype, and was heterologous with the other. Figure 1 shows spurs typical of each type isolate with each antiserum. SMV-M and SMV-P were serologically identical to SMV-C and produced reactions against type antisera identical to those shown in Fig. 1-B and 1-C. SMV-W isolates were also serologically with SMV-W (10-28-4) and produced reactions typical for this serotype.

This split between serotypes was consistent with the results of host range studies (Table 1). No isolate tested and found serologically identical to SMV-C was infectious to watermelon. Conversely, no isolate which infects watermelon is serologically identical with SMV-C.

DISCUSSION

Lindberg, Hall and Walker (12) relate that several isolates studied are similar antigenically to the common SMV strain obtained from Freitag (6). They indicate that these isolates are also similar on the basis of physical properties, insect transmission and host range. Studies of Nelson, Matjeka and McDonald show a close serological relationship between the common and watermelon strains of SMV. Proof of the relationship is substantial; the main differences between these strains are symptomatology and host range.

Serological data obtained during the course of this study confirm a strong relationship between these two strains, but also indicate that there is a certain degree of antigenic difference.

On the basis of cross reaction studies, these two strains are found to be serologically indistinguishable when dilutions of antiserum are reacted against a constant concentration (0.1 mg/ml) of either strain.

The absorption of common SMV antiserum by the watermelon strain and the subsequent development of a precipitate about 1/16 of the original titer, when used to react

to the common SMV antigen, indicates that certain antibodies remain which are not common to the watermelon strain of SMV; the absorbed antiserum retains a titer of 1/16 of the original.

The cross reactions obtained in the Ouchterlony immunodiffusion studies confirm the results of the cross absorption studies. Both virus strains appear to be antigenically incomplete with regard to the other when tested against the heterologous antiserum. The earlier development of a denser spur by the watermelon strain suggests the possibility that the watermelon strain has more unshared antigenic sites than the common strain. The fact that each strain protein has antigenic sites that are not common to the other indicates that at least certain features of the chemistry or spatial arrangement of the coat protein of these strains is different (17).

The presence of only one distinct precipitin band in the Ouchterlony immunodiffusion patterns (Fig. 4), or one precipitin arc found during the immunoelectrophoretic studies confirms the purity of the virus suspensions and their suitability for use as antigens in this study.

Electrophoretic mobilities of the type virus strains when compared in the same gel are nearly identical; thus it is apparent that these strains of SMV are also

very similar chemically. As noted by Rappaport (17), subtle chemical substitutions or differences in spatial arrangement of viral protein subunits which might not be detectable by this method can play an important role in the determination of the serotype, and aid in the differentiation between virus strains.

The segregation into serotypes is consistent with the results of host range studies (Table 1). No isolate tested and found to be serologically identical to SMV-C infects watermelon. Conversely, no isolate which infects watermelon is serologically identical with SMV-C.

These data suggest that the changes in the surface structure of this virus, and the associated change in antigenic properties, are related to the host range difference and not differences in symptomatology.

The phenotypic traits of serological specificity and host range are well defined, and for separation of these isolates into strains, are selected for their dependability as opposed to symptomatology which too often may be confused by overlapping characteristics.

Results presented in this thesis show that there are at least two distinct serotypes among isolates of SMV, one which infects watermelon and one which does not. Thus, the commonly established criteria for identification of isolates of SMV, and distinction of these

isolates from wild cucumber mosaic virus (WCMV) as established by Lindberg, Hall and Walker (12), based on host range, may lead to erroneous results.

SUMMARY

This study commences with the intent to extend the serological work of Lindberg, Hall and Walker (12) and Nelson, Matjeka and McDonald (14); an attempt is made to determine whether the two serologically related strains taken under consideration are antigenically different and the extent of this difference.

1. Purification of both virus strains may be accomplished by a simplified technique utilizing heat for clarification and removal of plant proteins. Virus yield by this technique ranges from 0.08 to 0.1 mg of virus per gram of tissue (wet weight).

2. An antiserum of high titer (1:1028) is produced by a simple injection schedule. Antiserum produced by this technique is exceptionally free from antibodies to contaminating plant antigens.

3. Antiserum to both virus isolates cross reacts strongly with the heterologous antigen. When these diluted antisera are reacted against a constant concentration of either virus strain, there is no appreciable depression in the titer of either antiserum.

4. Each virus strain has common antigenic sites with the other but also has some antigenic sites which are not common to the other strain. This result is substantiated by the formation of spurs in the Ouchterlony double diffusion tests.

5. The electrophoretic mobilities of the viruses are about equal although SMV common may move slightly faster toward the anode than the watermelon strain. One precipitin arc in the vicinity of either virus zone indicates freedom from contamination by plant proteins.

6. Of four isolates tested, only two serotypes are identified among the SMV strains.

LITERATURE CITED

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

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

3. Aycock, R. 1951. Studies on a mosaic of cantaloupe. S. C. Agr. Exp. Sta. Rpt. 63:143-147.

4. Bancroft, J. B. 1958. Temperature and temperature light on the concentration of squash mosaic virus in the leaves of growing cucurbits. Phytopathology 48:93-102.

5. Dolittle, S. P., and W. W. Gilbert. 1949. Seed transmission of cucurbit mosaic by the wild cucumber. Phytopathology 9:326-327.

6. Freitag, J. H. 1941. A comparison of the transmission of four cucurbit viruses by cucumber beetles and aphids. Phytopathology 31:8 (Abstr.).

7. Freitag, J. H. 1941. Insect transmission, host range and properties of squash mosaic virus. Phytopathology 31:8 (Abstr.).

8. Freitag, J. H. 1952. Seven virus diseases of cucurbits in California. Phytopathology 42:8 (Abstr.).

9. Grogan, R. G., D. H. Hall, and K. A. Kimble. 1959. Cucurbit mosaic viruses in California. Phytopathology 49:366-376.

10. Keener, P. D. 1952. Virus diseases and interesting virus-like symptoms in melons in Arizona - 1950-1951. Plant Disease Reptr. 36:128-131.

11. Kendrick, J. B. 1934. Cucurbit mosaic transmitted by muskmelon seed. Phytopathology 24:820-823.

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

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

14. Nelson, M. R., J. C. Matejka, and H. H. McDonald. 1965. Systemic infection of watermelon by a strain of squash mosaic virus. Phytopathology 55:1362-1364.

15. Ouchterlony, O. 1949. Antigen-antibody reactions in gels. Acta. Pathol. Microbiol. Scand. 26:507.

16. Rader, W. E., H. F. Fitzpatrick, and E. M. Hildebrand. 1947. A seed-borne virus of muskmelon. Phytopathology 37:809-816.

17. Rappaport, I. 1961. Analysis of the cross-reaction between two strains of tobacco mosaic virus. Nature 189:986-990.

18. Scheidegger, J. J. 1955. Une micro-methode de l'immunoelectrophorese. Intern. Arch. Allergy Appl. Immunol. 7:103-110.

19. Wright, N. S. and R. Stace-Smith. 1966. A comparison of the sensitivity of three serological tests for plant viruses and other antigens. Phytopathology 56:944-948.