

A STUDY OF THE EFFECT OF TOBACCO MOSAIC VIRUS
INOCULATIONS ON CERTAIN GREENHOUSE
TOMATO VARIETIES AND SELECTIONS

By

KWEE-CHONG PAN

Bachelor of Science

Chung-Hsing University

Taiwan, China

1962

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
July, 1968

JAN 30 1989

A STUDY OF THE EFFECT OF TOBACCO MOSAIC VIRUS
INOCULATIONS ON CERTAIN GREENHOUSE
TOMATO VARIETIES AND SELECTIONS

Thesis Approved:

F. A. Romshel

Thesis Adviser

W. R. Kays

N. N. Durham

Dean of the Graduate College

696419

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to the late Dr. H. B. Cordner, Professor of Horticulture, for his kind interest and many valuable instructions and assistances during the progress of the experiment.

Acknowledgment is due Professor W. R. Kays, Head of Horticulture Department and F. A. Romshe, Professor of Horticulture in the preparation of this thesis.

TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION.	1
II. REVIEW OF LITERATURE.	3
Physical Properties and Other Characteristics of TMV Disease Cycle.	3 3
Infection of TMV in Tomato	4
Symptoms	5
The Effect of TMV on Tomato Yield.	6
Inheritance of Resistance to TMV in Tomato	7
Method of Inoculation of TMV to Tomato	10
Double Virus Inoculation Method.	11
III. MATERIALS AND METHODS	13
Reaction of Double Virus Inoculation	14
Experiment A-1.	14
Experiment A-2.	15
Experiment A-3.	15
Experiment A-4.	16
Experiment A-5.	16
Experiment A-6.	17
Effect of TMV Infection on Yield	18
Experiment B-1 Spring 1966.	18
Experiment B-2 Fall 1966.	18
IV. EXPERIMENTAL RESULTS.	19
Reaction of Double Virus Inoculation	19
Experiment A-1.	19
Experiment A-2.	20
Experiment A-3.	20
Experiment A-4.	21
Experiment A-5.	22
Experiment A-6.	25
Effect of TMV Infection on Yield	26
Experiment B-1 Spring 1966.	26

TABLE OF CONTENTS (Continued)

Chapter	Page
Experiment B-2 Fall 1966.	29
V. DISCUSSION AND CONCLUSION	42
VI. SUMMARY	47
LITERATURE CITED.	49

LIST OF TABLES

Table	Page
I. Reaction of Sioux Tomato Plants to Single Inoculations of TMV or with PVX	19
II. Reaction of Sioux Tomato Plants to Double Inoculations of TMV and PVX	20
III. Reaction of Certain Tomato Lines to Double Virus Inoculations	21
IV. Reaction of Tomato Lines STEP 390 and 431 and Derivatives of 431 to Double Virus Inoculation with TMV and PVX . .	22
V. Reaction of Certain Tomato Varieties, Lines and Hybrids to TMV Inoculation Obtained from Various Sources with 1:5 Solution.	23
VI. Reaction of Certain Tomato Varieties, Lines and Hybrids to TMV Inoculation Obtained from Various Sources with 1:25 Solution	24
VII. The Effect of Two Sources of TMV Inoculum on Certain Varieties, Lines, and Hybrids of Tomatoes	25
VIII. The Effect of TMV Inoculation on the Yield of Fruit of Certain Varieties, Lines, and Hybrids of Tomatoes, Spring 1966	26
IX. The Effect of TMV Inoculation on the Total Yield of Certain Varieties, Lines, and Hybrids of Tomatoes for 9-Harvests, Spring 1966	27
X. Average Weight of Fruit Per Plant and Shape Index of Certain Varieties, Lines, and Hybrids of Tomatoes After Inoculation with TMV, Spring 1966	28
XI. The Effect of Natural Dissemination of TMV on the Yield of Fruit of Certain Varieties, Lines, and Hybrids of Tomatoes, Fall 1966	29
XII. The Effect of Natural Dissemination of TMV on the Yield of Fruit of Certain Varieties, Lines, and Hybrids of Tomatoes for 9-Harvests, Fall 1966.	30

LIST OF TABLES (Continued)

Table	Page
XIII. The Effect of Natural Dissemination of TMV on Average Weight of Fruit Per Plant and Shape Index of Certain Varieties, Lines, and Hybrids of Tomatoes, Fall 1966. .	31
XIV. The Effect of Natural Dissemination of TMV on the Percent of Infection of Certain Varieties, Lines, and Hybrids of Tomatoes Grown in the Greenhouse, Fall 1966. .	32
XV. Summary of Tomato Plant Characters of Certain Non-Inoculated Varieties, Lines, and Hybrids, Fall 1966.	33

LIST OF FIGURES

Figure	Page
1. Comparison of TMV 1 and TMV 2 Symptoms on Leaves of TMV Susceptible Variety Michigan State Forcing.	34
2. Comparison of TMV 1 and TMV 2 Symptoms on Leaves of TMV Resistant Line STEP 431	34
3. Comparison of TMV 1 and TMV 2 Symptoms on Leaves of TMV Resistant Line Selection STEP 431-2-2	35
4. Comparison of TMV 1 and TMV 2 Symptoms on Leaves of F ₁ Hybrid STEP 431 x Nemared	35
5. Comparison of TMV 1 and TMV 2 Symptoms on Leaves of F ₁ Hybrid STEP 390 x Nemared	36
6. Comparison of TMV 1 and TMV 2 Symptoms on Leaves of F ₁ Hybrid STEP 431 x Michigan State Forcing.	36
7. Comparison of TMV 1 and TMV 2 Symptoms on Leaves of F ₁ Hybrid STEP 431-2-2 x Michigan State Forcing.	37
8. Comparison of TMV 1 and TMV 2 Symptoms on Leaves of F ₁ Hybrid STEP 431-2-2 x Nemared	37
9. Symptoms on Leaves, Showing Curling, Twisting and Necrosis	38
10. Fruit Shape of F ₁ Hybrid of STEP 431 x Nemared in Comparison with Mich.-Ohio Hybrid, STEP 390 and 431	38
11. The Healthy Plant of STEP 431 with Fruits	39
12. The F ₁ Hybrid of STEP 431 x Nemared, Showing High Degree of TMV Resistance	39
13. The F ₁ Hybrid of STEP 431 x Michigan State Forcing (center), Showing the Level of TMV Resistance	40
14. Comparison of Plant of STEP 431 x Nemared (left) and Mich.-Ohio Hybrid (right) which Showed Stunted and Severe Damaged by TMV	40

LIST OF FIGURES (Continued)

Figure	Page
15. Comparison of TMV Resistance. From Left to Right: First Row, STEP 390 x 431; Second Row, STEP 390; Third Row, Michigan State Forcing; Fourth Row, Mich -Ohio Hybrid. .	41
16. TMV Susceptibility of Mich -Ohio Hybrid and Michigan State Forcing Showing Severe Symptoms on Leaves.	41

CHAPTER I

INTRODUCTION

Greenhouse tomatoes are subject to a number of virus diseases which cause loss to the grower. Of the virus diseases, the most common and serious mosaic is known as tobacco mosaic virus (herein after to be designated TMV) which entity can be detected only by means of electron microscope. A great deal of research has been carried out concerning their properties.

TMV is highly infectious on tomatoes. It can be readily transmitted from plant to plant by means of animals, insects, debris in the soil and various cultural practices. Therefore, among other factors, the highly infectious nature results in widespread contamination and serious damage to greenhouse tomatoes.

The symptoms of TMV are varied depending upon environmental conditions such as temperature and light. In some instances it is difficult to distinguish the difference between healthy and infected plants. The combination of TMV with potato mosaic virus or cucumber mosaic virus is known as "double virus." This combination, streak, can cause most serious loss to greenhouse tomatoes, especially when combined with the potato mosaic virus. The symptoms of double virus on leaves are more conspicuous and show clear-cut symptoms at the seedling stage.

Walter (39) and Murakakishi and Honma (28) stated that to determine the clear-cut symptoms of TMV infected tomato seedlings is difficult.

Some of the susceptible seedlings did not show symptoms until they had been transplanted into the ground bed.

As a matter of fact, it is necessary to develop techniques to determine TMV susceptibility at an early age in order to save labor, time, and land for greenhouse tomato production. An advantage of double virus is that it exhibits severe and clear-cut symptoms and can be used as a method for screening the seedlings. Sources of virus and concentration of inoculum may also affect the expression of symptoms.

Since TMV has become such a destructive disease in greenhouse tomatoes, the need for breeding of resistant varieties and F₁ hybrids has become obvious and important. Many attempts have been made to combine TMV--resistance and high yield by using Lycopersicon esculentum Mill. in combination with the mosaic--tolerant species, such as L. hirsutum Humb. and Bonpl., L. chilense Dun., L. peruvianum (L.) Mill., and L. pimpinellifolium Mill.

The purposes of these experiments were to screen the TMV tolerant lines STEP* 390, STEP 431 and their hybrids and to estimate the effect of TMV on yields.

*STEP designates Southern Tomato Exchange Program.

CHAPTER II

REVIEW OF LITERATURE

Physical Properties and Other Characteristics of TMV

Research with regard to TMV has been carried out by various workers. A considerable amount of information concerning the physical properties and certain other characteristics of TMV was stated by Smith (34). He identified the principal chemical, physical, and physiological characteristics of TMV. He pointed out that the virus is a complex protein, affected by pH, inactivated by the enzymes trypsin and pepsin, is inactivated by thermal treatments, may remain virulent for long periods of time and is infectious at a dilution of 1 : 1,000,000.

Disease Cycle

Walker (36) reported the debris of diseased plants which remain in the soil is the sources of primary inoculum. Johnson (21) found that cigar, cigarette and pipe tobacco carry the virus 67, 81, and 62 per cent respectively.

Dissemination of virus from plant to plant is readily affected through mechanical transmission. Transplanting of the seedlings, suckering and pruning of the plants, contact by man, animals and machinery are various means or modes of transmission. The false potato aphid, Myzus pseudosolanii Theob., can transmit the virus readily from plant to plant.

Infection of TMV in Tomato

Reports of TMV infection of tomatoes have come from Europe, Australia, Canada and the United States. It is recognized as one of the more serious diseases affecting greenhouse tomatoes, due in part, no doubt, to the improved control methods available for certain other (fungus) diseases.

In 1880, the infection of mosaic disease in tomato was studied. The juice extracted from the diseased leaf was used to inject into the midrib of a healthy plant. After 10 days, the youngest leaves of the healthy plant showed typical symptoms (36).

In 1907, Clinton (3) observed tomato mosaic under field conditions in Westville, Connecticut. The leaves of the plants showed mottling with yellowish-green which is now known as one of the most common symptoms of tomato mosaic.

It was known that this tomato disease was readily transmissible by the method of inoculation in 1910. In 1914, Norton (30) reported that mosaic disease had been observed in Maryland and was distributed in both field and greenhouse.

That tomato mosaic was transmissible by aphids and also that the disease was not transmitted by the seed was reported in 1914 (12). It also can be transmitted to some species of Solanaceae, and several species of Nicotiana (36). In 1934, Grant (10) investigated the host range of TMV and found 29 species in 14 widely separated families were susceptible to the virus.

Soil has been considered as a carrier of TMV. Johnson (20) reported that TMV might remain virulent in the soil for several months.

It remained viable for a longer period of time in clay or organic soils than in sandy soil. He also found TMV in high concentrations in roots under field conditions. Lehman (23) reported that soil contaminated with TMV was a hazard to future crops.

Cook (4) reported transmission of TMV from affected plants to healthy plants by laborers, even with very gentle handling. Allard (1) found that dropping juice extract from a diseased plant on an uninjured healthy plant would not transmit the disease.

The debris of old plants that remain in the soil appears to be a source of TMV infection. It is possible that the transmission through the roots is a result of injuries by cultivation. Mosaic also can be transmitted through various plant parts which can be adopted for vegetative propagation. Virus exists in all parts of the plant except the seeds. Some workers reported that an infected plant sometimes produces healthy plants from cuttings due to the virus not being distributed in all plant parts. A great deal of work has been done regarding infection of TMV in tomato since 1916 by various workers (4).

Symptoms

The sequence of the symptoms is varied with the presence of light. Usually, dark green areas appear in the affected leaf tissue (24). In young plants, malformation of the leaflets is the most conspicuous symptom, and in some cases, instead of mottling. The young leaflets are often smaller, constricted and tend to be "shoestring-like." The marked symptom in other plants is a mottling of the leaves (31). When the infected plant is in an early stage, the plant will be stunted. The symptoms shown on the fruit are quite variable. Irregular size and shape

occur and the skin of the fruit soon turns brown followed by cracking of the skin due to the fruit growth. Fruit symptoms may be combinations with viruses other than TMV (12).

Symptoms of the tobacco and potato mosaic virus combinations which is known as double virus streak, or combination streak in tomatoes, is somewhat difficult to describe. The latent virus of potato will cause several forms of symptoms when combined with TMV. When the TMV is combined with a mild form, the symptoms are less severe; conversely, the combination of a severe form of latent virus will make very severe symptoms. The symptoms vary with a difference in light intensity, the age of the plant and some other factors. Double virus streak can spread rapidly. Generally, it only affects the young leaf and the leaf curls downward. The lesions on the leaf are small, brown, and necrotic. The lesions on the stem are spoken of as pencil-lines. Sometimes numerous stem lesions combine and form large necrotic spots. Lesions may occur any place on the fruit, dark-brown in color and usually small. Tobacco and cucumber mosaic combinations cause less damage than the tobacco and potato mosaic combinations in tomatoes. The symptom of this disease is stunting of the plant. The growing point of the plant tends to be a rosette and usually yellowing of the uppermost portion occurs. In some cases, necrotic spots may develop on the leaves (31).

The Effect of TMV on Tomato Yield

Norton (29) reported that the effect of mosaic on the yield of tomatoes was not certain. He found under greenhouse conditions that 33 per cent more fruit was set on healthy than diseased plants.

According to the U.S. plant disease survey in 1919, mosaic diseases

caused a heavy loss in Florida, California, and Pennsylvania. In 1920, the total crop loss due to mosaic was between 5 to 10 per cent for Louisiana, 9 per cent for Iowa and 7 per cent for California (9).

Heuberger and Moyer (11) reported that the early infection of plants resulted in more than a 50 per cent loss in yield whereas a late infection reduced yield by only 1.9 per cent in 1927 and 11.2 per cent in 1930.

In 1950, Walter (37) reported on tests conducted with two tomato lines, W 185-6 and the TMV tolerant MStW 210-5. He found that the time of inoculation was not important to MStW 210-5, but it resulted in a reduction of about 50 per cent in yield on W 185-6 from early inoculation.

In 1957, McRitchie and Leonard (27) reported that infected plants of Rutgers, W-R Globe, and W-R Brookston had a yield reduction of 13, 3, and 12 per cent, respectively. Tests were repeated during the following summer with a yield reduction of 27, 21, and 13 per cent, respectively. At the same time it was found that the TMV tolerant line CStMW-18 was also infected and yield reduced 38 per cent. They concluded that the different strains of TMV cause various degrees of severity as reflected in yield differences.

In 1966, Davis and Webb (5) reported the varieties Manalucie, Trellis 22, Bay Station Improved, and Tuckcross 0 were reduced in total fruit yield on the first 6 clusters by an average of 2.0 lbs. and 0.8 lbs. per plant in the fall and spring crops respectively when infected by TMV.

It is evident from the literature that TMV is a potentially serious disease of all edible varieties of tomato (*L. esculentum* Mill.). Considerable research has been conducted to study methods of control.

Porte and Doolittle (33) found resistance to TMV in F_1 hybrids of the wild tomato species *L. hirsutum* Humb. and Bonpl. crossed with the two commercial varieties, Marglobe and Bonny Best. Holmes (15) produced hybrid tomatoes using the wild Chilean tomato (*L. chilense* Dun.) as one parent, in his search for a source of TMV resistance.

In 1943, Holmes (16,17) reported hybrids of *L. esculentum* x *L. chilense* showed intermediate characteristics of the parental species; to be less affected than ordinary tomato and less resistant than *L. chilense*. A few seedlings in subsequent generations were obtained which showed a similar level of TMV resistance as *L. chilense*. These seedlings were not evaluated regarding quality or yield.

TMV-resistant strains of *L. hirsutum*, *L. chilense*, and *L. peruvianum* have been grown for observation at the Hawaii Agriculture Experiment Station. Several hybrids have been made using *L. esculentum* x *L. hirsutum* or their derivatives; *L. esculentum* x *L. chilense* derivatives; *L. hirsutum* derivatives x *L. chilense* derivatives, and *L. esculentum* x (*L. hirsutum* x *L. chilense* derivatives). They found the F_1 hybrids of *L. esculentum* x *L. hirsutum* were not completely dominant with respect to TMV resistance whereas several segregates of *L. esculentum* (HES-2269) x [*L. peruvianum* x MSF x *L. pimpinellifolium*] x *L. hirsutum*] showed a high degree of TMV-resistant (22). It was known that the genetic material was highly heterozygous.

On this premise tests were carried out by Frazier and Dennett (8)

with the most promising lines. They found that plum or cherry type tomatoes possessed the greatest level of TMV-resistance. Lines which had better horticultural characteristics were less resistant. More than one gene or gene modifier, or possibly both, may have been involved.

Frazier's selected lines were very complex in that they contained L. chilense and derivatives. The wild species, L. hirsutum Humb. and Bonpl., L. peruvianum (L.) Mill., and L. pimpinellifolium Mill. were also used. It is difficult to determine which species contributed the gene for resistance (18). Holmes (18) studied the dominant characteristics of resistance to infection based on Frazier's selected lines and found no homozygous condition in resistance. This may be due to incomplete dominance.

Walter (39) reported that Bechenbach began a program of breeding for TMV-resistant at Gulf Coast Experiment Station in 1942. He used a three way cross (Rutgers x L. hirsutum, P.I. 126445) x Indiana Baltimore. Homozygous conditions for resistance to the combination of green and yellow strains of the virus were obtained. Transfer of resistance to tomatoes with acceptable horticultural characteristics has been obtained.

In 1956, Walter (38) reported that P.I. 183692 (L. esculentum var. Scarlentawen) was susceptible to every pathogen but resistant to tobacco-etch virus. Crossing the P.I. 183692 with TMV-resistant stock, CStMW-18, produced progeny resistant to tobacco-etch virus, and TMV as well as the viruses in combination.

In 1957, Holmes (19) reported a tomato selection which had homozygous resistance to TMV. This line possessed horticultural characteristics of commercial varieties. The same year, McRitchie (26) reported the

resistant line CStMW-18 and certain hybrid derivatives sometimes became infected after inoculation. He concluded these erratic results were due to different strains of virus.

Scoost (35) studied the complex hybrid of L. esculentum, L. hirsutum, L. peruvianum and L. pimpinellifolium and found the resistance was due to a single dominant gene linked to the recessive character. He also concluded that the resistance gene or genes could be the same as reported by Frazier and Dennet.

Phillip, Honma and Murakishi (32) studied the inheritance of resistance to TMV by the use of P.I. 235673, known to have TMV-resistance, in crosses with the susceptible variety Fireball. They suggested that multigenic factors govern resistance or susceptibility to the virus. According to the virus assay, they stated that the resistant expression was due to the suppression of virus multiplication.

TMV-resistant line, 63 G 463, which was developed by the U.S.D.A. at Beltsville, was hybridized with four susceptible commercial varieties. Most F₁ plants appeared to possess resistance while half of them remained symptomless throughout the crop life. This apparent genetic difference may have involved one or more genes. Therefore, the similarity of 63 G 463 to present horticultural varieties of tomatoes makes it valuable in the development of TMV-resistant commercial varieties (5).

Method of Inoculation of TMV to Tomato

It is known that TMV can be transmitted by various methods. Numerous reports regarding the transmission of TMV by juice extracted from diseased plants were published.

Various methods of inoculating plants with TMV were conducted by

Holmes (13,14). He used a set of five insect pins bound on a handle to inoculate tobacco plants. He also demonstrated that scratching the surface of the leaf was a less effective method of inoculation than rubbing the leaf with a saturated cheesecloth. The virus did not readily attack healthy tobacco plants if the extracted juice was applied after the wound was made. Lesions developed abundantly on a leaf which was rubbed with freshly extracted and immediately used juice. Washing away the excess virus does not decrease the total number of infections. In some cases, washing with a stream of water favored the inoculation.

Another method was developed by Lorin and Munger (25). They transmitted certain plant viruses by the use of a high-velocity spray stream.

Frazier and Dennett (8) in their tomato breeding program adopted the inoculation method suggested by Holmes (13). Other methods were employed by Boyle and Wharton (2) and Weber (40).

Double Virus Inoculation Method

The conventional method in inoculating of TMV to tomato usually does not show the clear-cut symptoms of infection during the seedling stage. In view of this, a method of double inoculation by the use of TMV and potato virus X (hereafter to be designated PVX) was adopted.

In 1925, Dickson (6) described a disease known as streak by using double inoculation with TMV and PVX. Murakishi and Homa (28) reported that plants inoculated singly with TMV and PVX and doubly with both viruses simultaneously to TMV susceptible varieties, experimental lines and resistant lines resulted in different levels of infection. The four susceptible tomato varieties were infected 100 per cent due to the double viruses while only 63 to 83 per cent became affected when

inoculated singly with TMV. The TMV-resistant lines remained free from both viruses.

Doolittle and Portę (7) studied the effect of different concentrations of extracted juice used for inoculation of tomatoes. They found the higher concentration of inoculum caused a higher percentage of infection.

CHAPTER III

MATERIALS AND METHODS

The studies reported herein were conducted in the Oklahoma State University, Department of Horticulture greenhouse during the spring, summer and fall of 1966 and early spring of 1967.

Plant materials in these trials included STEP 390, STEP 431 and F₁ hybrids as well as Nemared, Michigan State Forcing (here after to be designated MSF), Mich-Ohio hybrid, Sioux and Y91 which were susceptible. It was the opinion of Dr. H. B. Cordner that lines STEP 390, and STEP 431 were derived from Lycopersicon esculentum and L. hirsutum crosses. The original designation of STEP 390 is CASTMW 258-4-2-Bk-Bk-Bk-Bk and STEP 431 as CASTMW 258 479-6-1 Bk. Both of these lines were developed at Gulf Coast Experimental Station, Bradenton, Florida. Walter prepared the following description of these two lines.

STEP 390 - Normal, with rangy vine. Fruits have extra depth, weight, firmness. Res. to Fus. wilt, gray leaf spot, early blight, leafmold, TMV, blossom-end rot and growth cracks and probably resistant to blotchy ripening.

STEP 431 - Normal, prolific of large fruits, with resistant to growth cracks, blotchy ripening, blossom-end rot, cat-face, leaf-mold, early blight, gray leaf spot, Fus. wilt and TMV.

Seeds of crosses of F₁ hybrids made in the fall crop of 1965 were

collected, stored and kept dry for at least two months prior to planting. An inoculum of TMV was obtained from infected plants of the Bradley variety from the fall crop of 1965. The virus was maintained in MSF and 431-2-2. Potato virus X was derived from plants of the commercial potato variety **Cobbler**.

Plants were inoculated with virus by rubbing the leaves. The material was ground in a mortar to make the crude sap extract just prior to inoculation. Potato virus infected **Cobbler** variety potato plants were the source of PVX which was prepared by the method described above. The crude sap was diluted with distilled water to 1 : 5 and 1 : 25. A spongy rubber pad was dipped in the inoculum and rubbed on the tip of one new leaflet of each of two leaves.

Inoculated plants were maintained in 2½-inch clay rose pots or 3-inch standard clay plots in a greenhouse section with a night to day temperature range of 68° to 75°F.

Symptoms were checked 7 days following inoculation.

Treated plants which were grown for yield tests were set in soil in steam sterilized beds in the tomato house. Temperature ranged between 65° and 80°F during a 24 hour period of the fruit production tests.

Reaction of Double Virus Inoculation

Experiment A-1

The Sioux variety was selected to determine the effect of double virus inoculation on a TMV-susceptible variety. Seeds were sown Jan. 29 and the seedlings transplanted into 2½-inch clay rose pots Feb. 9, 1966.

Five groups of twelve plants each were used per test. All plants were located on the center of a sand bench.

The first inoculation, with either TMV or PVX, was made two weeks following transplanting. The concentration of inoculum was made up of 1 : 5 dilutions of crude sap with distilled water. A second inoculation was made 9 days following the first inoculation.

Experiment A-2

The objective of this study was also to determine the effect of double virus inoculation on a TMV-susceptible variety. In this instance the crude sap of TMV and PVX was mixed together immediately prior to inoculation.

Plants of the same variety as used in experiment A-1 were used in these tests. The seedlings were divided into four groups of twelve plants each. Inoculation was made Mar. 8, 1966, using equal volumes of crude sap of TMV and PVX mixed together and diluted to 1 : 5 with distilled water. Groups No. 1, No. II and No. III were inoculated with such inoculum while Group IV was inoculated only with TMV and used as a check.

Experiment A-3

The main purpose of this study was to evaluate the susceptibility or tolerance of STEP 390 and 431 and their F₁ hybrids to TMV inoculations in comparison with two TMV susceptible lines, MSF and Mich.-Ohio hybrid. The F₁ hybrids of STEP 390, and 431 were 390 x Nemared (here after to be designated N), 390 x MSF, 390 x Y91, 431 x N, 431 x MSF, and 431 x Y91. The F₁ hybrids of both TMV resistant parents 390 x 431

and selection 431-2-2 were also included. Thirty two plants were used per plot per variety and F₁ hybrid.

Seeds were sown Feb. 16 and 26 and seedlings transplanted into 3-inch standard clay pots Mar. 2, 7, and 8, 1966. The source of double virus inoculum was obtained from diseased plants of the Sioux variety which exhibited streak symptoms. The first inoculation was made Mar. 22, and the second Mar. 31. The method of inoculation and the concentration of inoculum were the same as previously described (A-1).

Experiment A-4

TMV resistant lines STEP 390, 431 and derivatives of 431 were used to test the reaction for leaf symptoms of double virus. Seeds were sown May 11 and seedlings transplanted into 3-inch standard clay pots May 25 and 26. The inoculum was prepared of equal parts of infected leaves of Sioux plus the leaves of severely infected plant of Mich-Ohio hybrid. Juice of this mixture was diluted 1:1 with distilled water.

Experiment A-5

The purpose of this study was to determine the response of the previously tested TMV-resistant line to various sources of TMV and degree of concentration of inoculum. The sources of TMV were as follows:

TMV1 - Mixture with the equal parts of infected leaves from TMV2 plus TMV3.

TMV2 - From 431-2-2, showing very slight symptoms at the end of the spring crop.

TMV3 - From 431-2-2, showing severe infection at the end of the spring crop.

TMV4 - Nemared, showing severely infected plant at the end of the spring crop.

Seeds were sown Sept. 19, and transplanted into 3-inch standard clay pots Oct. 10, 1966. The first inoculation was Oct. 15. After one week, the second inoculation followed.

Each inoculum source was made up to two levels of concentration. These were 1 : 5 and 1 : 25 diluted with distilled water. Six plants were inoculated per treatment.

Experiment A-6

The objective of this test was to screen the F₂ hybrids of STEP 390 and 431. Seeds were collected from the 1966 spring crop in the greenhouse. Sources of virus were from the test plants in experiment A-5 which were designated as follows:

TMV1 - From MSF which showed severe symptoms after having been inoculated with TMV1.

TMV2 - From MSF which showed severe symptoms after having been inoculated with TMV2.

Double virus - From 431-2-2 x N of the fall crop, 1966 which showed severe symptoms.

Seeds of STEP 431 and its F₂ hybrids were sown Nov. 6 and seedlings transplanted into 3-inch standard clay pots Nov. 21, 1966. Inoculation was made Dec. 7, 1966. A second seeding was made of STEP 390 and its hybrids Dec. 13, 1966. Seedlings were transplanted Dec. 28, 1966, into 3-inch standard clay pots. Inoculations were made two weeks after transplanting.

The method of inoculation, number of plants per treatment and con-

centration of inoculum was the same as in experiment A-5.

Effect of TMV Infection on Yield

Experiment B-1 Spring 1966

Seedlings which had been tested in experiment A-3 were transplanted on April 1, 1966, to ground beds for yield trials. A randomized block design with four replications was employed in this test. Twelve varieties or lines were planted in rows spaced 24" x 22" with 7-plant plots. Normal greenhouse cultural practices were followed.

Experiment B-2 Fall 1966

Seeds were sown during July, seedlings potted and later transplanted to the ground bed in Sept. The soil had been sterilized and saw-dust added to tie up a high level of nitrogen. The randomized block design, plant spacing and number of plants per plots were the same as in experiment B-1. Plants were not inoculated with TMV, but became infected by natural contamination.

Data were obtained on the following characters;

- (1) Number of flowers per inflorescence.
- (2) Number of leaves between inflorescences.
- (3) Length (inch) of stem between inflorescences.
- (4) Per cent of leafy inflorescences.
- (5) Time lapse for development of clusters (1st bloom).
- (6) Number of inflorescences per plant.
- (7) Classes of severity of infected plants.
- (8) Date of receptivity of first flower blossom.

CHAPTER IV

EXPERIMENTAL RESULTS

Reaction of Double Virus Inoculation

Experiment A-1

This experiment was established to determine the effect of double virus on TMV susceptible variety Sioux. All plants were examined at two-day intervals. Symptoms were evident on leaves two weeks following inoculation with either TMV or PVX. Streak symptoms were not evident. All of the inoculated plants shown in Table I became affected.

TABLE I
REACTION OF SIOUX TOMATO PLANTS TO SINGLE
INOCULATIONS OF TMV OR WITH PVX

Group No.	No. of Plant	Infection Classes*			Per Cent of Infection
		0	1	2	
I	12	0	12	0	100
II	12	0	12	0	100
III	12	0	12	0	100
IV	12	0	12	0	100
V(ck)	12	0	0	0	0
Total	60	0	48	0	400
Average	12	0	12	0	100

*Arbitrary infection classes of severity of mosaic symptoms.

0: No symptoms.

1: Slight distortion of the leaflets, no necrosis and chlorotic mottling.

2: Severe chlorotic mottling and distortion.

Experiment A-2

Symptoms on leaves appeared 12 days following inoculation. These effects were evident earlier than those singly inoculated with TMV and with PVX. In Table II, plants rated in class 2 were 66.6%, 75%, 66.6% respectively for groups I, II, and III. The symptoms became severe and necrotic as a result of inoculation with combination of double virus. Slight symptoms appeared on the leaves of check plants. All plants became infected with mosaic virus.

TABLE II
REACTION OF SIOUX TOMATO PLANTS TO
DOUBLE INOCULATIONS OF TMV AND PVX

Group No.	No. of Plant	Infection Classes*			Per Cent Infected in Class 2	Per Cent of Total Infection
		0	1	2		
I	12	0	4	8	66.6	100
II	12	0	3	9	75.0	100
III	12	0	4	8	66.6	100
IV(ck)	12	0	12	0	0	100
Total	48	0	23	25	208.2	400
Average	12	0	5.75	6.25	52.05	100

*Arbitrary infection classes of severity of mosaic symptoms.

0: No symptoms.

1: Slight distortion of the leaflets, no necrosis and chlorotic mottling and distortion.

2: Severe chlorotic mottling and distortion.

Experiment A-3

The symptoms were evident 9 days following the first inoculation. Symptoms on leaves of both susceptible varieties, MSF and Mich-Ohio

hybrid appeared extremely severe and 100 per cent were infected. No symptoms were found on plants of the TMV-resistant lines STEP 390, 431 and 431-2-2. They appeared to be resistant. The F₁ hybrids, involving STEP 390 and 431, and susceptible varieties, showed moderate susceptibility to the virus.

TABLE III
REACTION OF CERTAIN TOMATO LINES
TO DOUBLE VIRUS INOCULATIONS

Variety, Line, or Hybrid	No. of Plants	Plants		Per Cent with Symptoms
		With Symptoms	No Symptoms	
MSF	32	32	0	100.0
Mich -Ohio hyb.	32	32	0	100.0
390	32	0	32	0.0
390 x Y91	32	3	29	9.3
390 x N	32	4	28	12.5
390 x MSF	32	9	23	28.1
390 x 431	32	0	32	0.0
431	32	0	32	0.0
431-2-2	32	0	32	0.0
431 x Y91	32	3	29	9.3
431 x N	32	4	28	12.5
431 x MSF	<u>32</u>	<u>13</u>	<u>19</u>	<u>40.6</u>
Total	384	100	284	312.6
Average	32	8.33	23.66	26.02

Experiment A-4

Symptoms resulting from inoculations in this test were considerably

more severe than those in former tests. Almost all the plants in all of the lines showed leaf symptoms 9 days following inoculation.

TABLE IV
REACTION OF TOMATO LINES STEP 390 AND 431 AND DERIVATIVES
OF 431 TO DOUBLE VIRUS INOCULATION WITH TMV AND PVX

Lines and Derivatives	No. of Plants	With Symptoms	No Symptoms	Per Cent with Symptoms
390	10	9	1	90
431	10	10	0	100
431-1	27	27	0	100
431-1-2	30	29	1	97
431-2-1	30	29	1	97
431-2-2	10	10	0	100
431-3-1	10	10	0	100
431-3-2	10	10	0	100
Total	137	134	3	784
Average	17	16.7	0.3	98

Experiment A-5

For plants treated with a 1 : 5 diluted TMV2 inoculum, the first symptoms appeared on leaves and all plants were infected in 17 days. The inoculation of TMV1, TMV3, and TMV4 caused symptoms to develop a few days later than TMV2. Almost all of the plants were infected within five weeks.

The time for symptoms to show was delayed about 10 days by the use of 1 : 25 diluted inoculum. TMV2 caused more severe and earlier

occurrence of symptoms.

TABLE V

REACTION OF CERTAIN TOMATO VARIETIES, LINES AND HYBRIDS TO TMV
INOCULATION OBTAINED FROM VARIOUS SOURCES WITH 1:5 SOLUTION

Variety, Line, or Hybrid	No. of Plants											
	With Symptoms				Without Symptoms				Per Cent with Symptoms			
	TMV1	2	3	4	TMV1	2	3	4	TMV1	2	3	4
MSF	6	6	6	6	0	0	0	0	100	100	100	100
Mich.-Ohio hyb.	6	6	6	6	0	0	0	0	100	100	100	100
390	1	6	6	6	5	0	0	0	16.2	100	100	100
390 x N	6	6	6	6	0	0	0	0	100	100	100	100
431	6	6	6	6	0	0	0	0	100	100	100	100
431-2-2	1	6	6	6	5	0	0	0	16.2	100	100	100
431 x N	6	6	6	6	0	0	0	0	100	100	100	100
431 x MSF	6	6	6	6	0	0	0	0	100	100	100	100
431-2-2 x N	6	6	6	6	0	0	0	0	100	100	100	100
431-2-2 x MSF	6	6	6	6	0	0	0	0	100	100	100	100
Total	50	60	60	60	10	0	0	0	832.4	1000	1000	1000
Average	5	6	6	6	1	0	0	0	83.2	100	100	100

TABLE VI

REACTION OF CERTAIN TOMATO VARIETIES, LINES AND HYBRIDS TO TMV
 INOCULATION OBTAINED FROM VARIOUS SOURCES WITH 1:25 SOLUTION

Variety, Line, or Hybrid	No. of Plants											
	With Symptoms				Without Symptoms				Per Cent with Symptoms			
	TMV1	2	3	4	TMV1	2	3	4	TMV1	2	3	4
MSF	6	6	6	6	0	0	0	0	100	100	100	100
390	1	6	6	6	5	0	0	0	16.2	100	100	100
390 x N	6	6	6	6	0	0	0	0	100	100	100	100
431	6	6	6	6	0	0	0	0	100	100	100	100
431-2-2	1	6	6	6	5	0	0	0	16.2	100	100	100
431-2-2 x N	6	6	6	6	0	0	0	0	100	100	100	100
Total	26	36	36	36	10	0	0	0	432.4	600	600	600
Average	4	6	6	6	2	0	0	0	72.1	100	100	100

TABLE VII
 THE EFFECT OF TWO SOURCES OF TMV INOCULUM ON CERTAIN
 VARIETIES, LINES AND HYBRIDS OF TOMATOES

Variety, Line, or Hybrid	Inoculum Source	Date Symptoms Became Evident					
		1	2	Plant No.		5	6
				3	4		
MSF	TMV 1	10/28	10/28	10/28	10/26	10/26	10/26
	TMV 2	10/24	10/28	10/24	10/26	10/24	10/28
Mich.-Ohio hyb.	TMV 1	10/26	10/26	10/26	10/28	10/28	10/28
	TMV 2	10/24	10/26	10/26	10/26	10/28	10/28
390	TMV 1	-*	11/14	-	-	-	-
	TMV 2	10/28	10/28	10/26	10/28	10/28	10/28
390 x N	TMV 1	11/14	11/14	11/14	11/6	11/14	11/14
	TMV 2	10/26	10/26	10/26	10/26	10/28	10/26
431	TMV 1	11/13	11/13	11/13	11/13	11/13	11/13
	TMV 2	10/26	10/26	10/28	10/28	10/26	10/26
431-2-2	TMV 1	-	-	11/14	-	-	-
	TMV 2	10/28	10/29	10/28	10/24	10/26	10/24
431 x N	TMV 1	11/4	11/4	11/14	11/6	11/14	11/14
	TMV 2	10/26	10/26	10/26	10/26	10/28	10/26
431 x MSF	TMV 1	11/14	11/6	11/4	11/4	11/14	11/4
	TMV 2	10/26	10/24	10/26	10/26	10/26	10/24
431-2-2 x N	TMV 1	10/28	11/6	11/6	11/6	11/14	11/14
	TMV 2	10/26	10/26	10/26	10/26	10/28	10/26
431-2-2 x MSF	TMV 1	11/14	11/4	11/4	11/6	11/14	11/6
	TMV 2	10/24	10/26	10/24	10/26	10/24	10/26

*- Indicates no symptoms.

Experiment A-6

The results were the same as experiment A-5. Plants which were inoculated with TMV 2 showed a more severe level of infection and symp-

toms appeared earlier than with TMV 1. All of the plants were infected. For those plants which were inoculated with a 1:25 dilution inoculum there was a delay of a few days in the appearance of symptoms.

Effect of TMV Infection on Yield

Experiment B-1 1966

On April 1, all plants of MSF and Mich-Ohio hybrid had TMV symptoms, MSF hybrids had 34.4%, Nemared hybrids had 12.5% and Y91 hybrids had 9.3%. Susceptible varieties, MSF and Mich-Ohio hybrid were severely injured by TMV and as a result, fruiting was delayed and yield was reduced by approximately one-half. The harvest period was from June 16 to Aug. 8, 1966. Crop yield, quality index and average weight of fruit per plant are given in Table VIII, Table IX and Table X.

TABLE VIII
THE EFFECT OF TMV INOCULATION ON THE YIELD OF FRUIT OF CERTAIN
VARIETIES, LINES, AND HYBRIDS OF TOMATOES, SPRING 1966

Variety, Line or Hybrid	Mean Yield* lbs.	
431 x N	62.0	a**
390 x N	58.0	a b
431-2-2	53.1	a b c
431 x MSF	52.1	a b c
390 x 431	49.3	b c d
390	47.8	b c d
390 x Y91	46.3	b c d
431 x Y91	41.8	c d
431	41.2	c d
390 x MSF	39.6	d e
Mich-Ohio hyb.	29.3	e f
MSF	19.9	f

*Mean yield per replicate with 7 plants.

**Means followed by the same letter are not significantly different at 0.05 level of probability.

TABLE IX
 THE EFFECT OF TMV INOCULATION ON THE TOTAL YIELD OF CERTAIN VARIETIES,
 LINES, AND HYBRIDS OF TOMATOES FOR 9-HARVESTS, SPRING 1966

Variety, Line, or Hybrid	Date 6/16	To 6/23	To 6/30	To 7/6	To 7/12	To 7/18	To 7/25	To 8/1	To 8/8
MSF	-*	-	6.6	17.5	28.0	40.4	54.7	68.3	79.5
Mich-Ohio hyb.	-	5.0	15.2	31.0	44.7	63.3	84.0	104.0	117.1
431 x N	13.0	39.3	77.4	118.4	152.2	117.7	200.4	222.8	247.8
431 x MSF	9.7	36.8	73.3	105.6	134.6	157.6	183.6	197.2	208.3
431 x Y91	16.6	39.4	69.4	90.8	113.3	130.3	141.6	150.0	167.0
431	2.5	31.8	60.7	90.7	107.7	126.8	140.7	149.1	164.2
390	5.0	31.7	65.3	103.6	136.2	151.0	168.3	177.1	191.3
390 x Y91	11.4	40.0	70.2	95.0	123.0	145.5	162.6	171.6	185.0
390 x N	11.4	31.6	67.9	112.0	144.4	174.6	200.1	214.0	232.4
390 x MSF	2.8	18.3	48.8	66.5	95.5	116.5	139.0	148.2	158.2
390 x 431	8.9	37.8	73.6	106.6	136.8	155.3	169.5	178.2	197.2
431-2-2	8.3	37.6	72.7	111.6	141.4	159.7	180.4	187.8	212.3

* Not harvested

TABLE X
 AVERAGE WEIGHT OF FRUIT PER PLANT AND SHAPE INDEX OF
 CERTAIN VARIETIES, LINES, AND HYBRIDS OF TOMATOES
 AFTER INOCULATION WITH TMV, SPRING 1966

Variety, Line, or Hybrid	Avg. Yield/Plant in lbs.	Avg. Shape Index*
MSF	2.8	1.02
Mich-Ohio hyb.	4.2	1.12
431 x N	8.9	1.51
431 x MSF	7.4	1.28
431 x Y91	6.0	1.56
431	5.9	1.53
390	6.8	1.47
390 x Y91	6.6	1.59
390 x N	8.3	1.49
390 x MSF	5.7	1.20
390 x 431	7.0	1.60
431-2-2	7.6	1.63

*Avg. Shape Index: Range from 1 (best-shape), 3 (with fasciation, rough blossom scars) and 5 (fruits - not marketable). Calculation: total number of fruits in each class multiplied by the class range, the sum of three classes divided by the gross total of fruit number.

Experiment B-2 1966

Susceptible varieties MSF and Mich-Ohio hybrid were the first to show TMV symptoms. The virus spread rapidly from row to row to healthy plants as a result of handling during pruning, pollination and harvest. Double virus was first evident in replicate IV and then gradually infected the entire crop. Nine harvests were made between Dec. 12, 1966, and Feb. 3, 1967. Following the fourth harvest, it was observed that some plants of 431-2-2 x N, 431 x N, 431-2-2, 431, 431-2-2 x MSF, 431 x MSF, 390 x N and 390 were infected by streak virus. This caused a severe reduction in yield. Crop yield, average fruit weight, shape index, infection classes, and the summary of certain plant characters are given in Table XI, Table XII, Table XIII, Table XIV, and Table XV.

TABLE XI

THE EFFECT OF NATURAL DISSEMINATION OF TMV ON THE YIELD OF FRUIT OF CERTAIN VARIETIES, LINES, AND HYBRIDS OF TOMATOES, FALL 1966

Variety, Line, or Hybrid	Mean Yield* lbs	
Mich-Ohio hyb.	36.5	a**
390 x N	36.0	a
431 x MSF	35.2	a
431 x N	33.9	a
MSF	30.6	a
431-2-2 x MSF	29.9	a
431-2-2 x N	29.7	a
431-2-2	28.5	a
390	26.5	a
431	23.7	b

*Mean yield per replicate with 7 plants.

**Means followed by the same letter are not significantly different at the 0.05 level of probability.

TABLE XII

THE EFFECT OF NATURAL DISSEMINATION OF TMV ON THE
YIELD OF FRUIT* OF CERTAIN VARIETIES, LINES, AND
HYBRIDS OF TOMATOES FOR 9-HARVESTS, FALL 1966

Variety, Line, or Hybrid	Date 12/12	To 12/16	To 12/21	To 12/28	To 1/5	To 1/10	To 1/18	To 1/24	To 2/3
431-2-2 x N	3.2	6.5	21.3	43.3	57.7	67.2	84.6	93.5	118.8
431 x N	13.1	19.9	37.1	61.6	76.6	88.0	102.8	110.8	134.8
431-2-2	11.9	19.3	36.2	67.1	79.3	86.1	96.2	100.7	113.8
431	6.7	9.2	22.2	52.0	67.0	71.9	79.8	86.6	94.7
431-2-2 x MSF	4.3	6.1	24.0	48.8	64.9	71.8	88.1	95.5	119.5
431 x MSF	7.9	10.7	30.9	59.3	74.4	83.9	106.5	112.5	140.6
390 x N	12.8	21.6	46.9	86.5	103.2	115.1	123.2	126.0	144.0
390	5.9	10.6	28.1	61.8	75.2	81.1	89.1	93.8	105.8
MSF	1.7	3.0	9.7	28.0	41.3	54.9	77.5	89.0	122.2
Mich-Ohio hyb.	0.6	3.2	14.3	35.2	49.2	68.3	97.3	105.2	145.9

*Total yield in pounds of four, seven plant replicates.

TABLE XIII

THE EFFECT OF NATURAL DISSEMINATION OF TMV ON AVERAGE WEIGHT
OF FRUIT PER PLANT AND SHAPE INDEX OF CERTAIN VARIETIES,
LINES, AND HYBRIDS OF TOMATOES, FALL 1966

Variety, Line, or Hybrid	Avg. Yield/Plant in lbs.	Avg. Shape Index*
431-2-2 x N	4.2	1.55
431 x N	4.8	1.51
431-2-2	4.1	1.46
431	3.4	1.21
431-2-2 x MSF	4.3	1.54
431 x MSF	5.0	1.38
390 x N	5.1	1.53
390	3.8	1.58
MSF	4.4	1.17
Mich-Ohio hyb.	5.2	1.16

*Avg. Shape Index: Range from 1 (best-shape), 3 (with fasciation, rough blossom scars) and 5 (fruit-not marketable). Calculation: total number of fruits in each class multiplied by the class range, the sum of three classes divided by the gross total of fruit number.

TABLE XIV
 THE EFFECT OF NATURAL DISSEMINATION OF TMV ON THE PERCENT
 OF INFECTION OF CERTAIN VARIETIES, LINES, AND HYBRIDS
 OF TOMATOES GROWN IN THE GREENHOUSE, FALL 1966

Variety, Line, or Hybrid	Total Plants	Per Cent of Infection Classes*				
		1	2	3	4	5
431-2-2 x N	28	0	18.2	40.9	36.4	4.5
431 x N	28	0	11.1	22.2	66.7	0
431-2-2	28	5.2	47.4	31.6	15.8	0
431	28	0	14.3	50.0	35.7	0
431-2-2 x MSF	28	0	4.4	30.4	65.2	0
431 x MSF	28	0	4.2	54.1	41.7	0
390 x N	28	0	5.2	89.6	5.2	0
390	28	0	15.4	61.5	23.1	0
MSF	28	0	0	0	0	100
Mich-Ohio hyb.	28	0	0	0	0	100

*Arbitrary infection classes of severity of mosaic symptoms:

1. Virus symptoms to only a few leaves.
2. Less than one third of the foliage show symptoms.
3. One third of the foliage show symptoms.
4. One-half of the foliage show symptoms.
5. More than one-half the foliage show symptoms.

TABLE XV
SUMMARY OF TOMATO PLANT CHARACTERS OF CERTAIN NON-INOCULATED
VARIETIES, LINES, AND HYBRIDS, FALL 1966

Variety, Line, or Hybrid	Date of First Flower	Avg. No. of Flowers per Cluster	Avg. No. of Clusters per Plant	Avg. No. of Leaves Between Clusters	Per Cent of Leafy Clusters	Interval of Flowering*	Avg. Distance in Inches Between Clusters
431-2-2 x N	10/5	6.4	8.1	3.0	9.4	11.4	9.0
431 x N	10/1	6.1	8.5	2.9	10.9	9.4	8.9
431-2-2	10/1	5.3	8.7	2.8	10.2	9.5	8.1
431	10/1	6.0	8.3	2.9	16.9	9.5	8.7
431-2-2 x MSF	10/3	6.7	8.6	3.1	14.4	9.3	9.5
431 x MSF	10/2	5.2	8.0	3.1	20.0	9.3	9.9
390 x N	10/5	5.5	8.1	2.7	11.0	7.8	9.4
390	10/2	5.8	8.2	3.0	14.7	9.0	9.4
MSF	10/9	6.7	8.3	3.0	14.5	9.0	9.9
Mich-Ohio hyb.	10/6	6.8	7.4	3.0	10.6	8.4	10.8

*Average number of days between opening of the first flower of adjacent clusters.

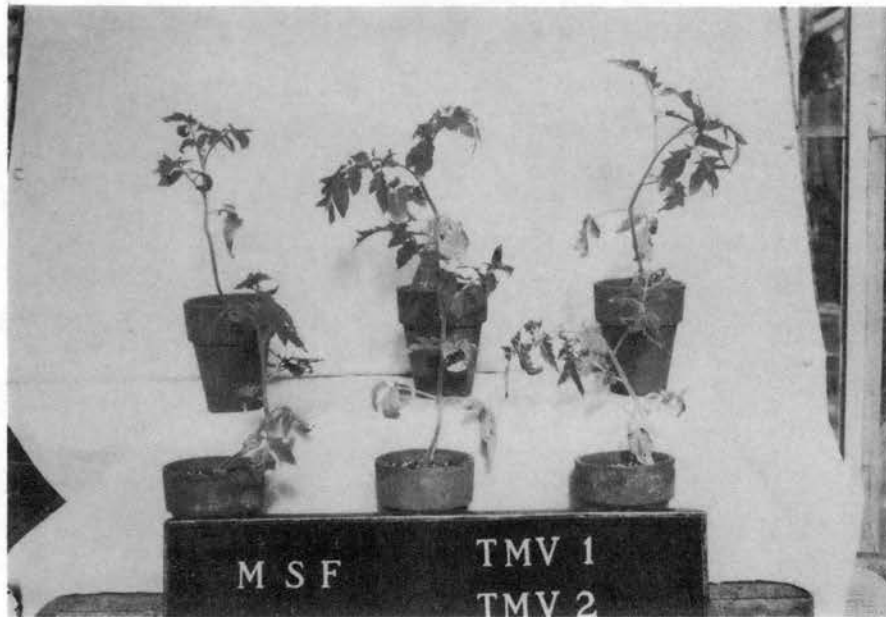


Figure 1. Comparison of TMV 1 and TMV 2 Symptoms on Leaves of TMV Susceptible Variety Michigan State Forcing.

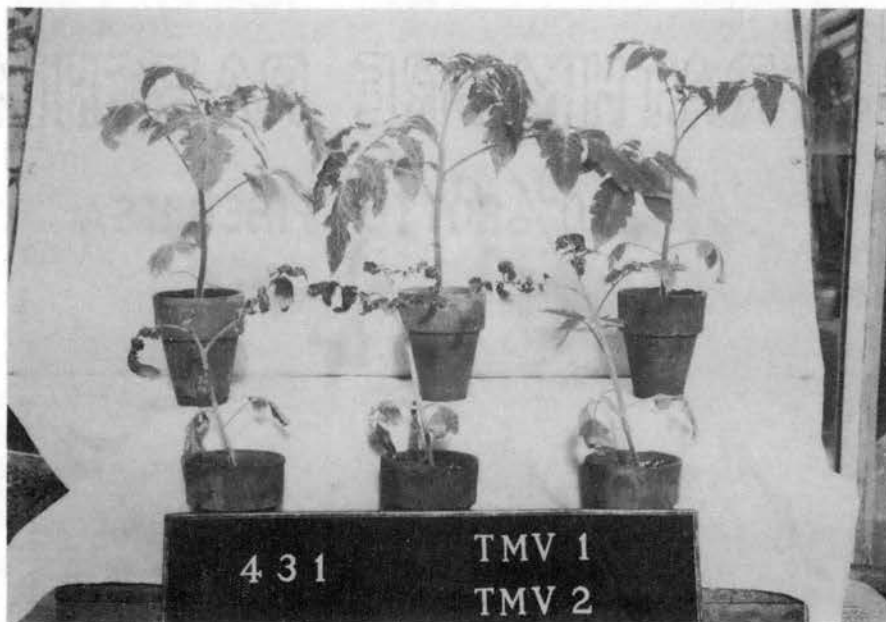


Figure 2. Comparison of TMV 1 and TMV 2 Symptoms on Leaves of TMV Resistant Line STEP 431.

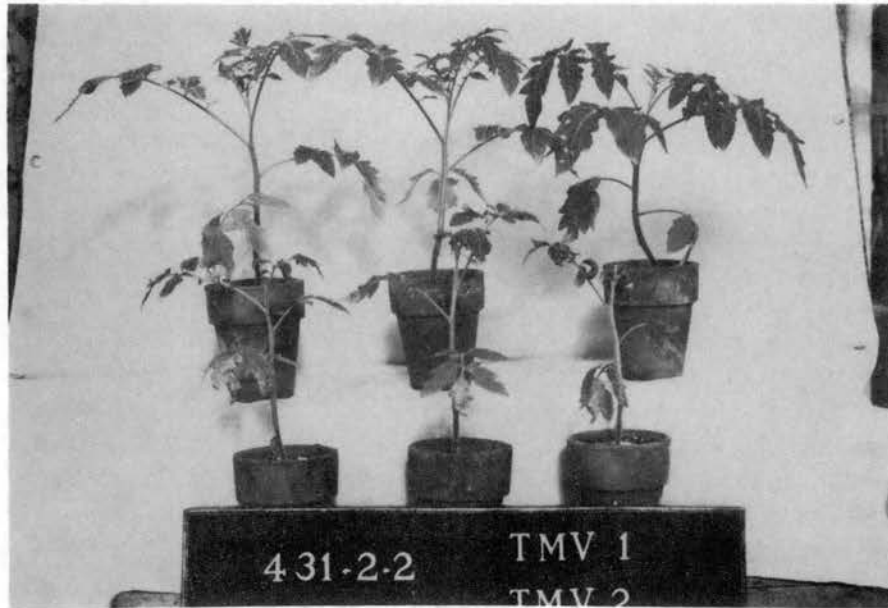


Figure 3. Comparison of TMV 1 and TMV 2 Symptoms on Leaves of TMV Resistant Line Selection STEP 431-2-2.

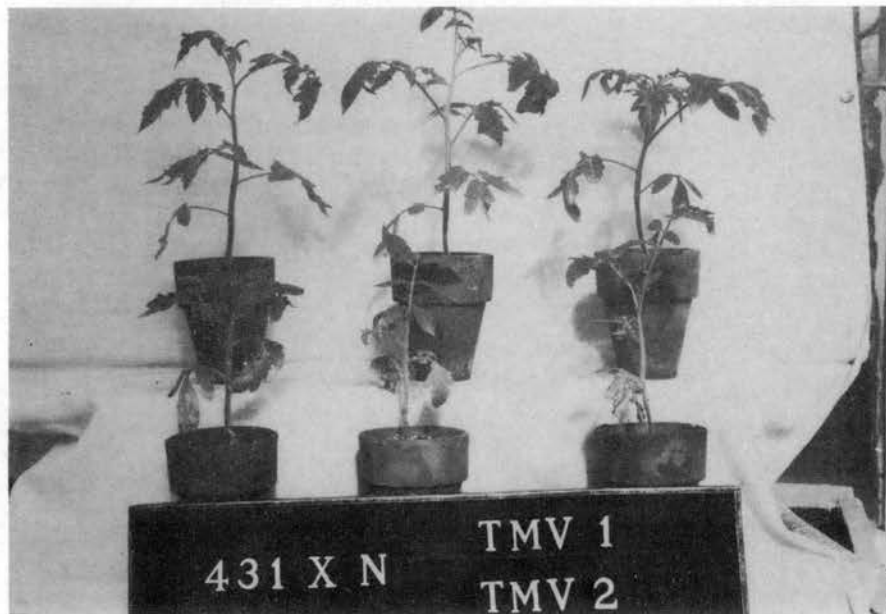


Figure 4. Comparison of TMV 1 and TMV 2 Symptoms on Leaves of F_1 Hybrid STEP 431 x Nemared.

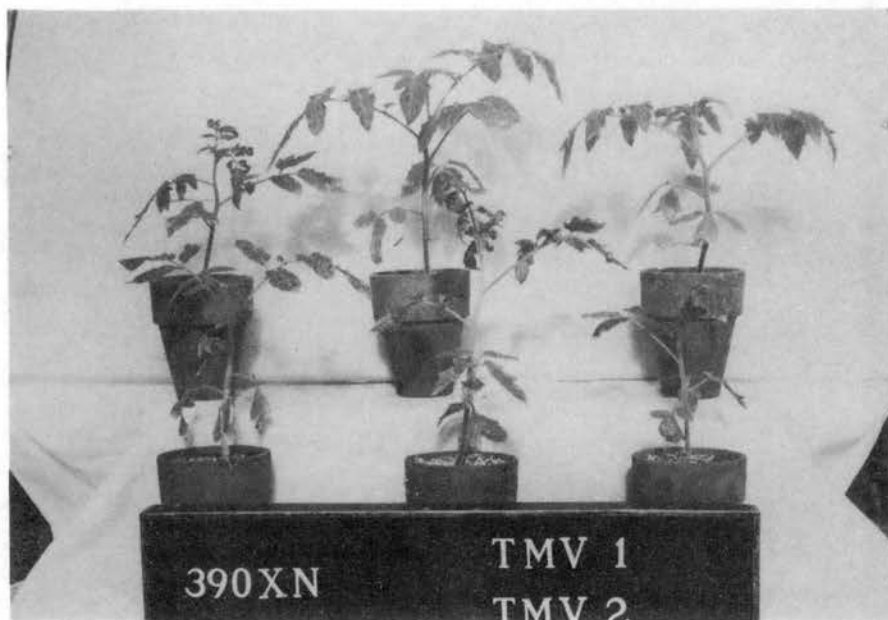


Figure 5. Comparison of TMV 1 and TMV 2 Symptoms on Leaves of F_1 Hybrid STEP 390 x Nemared.

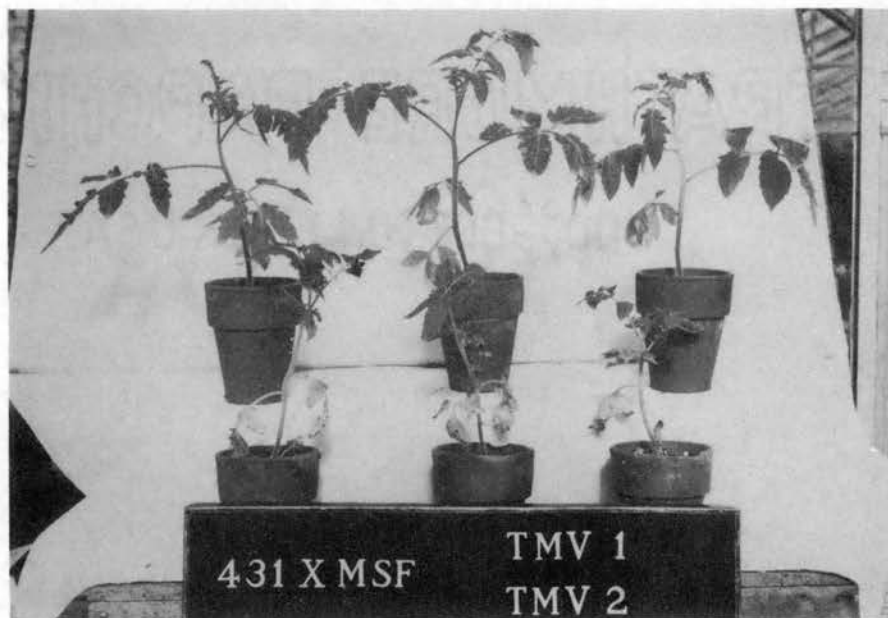


Figure 6. Comparison of TMV 1 and TMV 2 Symptoms on Leaves of F_1 Hybrid STEP 431 x Michigan State Forcing.



Figure 7. Comparison of TMV 1 and TMV 2 Symptoms on Leaves of F_1 Hybrid STEP 431-2-2 x Michigan State Forcing.

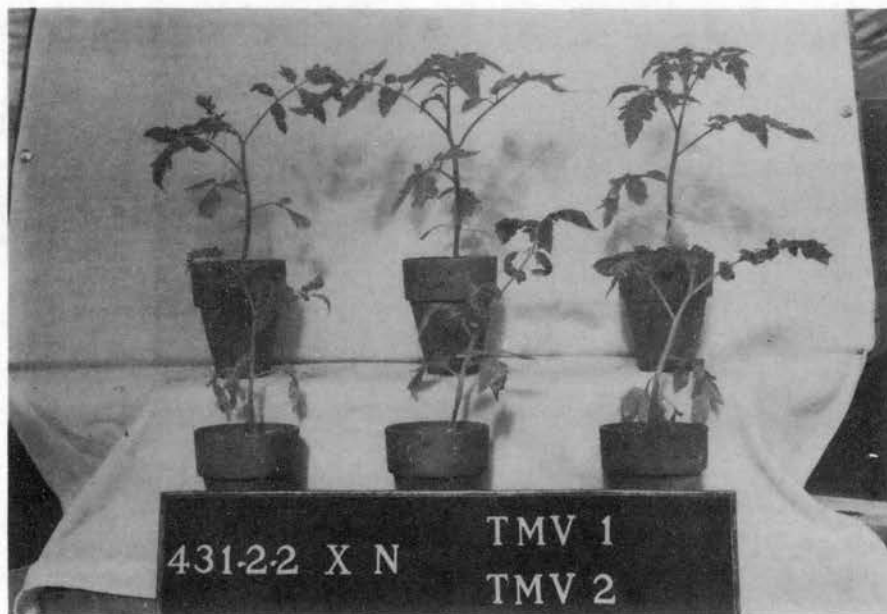


Figure 8. Comparison of TMV 1 and TMV 2 Symptoms on Leaves of F_1 Hybrid STEP 431-2-2 x Nemared.

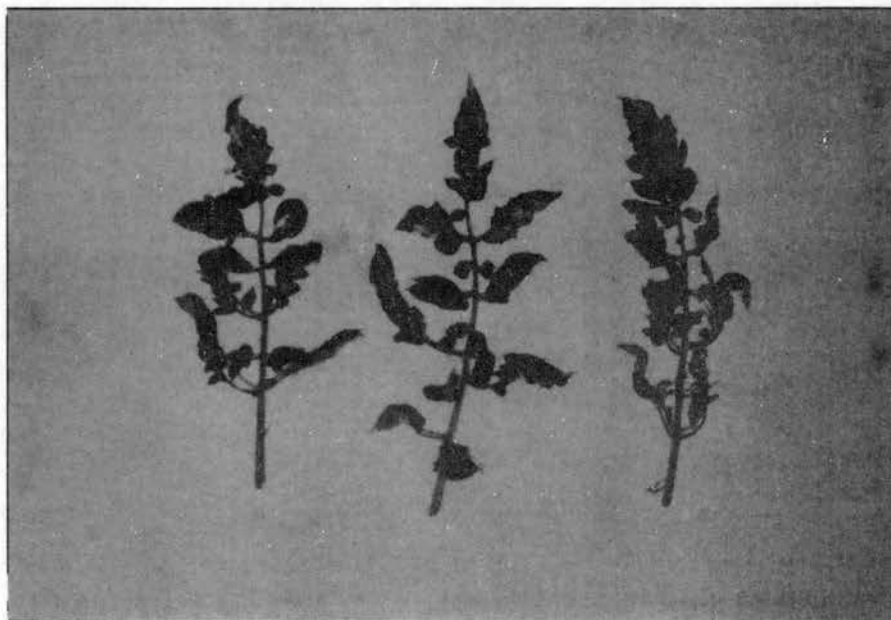


Figure 9. Symptoms on Leaves, Showing Curling, Twisting and Necrosis.

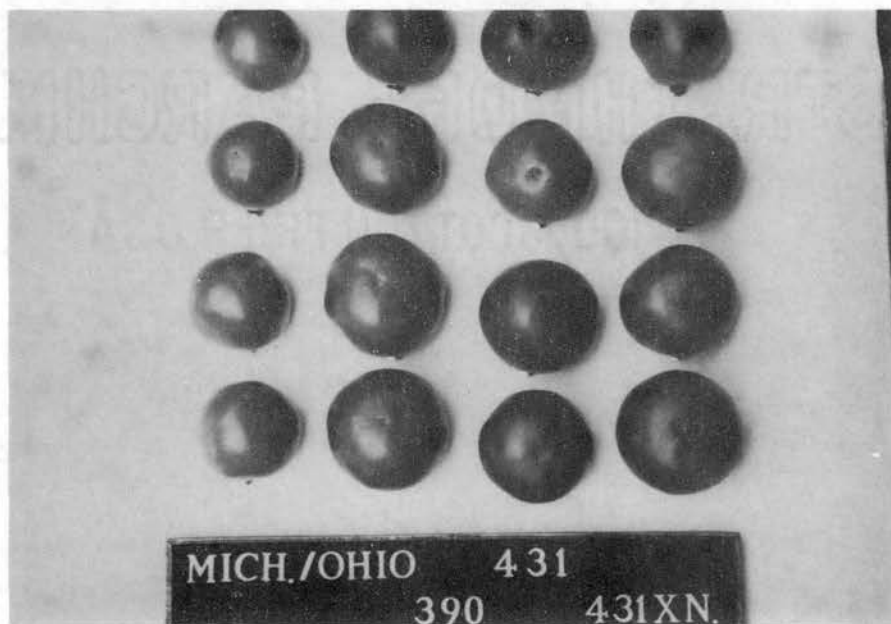


Figure 10. Fruit Shape of F_1 Hybrid of STEP 431 x Nema-
red in Comparison with Mich -Ohio Hybrid,
STEP 390 and 431.



Figure 11. The Healthy Plant of STEP 431 with Fruits.



Figure 12. The F_1 Hybrid of STEP 431 x Nemared, Showing High Degree of TMV Resistance.



Figure 13. The F_1 Hybrid of STEP 431 x Michigan State Forcing (center), Showing the Level of TMV Resistance.

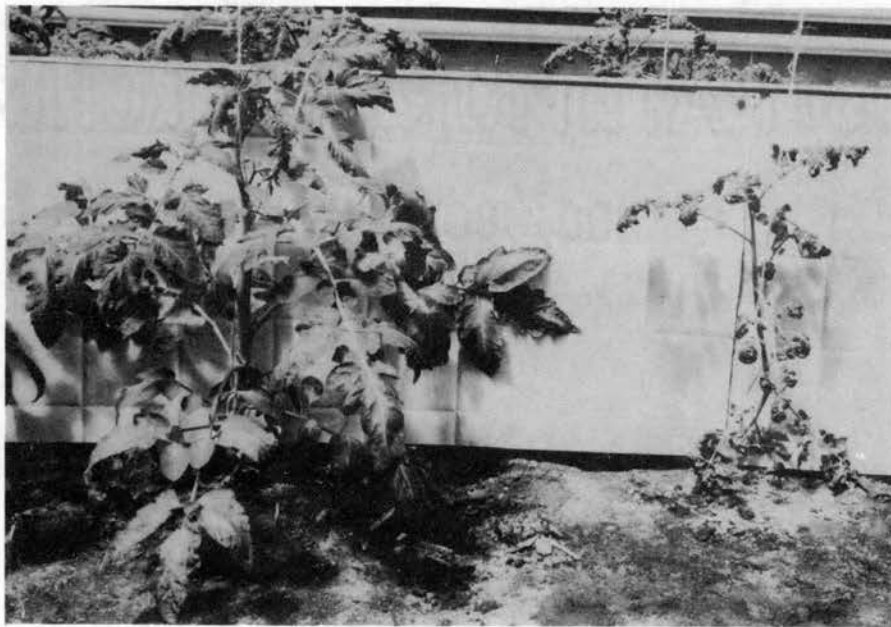


Figure 14. Comparison of Plant of STEP 431 x Nemared (left) and Mich -Ohio Hybrid (right) which Showed Stunted and Severe Damage by TMV.

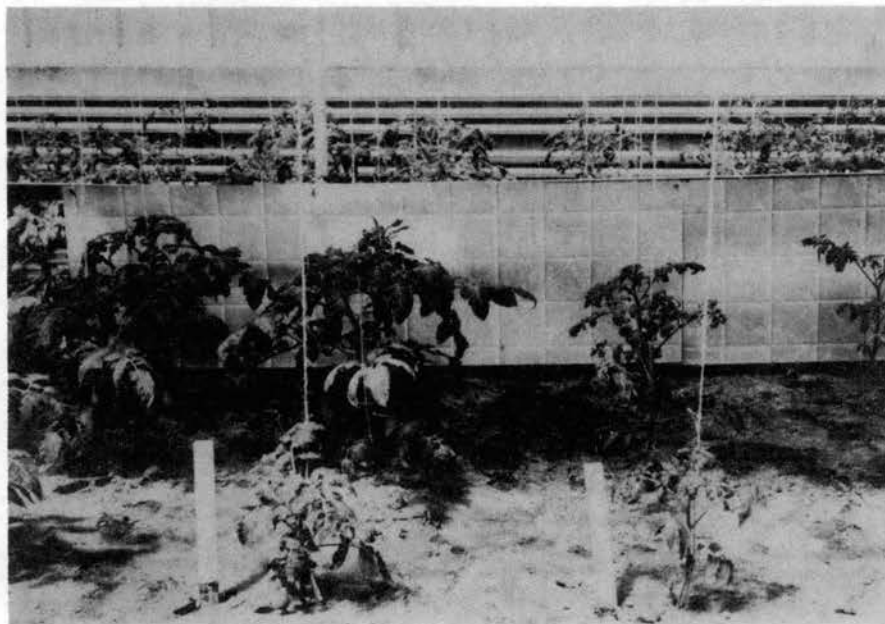


Figure 15. Comparison of TMV Resistance. From left to Right: First Row, STEP 390 x 431; Second Row, STEP 390; Third Row, Michigan State Forcing; Fourth Row, Mich.-Ohio Hybrid.



Figure 16. TMV Susceptibility of Mich -Ohio Hybrid and Michigan State Forcing Showing Severe Symptom on Leaves.

CHAPTER V

DISCUSSION AND CONCLUSION

Owing to the rapid infection and marked reduction of crop yield, TMV is one of the most important diseases of greenhouse tomatoes. Many attempts have been made to prevent disease contamination, the results in general have been erratic and/or unsuccessful. The development of TMV-resistance has become the obvious solution.

It is important to determine if young seedlings are contaminated by and show symptoms of virus prior to transplanting into the production bed. This would save labor, greenhouse space and other expenses if infected plants could be detected and discarded during the seedling stage.

Workers (28,39) have stressed that it is difficult to obtain clear-cut symptoms of the disease on the plant in the seedling stage. Preliminary tests were made to determine the effect of double virus inoculation on early symptom detection. The TMV-susceptible variety Sioux was used in this test. Data in Table I showed that only slight symptoms appeared on leaves within 14 days after inoculation with either TMV or PVX. On the other hand, when the seedlings were inoculated with double virus, severe chlorotic mottling and distortion occurred on the leaves within 12 days. From the data in Table II, it is evident that the double virus inoculation provided the earliest and most reliable symptoms for tomato plants.

In screening TMV-resistant lines STEP 390 and 431 and hybrids, symptoms of double virus were evident on hybrids. Data on the appearance of symptoms on leaves in the young seedling stage are presented in Table III. This suggests that the resistant lines were 100 per cent resistant to double virus. STEP 390 and 431 produced resistant hybrids when used in combination with Y91 and Nemared with only 12.5% showing infection. The TMV-susceptible varieties MSF and Mich.-Ohio hybrid were 100 per cent infected and severe symptoms appeared on leaves within 9 days.

In further screening tests most of the seedling plants of TMV-resistant lines STEP 390, 431 and derivatives of 431 were infected. Obviously, a strain of virus not present in previous tests was present at this time. The apparent resistant lines, STEP 390, 431 and the derivatives of 431 were essentially susceptible to the new race of inoculum. Data from these tests are presented in Table IV. Three escaped seedlings of STEP 390, 431-1-2 and 431-2-1 exhibited TMV symptoms after three months.

Results of tests with tomato plants inoculated with different sources and concentration of TMV virus are given in Table V, Table VI and Table VII. Inoculum obtained from tomato selection 431-2-2 which survived the spring crop, caused earlier and more severe symptoms than other inocula used. Three sources of inoculum TMV 1, TMV 3, and TMV 4 caused no apparent difference in either the time lapse or severity of symptoms.

The higher concentration of inoculum produced the earliest symptoms. It is noted that plants treated with inoculum of a 1:5 dilution had visible symptoms about ten days prior to those inoculated with a 1:25 dilution. There was no difference in per cent of infection finally

obtained between plants treated with the two levels of concentration of inoculum.

The F₂ hybrids of STEP 390 and 431 were screened with different sources of virus at two levels of concentration of inoculum. A source of TMV 2 obtained from MSF, which originated from 431-2-2, produced disease symptoms. Apparently, this was due to the combination of other strains of virus and less hybrid vigor for resistance as in F₂. It was also noted that the higher concentration of inoculum produced the earlier symptoms in the F₂ plants. None of the F₂ hybrids or TMV-resistant lines were resistant to the virus. The concentration of inoculum of a 1:5 dilution caused symptoms to be evident at an earlier date for the F₂ hybrids than was the case with the F₁ hybrids of STEP 390 and 431.

It has been reported that the F₁ hybrids of TMV-resistant lines have a wider range of resistance to other tomato diseases and, therefore, may be more productive than other lines or varieties. This may be due to the level of heterosis. The lack of resistance of F₁ hybrids involving STEP 390 and 431 may be due to incomplete dominance as suggested in the work carried out by Kikuta and Frazier (22). It may also be possible that the difference is due to a gene modifier.

The reaction of plants of the combination STEP 390 x 431, which showed only partial resistance to TMV, may have been due to the fact that the parents originated from similar stocks. The more similar the characters possessed by the parents the less the potential for heterosis in the hybrids.

Data on the effect of TMV on yield of tomato lines and hybrids for the spring of 1966 are given in Table VIII, Table IX and Table X.

TMV-susceptible variety MSF, and Mich.-Ohio hybrid which were infected early were markedly delayed in fruiting and yield was substantially reduced. Plants of F_1 hybrids which showed symptoms at later times were not as severely affected nor were there similar reduction in yield. Later in the spring, temperatures were high enough to inactivate the virus, thereafter new growth appeared free of virus.

Data on the effect of TMV on total yield and other characters of tomatoes grown in the fall of 1966 are given in Table XI, Table XII, Table XIII, Table XIV, and Table XV. Virus was evident when the second cluster was in bloom and spread rapidly from plant to plant due to pruning and other cultural practices. Streak virus occurred following the TMV and rapidly infected the entire crop. This resulted in severe damage to the plants and greatly reduced yield following the fourth harvest. A number of plants died. When the total yield of the spring crop of 1966 is compared with the total yield of the fall crop of 1966, the severity of the disease becomes evident. The temperatures were more favorable for virus development in the fall and all plants showed virus symptoms by the date of the last harvest.

Of the several varieties tested, Nemared appeared to provide the greatest amount of TMV-resistance when combined with STEP 390 or 431. This combination also provides southern root knot resistance in the F_1 in addition to an increase in TMV resistance. When Y91 was used as a parent in the hybrid combination, it performed in a similar fashion to Nemared. Its F_1 hybrids were earlier in production but lower in total yield than the hybrids with Nemared. The selection 431-2-2 was more productive than the original 431. Plants of the MSF x 431 F_1 hybrid produced good yields and at the same time MSF contributed much

to fruit smoothness.

CHAPTER VI

SUMMARY

1. The effectiveness of double virus inoculation in screening TMV-resistant lines and their F_1 hybrids and the influence on total yield of tomatoes were studied.
2. Double virus inoculation caused clear-cut symptoms when young seedling tomatoes were treated. It was evident that double virus inoculation provided an effective screening of seedlings before time to transplant to the ground bed. This should save labor, greenhouse space and other expenses.
3. TMV-resistant lines STEP 390 and 431 appeared to be 100 percent resistant to double virus. They were tolerant to the virus when used in combination with Y91 or Nemared.
4. The exceptions of STEP 390, 431 and derivatives of 431 were obviously due to the presence of other strains of virus which were not present in previous trials.
5. Similar reactions were observed by use of different sources of virus. TMV 2 caused more severe and earlier appearance of symptoms which may be due to a combination with other strains of virus.
6. A 1:5 dilution of inoculum caused symptoms to appear a few days earlier than those of a 1:25 dilution.
7. Due to heterosis, the F_1 hybrids of TMV-resistant lines appear to have a wider range in disease resistance. The exception, involving

STEP 390 and 431, may be due to incomplete dominance or to a gene modifier.

8. TMV-susceptible variety, MSF and Mich.-Ohio hybrid were markedly delayed in fruiting and yields were substantially reduced as a result of early infection. Nemared combined with STEP 390 or 431 appeared to be the best of the material tested with respect to TMV-resistance.

9. The selection 431-2-2 appeared to be more productive than the original 431.

10. Resistant varieties and F₁ hybrids involving resistant and susceptible parents show TMV symptoms late and in such cases production was not substantially reduced.

LITERATURE CITED

1. Allard, H. A. 1917. Further Studies of the Mosaic Disease of Tobacco. Jour. Agri. Res. 10:615-632.
2. Boyle, J. S. and D. C. Wharton. 1957. The Experimental Reproduction of Tobacco Internal Browning by Inoculation with Strains of Tobacco Mosaic Virus. Phytopath. 47:199-207.
3. Clinton, G. P. 1907. Chlorosis of Tomatoes. Conn. Agri. Sta. Rep. 31:362.
4. Cook, M. T. 1947. Virus and Virus Diseases of Plant. Burgess Publishing Company. Minneapolis, Minn.
5. Davis, D. W. and R. E. Webb. 1966. First Generation Crosses Between a New Virescence-free Tobacco Mosaic Resistant Tomato Line and Susceptible Commercial Varieties. Proc. Amer. Soc. Hort. Sci. 88:557-567.
6. Dickson, B. T. 1925. Tobacco and Tomato Mosaic. Science 62:398.
7. Doolittle, S. P. and W. S. Porte. 1949. Resistance of Lycopersicon hirsutum x L. esculentum Hybrids to Infection with Tobacco Mosaic Virus by Handling and Pruning. Phytopath. 39:503.
8. Frazier, W. A. and K. Dennett. 1949. Tomato Lines of Lycopersicon esculentum Type Resistant to Tobacco Mosaic Virus. Proc. Amer. Soc. Hort. Sci. 54:265-271.
9. Gardner, M. W. and J. B. Kendrick. 1922. Tomato Mosaic. Indiana Agri. Expt. Sta. Bull. No. 261-6.
10. Grant, T. J. 1934. The Host Range and Behavior of the Ordinary Tobacco-Mosaic Virus. Phytopath. 24:311-336.
11. Heuberger, J. W. and A. J. Moyer. 1931. Influence of Mosaic Infection on Tomato Yields. Phytopath. 21:745-749.
12. Heuberger, J. W. and J. B. Norton. 1933. The Mosaic Diseases of Tomato. Maryland Univ. Agri. Expt. Sta. Bull. No. 345, Part I. 447-486.
13. Holmes, F. O. 1928. Accuracy in Quantitative Work with Tobacco Mosaic Virus. Bot. Gazette. 86:66-81.

14. Holmes, F. O. 1929. Inoculating Methods in Tobacco Mosaic Studies. Bot. Gazette. 87:56-63.
15. Holmes, F. O. 1939. The Chilean Tomato, Lycopersicon chilense, as a Possible Source of Disease Resistance. Phytopath. 29:215-216.
16. Holmes, F. O. 1943. A Tendency to Escape Tobacco-Mosaic Disease in Derivatives from a Hybrid Tomato. Phytopath. 33:691-697.
17. Holmes, F. O. 1943. Derivatives of Tomato that Tend to Escape Tobacco-Mosaic Disease. Phytopath. 33-19.
18. Holmes, F. O. 1954. Inheritance of Resistance to Infection by Tobacco-Mosaic Virus in Tomato. Phytopath. 44:640-642.
19. Holmes, F. O. 1957. True-Breeding Resistance in Tomato to Infection by Tobacco-Mosaic Virus. Phytopath 47:16-17.
20. Johnson, F. 1926. The Attenuation of Plant Virus and the Inactivating Influence of Oxygen. Science. 64:210.
21. Johnson, J. 1937. Factors Relating to the Control of Ordinary Tobacco Mosaic. Jour. Agri. Res. 54:239-273.
22. Kikuta, K. and W. A. Frazier. 1947. Preliminary Report on Breeding Tomatoes for Resistance to Tobacco Mosaic Virus. Proc. Amer. Soc. Hort. Sci. 49:256-262.
23. Lehman, S. G. 1934. Contaminated Soil and Cultural Practices as Related to Occurrence and Spread of Tobacco Mosaic. North Carolina Agr. Expt. Sta. Tech. Bull. 46.
24. Leon, K. J. and G. Burnett. 1935. Virus Diseases of Greenhouse-Grown Tomatoes. Washington Agri. Expt. Sta. Bull. No. 308:11.
25. Lorin, R. B. and H. M. Munger. 1944. A Rapid Method for Mechanically Transmitting Plant Virus. Phytopath. 34:1010.
26. McRitchie, J. J. 1957. Pathogenic Strains of Tobacco Mosaic Virus on Tomato. Phytopath. 47:23-24.
27. McRitchie, J. J. and J. A. Leonard. 1957. Effect of Strains of Tobacco Mosaic Virus on Yield of Certain Tomato Varieties. Phytopath. 47:24.
28. Murakishi, H. H. and S. Honma. 1963. Resistance to Tobacco Mosaic Virus in Lycopersicon Hybrids Evaluated by Potato Virus x Synergy. Euphytica 12:27-31.
29. Norton, J. B. S. 1914. Loss from Mosaic Disease of Tomato. Phytopath. 4:398.

30. Norton, J. B. S. 1914. Tomato Disease. Md. Agri. Expt. Sta. Bull. 180:102-114.
31. Partyka, R. E. and J. E. Leonard. 1914. Greenhouse Tomatoes Disease Control. Ohio State Univ. and U.S.D.A. Cooperative Ext. Serv. SB-16 21.
32. Phillip, M. J., S. Honma and H. H. Murakishi. 1965. Studies on the Inheritance of Resistance to Tobacco Virus in the Tomato. Euphytica 14:231-236.
33. Porte, W. S., S. P. Doolittle and F. L. Wellman. 1939. Hybridization of a Mosaic-Tolerant, Wilt-Resistance Lycopersicon hirsutum with Lycopersicon esculentum. Phytopath. 29:757-759.
34. Smith, K. M. 1957. Plant Virus Disease. Little, Brown and Company. Boston.
35. Soost, R. K. 1963. Hybrid Tomato Resistant to Tobacco Mosaic Virus. Inheritance for Resistance in Derivatives of a Complex Species Hybrid. Jour. Hered. 54:241-244.
36. Walker, J. C. 1950. Plant Pathology. McGraw-Hill Book Company, Inc., New York, N. Y.
37. Walter, J. M. 1950. The Influence of Mosaic on Yield of Staked Tomatoes. Phytopath. 40:791.
38. Walter, J. M. 1956. Combination of Resistances to Tobacco-etch and Tobacco-Mosaic Virus in Tomato Breeding Stocks. Phytopath. 46:517-519.
39. Walter, J. M. 1956. Hereditary Resistance to Tobacco-Mosaic Virus in Tomato. Phytopath. 46:513-516.
40. Weber, P. V. 1960. The Effect of Tobacco Mosaic Virus on Tomato Yield. Phytopath. 50:235-237.

VITA

Kwee-Chong Pan

Candidate for the Degree of

Master of Science

Thesis: A STUDY OF THE EFFECT OF TOBACCO MOSAIC VIRUS INOCULATIONS ON
CERTAIN GREENHOUSE TOMATO VARIETIES AND SELECTIONS

Major Field: Horticulture

Biographical:

Personal Data: Born in Mantin, N.S., Malaya, August 12, 1937, the
son of Nyee Pan and Nam-Kwee Chang.

Education: Graduated from Chung-Chin High School, Singapore in
1957; received a Bachelor of Science degree from Taiwan Pro-
vincial Chung-Hsing University, with a major in Horticulture
in July, 1962; completed requirements for the Master of
Science degree in July, 1968.

Professional Experience: Teacher of Chung-Hwa School, Kuala Pilah,
N.S., Malaya, in 1958. Served as Primary Production Officer,
Primary Production Department, Singapore, from 1963 to 1965.