AN ABSTRACT OF THE THESIS OF

JUSTO LOPEZ-GARCIA for the M.S. in HORTICULTURE
(Name) (Degree) (Major)

Date thesis is presented December 14, 1963

Title EFFECT OF TEMPERATURE AND ZINC ON DEVELOPMENT OF TOBACCO MOSAIC VIRUS IN RESISTANT AND SUSCEPTIBLE TOMATOES

Abstract approved ____________________
(Major professor)

An investigation of the effects of temperature and of zinc on development of tobacco mosaic virus (TMV) in resistant and susceptible varieties of tomatoes was conducted under greenhouse conditions. The study included two experiments, one run during late winter and early spring, and the other during late spring and summer, 1963. Each experiment comprised four treatments and two temperatures.

Increase in temperature tended to increase virus concentration, although the differences were not significant. Fresh weights of the tomato plants were found to be greater at lower temperatures.

Zinc foliar sprays resulted in higher virus concentrations and in production of increased numbers of local lesions. Effects of zinc on fresh weights were not significant.

The varieties Hawaii 6832 and OSU-8 were found highly resistant to the common strain of TMV. California 62 PM 22 was shown to be heterozygous for resistance to this strain of TMV. OSU 435-4 was highly susceptible.

EFFECT OF TEMPERATURE AND ZINC ON DEVELOPMENT OF TOBACCO MOSAIC VIRUS IN RESISTANT AND SUSCEPTIBLE TOMATOES

by

JUSTO LOPEZ-GARCIA

A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1964

APPROVED:

____
Professor of Horticulture
In Charge of Major

____
Head of Department of Horticulture

____
Dean of Graduate School

Date thesis is presented December 14, 1963

Typed by Jolene Hunter Wuest

ACKNOWLEDGMENTS

The author wishes to express his very sincere and deep appreciation to his major professor, Dr. W. A Frazier, for his inspiring guidance, continuous encouragement, and invaluable assistance during this study and in the preparation of this manuscript.

Thanks are also expressed to my minor professor, Dr. E. K. Vaughan, for his assistance during the investigation, and in preparing the present thesis; to Dr. Harry J. Mack, for his assistance with the zinc treatment technique; to Dr. John A. Milbrath, for furnishing the strain of TMV used in this study; and to Dr. J. N. Singh, for his able assistance with the statistical analyses.

And finally, but not least, sincere appreciation is also extended to my wife for her continuous encouragement and invaluable assistance.

TABLE OF CONTENTS

LIST OF TABLES

EFFECT OF TEMPERATURE AND ZINC ON DEVELOPMENT OF TOBACCO MOSAIC VIRUS IN RESISTANT AND SUSCEPTIBLE TOMATOES

INTRODUCTION

The tomato, Lycopersicon esculentum Mill., is one of the most popular vegetable crops in the world. In the United States it is widely cultivated, being second only to potatoes in dollar value. Because of its significance to the agricultural economy, this crop has received major attention in research. Many studies have been conducted to attain a better understanding of the relationship between nutrition of the plant and disease development. It is generally acknowledged that a well nourished plant has better ability to resist infection by certain disease organisms than a weak, undernourished one. A well nourished plant should be considered as one that has the essential nutrients available at the appropriate time and has the ability to use them for optimal growth.

Assuming that all the required elements are available, other environmental factors must be at an optimum if plants are to make maximum growth. Temperature is one of the most important environmental factors, affecting both the physiologic and metabolic processes of the plant. Different temperatures may alter the resistance or susceptibility of the plant to certain diseases.

Although the relationships of nutrition and temperature to plant growth, infection, and disease development have been investigated by several workers, it appeared that new studies might lead to a better understanding of certain important diseases. The virus diseases constitute one of the serious threats to our horticultural crops. It is well known that tobacco mosaic is an important economic disease of the tomato plant. Tobacco mosaic virus (TMV) is one of the most, if not the most, widely distributed viruses affecting tomato. The purpose of this study was to determine the effects of temperature and of zinc on the development of tobacco mosaic virus (TMV) in resistant and susceptible varieties of tomato.

REVIEW OF LITERATURE

Adolf Mayer (22, vol. 32, p. 451-476) in 1886 described a disease of the tobacco plant, Nicotiana tabacum using the term "mosaic" which is now widely used for describing the mottling type of virus diseases. Iwanowsky (16, vol. 35, p. 67-70) in 1892 proved the filterability of the infected plant sap by passing it through a bacteria-proof filter candle. Beijerinck (5, vol. 65(2), p. 3-21) in 1898 first used the term "virus" to describe the causative agent of tobacco mosaic. It was not until 1935 that Stanley (32, p. 4-5) isolated the virus and described it as a glycoprotein. The same year Bawden and Pirie reported that the virus was a nucleoprotein. Since 1935 tobacco mosaic virus has been studied by hundreds of different investigators.

Although TMV attacks many hosts, certain members of the Solanaceae seem to be preferred. Within this family it is found commonly on tobacco. Nicotiana tabacum, L.; tomato, Lycopersicon esculentum, Mill.; and garden pepper Capsicum frutescens, L. In most susceptible varieties of tobacco the symptoms of the disease generally are light green spots, followed by vein clearing, distortion, and greenish-yellow mottling of newly formed leaves. Some strains of tobacco, in which TMV infection causes necrotic lesions, have been produced in an attempt to perform better and easier assays of the virus. The tomato plant does not, as a rule, show any visible primary lesions; the systemic disease is characterized by greenish-yellow mottling of foliage, moderate distortion of leaf shape, and a reduction of fruit yield. When potato virus X (PXV) is also present a more severe disease known as "double virus-streak" is induced. Tobacco mosaic virus is primarily transmitted by mechanical contact although it may be transmitted, to some extent, by aphids. It also may be transmitted through the seeds in tomato but not in tobacco (13, p. 1164-1166).

Numerous studies have been conducted in an attempt to clarify the role of host nutrition on TMV infection and multiplication. Bawden and Kassanis (3, vol. 37, p. 46-57) reported that the number of local lesions produced in leaves of Nicotiana tabacum and Nicotiana glutinosa by tomato aucuba mosaic virus and by TMV was increased by additions of both nitrogen and phosphorus, provided these also increased growth. In other experiments, Bawden and Kassanis (4, vol. 37, p. 215-228) found that the concentration of virus in the sap from potato plants systemically infected with two strains of potato virus X was not consistently affected by fertilizers; the chief effect of host nutrition on virus reproduction was indirect, by altering plant size. On the other hand, Weathers and Pound (39, vol. 44, p. 74) reported that with variations in the concentration of balanced nutrient solutions there was a close correlation of host growth and virus concentration. The highest lesion counts and optical density readings were obtained when both nitrogen and phosphorus were at the optimum levels for plant growth. Nitrogen and phosphorus are essential for virus synthesis and it may be expected that variations in these elements might affect virus multiplication.

Tobacco mosaic virus appears to affect the mineral composition of the leaves of virus-infected tomato plants, for Bergman and Boyle (7, vol. 52, p. 956-957) reported that TMV infection, although not significantly affecting P, K, Ca, Mg, and B in leaves, did affect Mn, Fe, Cu, Al, and Zn and the differences were significant. It is worthwhile to note that the elements that were significantly decreased were those metals involved in the activation of enzymes. These enzymes may be involved in virus synthesis.

Micronutrients have been considered, by several workers, to play key roles in host growth and virus multiplication. According to Loring and Waritz (21, vol 25, p. 646-648) Fe, Cu, Mg, and probably Ca are integral viral components. They also suggested that Fe and Mg are located in the nucleic acid rather than in the protein. Similarly, Pound and Welkie (28, vol. 5, p. 371-381) reported that marked deficiencies of Fe seemed to limit the effects of virus infection. This might be attributed to reduced virus multiplication in plants extremely deficient in Fe. Since Fe is essential for enzyme activation it is possible that Fe deficiencies would reduce virus synthesis and multiplication. Tobacco plants deficient in Mg (32, vol. 50, p. 195-198); Mo (25, vol. 52, p. 822-827); and S (20, vol. 52, p. 155-158), were found to have lower virus concentrations than plants having optimum levels of these elements. On the other hand, Mn-deficient tobacco plant tissue reached a higher level of virus concentration than normal plant tissue when samples were assayed on either dry or wet basis and by either ultra-violet absorption or local lesion assays (41, vol. 5, p. 92-109). Boron is probably not involved in TMV multiplication other than indirectly by favoring a more vigorous host plant, as reported by Shepherd and Pound (31, vol. 50, p. 26-30).

Zinc nutrition appears to have been studied to a great extent by several investigators. Helms and Pound (11, vol. 1, p. 408-423) working with tobacco plants, found that development of virus symptoms was more pronounced in plants grown in nutrient solutions having zinc levels optimal for growth than in plants grown at deficient levels. They also reported that TMV multiplication is associated with the utilization of zinc or with the withdrawal of the element from the leaves in which virus multiplication is occurring. Zinc seems to be essential for the general metabolic processes within the plant, for Skoog (33, vol. 27, p. 939-951) concluded that it is required for normal auxin activity in tomato plants. On the other hand, Weintraub, et al. (40, vol. 42, p. 417-419) suggested that the zinc ion was responsible for the inhibition of TMV in detached half-leaves of Nicotiana tabacum. They also suggested that to be effective the zinc ion must be in soluble form. Working with tomatoes, Smith (34, vol. 26, p. 737-749) found that the higher zinc concentrations tended to increase the resistance of the plants to late blight, Phytophthora infestans. Finally, Yarwood (42, vol. 44, p. 230-233) reported that the same zinc treatments which increased the number of local lesions on bean reduced the number of local lesions on Nicotiana tabacum.

Temperature has been found to exert considerable influence upon the growth of the host as well as on virus susceptibility and multiplication. Bancroft and Pound (1, vol. 44, p. 481-482) reported that four days after inoculation a sharp gradient of virus concentration existed in inoculated leaves of the susceptible variety of tobacco, the concentration increasing with increasing temperature. Similarly, in resistant tobacco varieties, the highest virus concentrations were always found in plants grown at the highest temperatures, when severe symptoms were produced (2, vol. 2, p. 29-43). Vaughan (37, vol. XX(3), p. 389-398) also found that in all tests most rapid TMV multiplication occurred at temperatures from 25°

to 30°C. On the other hand, it was reported by Ford and Ross (9, vol. 52, p. 71-77) working in tobacco plants with potato virus X and potato virus Y that, in general, symptom severity increased as temperature decreased. Meanwhile, it was found in another experiment by Hildebrandt, et al. (12, vol. 44, p. 492) that local lesion production was greatest from cultures at 24°, 26°, and 28°C; least at 36° and 37°C, and intermediate at 4°, 8°, 20°, and 42°C.

Sometimes, interaction occurs between unrelated viruses, and this interaction is affected by temperature. Murakishi (23, vol. 52, p. 22) working in tomatoes with TMV and potato virus X found that at 28°C TMV multiplication in doubly infected susceptible plants was rapid; multiplication of potato virus X was inhibited. According to Pound, (26, vol. 78, p. 161-170) the virus concentration was always higher in horseradish plants grown at 16°C than in those grown at 28°C. He also suggested the possibility of an inhibitor in horseradish plants occurring in greater concentrations at one temperature than at another. Pre-inoculation heating of bean leaves has been found to not only increase the number of local lesions but also to result in larger lesions (29, vol. 13, p. 338-347). Differences in photoperiods and light intensities have also been studied in tobacco plants in relation to virus multiplication. Long periods of diffuse light were found more favorable for virus multiplication than short periods of intense light (27, vol. 2, p. 44-56).

The evidence indicates that both nutrition and temperature are important factors in the interaction of plant growth and virus multiplication.

Plant breeders have conducted numerous studies of resistance to virus diseases, especially TMV. Although it was not the primary purpose of this study to deal with inheritance of resistance to TMV in tomatoes it was deemed advisable to use susceptible and resistant types, and a brief review is therefore given here. The wild species, _Lycopersicon pimpinellifolium_, _L. peruvianum_, and _L. hirsutum_ have been used as sources of resistance to TMV. Holmes (14, vol. 33, p. 691-697) reported a tendency to escape TMV in derivatives from a cross between _L. esculentum_ Mill. and _L. chilense_. His evidence suggests that this character is hereditary. In another experiment, Holmes (15, vol. 42, p. 467) found that in a series of backcrosses to susceptible lines, segregation occurred in the approximate ratio of 1:1. He concluded that the tendency to escape infection by TMV was dependent on a single dominant gene. Watson and Heinrich (38, vol. 44, p. 944) studied the inheritance of resistance to TMV in crosses of _L. esculentum_ var. Sioux x _L. hirsutum_. A ratio of three symptomless plants to one plant with symptoms was obtained in the backcross to _L. Hirsutum_. This indicated at least two factors for symptom expression. Frazier and Dennett (10, vol. 54, p. 265-271) and Kikuta and Frazier (17, vol. 49, p. 256-261) working in Hawaii, used *L. esculentum*, *L. hirsutum*, and *L. pimpinellifolium* as sources of resistance to TMV. The varieties Hawaii 6832 and OSU-8 used in the experiments reported here and the materials used by Holmes in his backcross study, were derived from these interspecific crosses.

MATERIALS AND METHODS

Materials

A. The tomato varieties used in this study were:

(1) OSU-435-4-(Susceptible check, developed in Oregon.)

(2) OSU-8-(Originated in Hawaii for resistance to TMV.)

(3) Hawaii 6832-(Developed in Hawaii for resistant to TMV.)

(4) California 62 PM 22-(Developed in California and reported as resistant to TMV.)

Varietal Background

Hawaii 6832

This is a derivative from a multiple cross (*L. esculentum*) x (*L. peruvianum* x *L. pimpinellifolium*) x (*L. hirsutum*). Because of the complexity of the cross, it is difficult to predict the specific origin of its resistance; however, it is well known that there is resistance to TMV in *L. peruvianum*, *L. pimpinellifolium*, and *L. hirsutum*.

California 62 PM 22

This variety was kindly furnished by Dr. Paul G. Smith of the University of California at Davis. This was an F_3 of the cross

Pearson x (Red Cherry x Solanum pennellii F_2).

OSU-435-4

The parentage of this variety is (Campbell Selection 10 x Pennheart) x Queens. The original cross was made in 1959; it was next crossed to Queens in 1953. It was selected as single plants in 1957, 1958 and 1959, then massed in 1960 and subsequent years.

OSU-8

Same parental background as Hawaii 6832.

B. Tobacco Variety:

The tobacco strain Necrotic Turk used in this study was derived from a cross of Nicotiana tabacum x Nicotiana glutinosa. It is the result of breeding work designed to secure a better test host for local lesion assays of TMV.

C. TMV Strain:

The common strain of TMV was used in the present study. The strain was inoculated to a tobacco plant of the variety Samsun and this systemically-infected plant was used to prepare the inoculum to inoculate the tomato plants.

D. Zinc:

Zinc sodium ethylenediamine tetracetate (E. D. T. A.), containing 14.2 percent zinc, was utilized for the foliar sprays at a rate of one gram per liter of water. This was a zinc concentration of about 142 parts per million.

Methods

First Experiment

Tobacco seeds were planted in the first week of December, 1962 in a one gallon metal can. The can was filled to within one inch of the top with ordinary greenhouse soil and irrigated. Then the seeds were spread over the soil and covered with about one-fourth inch of fine sand. The can was kept under normal greenhouse conditions. Individual tobacco seedlings were transplanted February 2, 1963, to one-gallon metal cans filled with greenhouse soil. One teaspoonful of complete fertilizer had been mixed with the soil prior to planting.

The tomato seeds were planted in one-gallon metal cans. Each variety was planted in a separate can filled with greenhouse soil to approximately one inch from the top. The seeds were pressed into the soil surface with a piece of wood, then covered with about one-fourth inch of fine sand. Enough moisture was provided for good germination and growth of the seedlings. The seeds were planted February 12, 1963. Symptoms of damping-off were observed a week or so after sowing. Zerlate sprays were applied in an attempt to control this condition. After the zerlate treatments mild to severe stunting was noticed in the tomato seedlings. Finally, fermate sprays were used to control damping-off. On March 12, 1963, seedlings were transplanted to one gallon metal cans filled with greenhouse soil to which one teaspoonful of complete fertilizer had been added. The fertilizer was thoroughly mixed prior to transplanting. Five seedlings were transplanted per can. Eighty plants of each variety were transplanted; there were sixteen cans of each variety.

The foliar sprays with zinc sodium E. D. T. A. were made with an atomizer about ten days before the seedlings were inoculated with TMV. Using a solution containing one gram per liter of water, the seedlings were uniformly wetted to the run-off point. Forty plants of each variety were treated with zinc foliar sprays.

About ten days after the zinc foliar sprays were applied the tomato seedlings were inoculated with the common strain of TMV. As a source of inoculum, one gram of leaf tissue from a systemically-infected Samsun tobacco plant was used. The tissue was ground in a mortar with pestle, and ten ml. of one percent K_2HPO_4 as a diluent buffer solution was added. The expressed sap was squeezed through cheesecloth. Two carborundum-dusted leaves of each tomato plant were inoculated by rubbing with the forefinger. Forty plants of each variety were inoculated. Twenty of these had been and 20 had not been treated with zinc.

Immediately after inoculation, the plants were divided into two lots and moved to the two temperature houses. Ten zinc-treated and TMV-inoculated plants of each variety were shifted to each temperature house. Ten zinc-treated but non-inoculated plants of each variety were moved to the high temperature and ten were placed in the low temperature house. Ten plants, without zinc treatment but TMV-inoculated, of each variety were moved to each temperature house. Ten plants, without zinc treatment and non-inoculated with TMV, of each variety were maintained in each house. Thus, in each house there were 40 plants of each variety.

In the high temperature house the temperature was kept approximately 75° F during the day and 65° F at night. On the other hand, in the low temperature house the temperature fluctuated with daily variations in outside temperature. Temperatures were recorded twice a day, in early morning and in mid-afternoon, from a thermometer located on the bench where the tomato plants were placed.

Because no symptoms appeared following the first inoculation with TMV, a second one was made about one week later. Twelve days after the second inoculation the virus titre was determined. One gram of TMV-infected tomato tissue was ground with a mortar and pestle with ten ml. of 0.5 percent Na_2HPO_4 buffer. The expressed sap was squeezed through cheesecloth and diluted to the following concentrations: 1:10; 1:100; 1:1,000; and 1:10,000. These dilutions were used to inoculate carborundum-dusted Necrotic Turk leaves and the number of local lesions was subsequently recorded. A concentration of 1:10 was found to be satisfactory in making the local lesion assays in this experiment.

The next step was to make the local lesion assay with the inoculum prepared from the tomato plants on the tobacco plants. The same procedure described above was used to prepare the inoculum. For each tomato plant one Necrotic Turk leaf was used. The tobacco leaf was tagged with the name of the variety, treatment, temperature, and number of the tomato plant.

The fresh weight of each tomato plant was determined by weighing the whole plant (leaves, stem, and roots). The weights in grams were recorded.

Second Experiment

For the second experiment essentially the same procedure was followed. Only three varieties of tomato were used in the second experiment. The variety OSU-8 was discarded because its apparent resistance was proved in the first experiment and one resistant type was considered sufficient.

Tobacco seeds were planted in the first week of May, 1963, following the same procedure used in the first experiment. The seedlings were transplanted June 20, 1963.

Tomato seeds were planted June 22, 1963, following the same procedure as in the first experiment, except that the soil in the cans was wetted, before spreading the seeds, with a fermate solution (one teaspoonful per gallon of water). No damping-off was noted in the second experiment. About two weeks later the seedlings were transplanted to one gallon cans, following the procedure used in the first experiment; however, in this test one tomato seedling was transplanted to each can. Eighty seedlings of each variety were planted.

The foliar sprays of zinc were applied ten days before the inoculation with TMV. By this time the seedlings were about one month old.

The tomato plants were inoculated with the same common strain of TMV from a systemically-infected Samsun tobacco plant, following essentially the same procedure as before. Forty plants of each variety were inoculated with TMV. Of these, 20 had, and 20 had not, received the zinc treatments.

Immediately following inoculation the plants were divided into two lots and shifted to the two temperature houses. Exactly the same procedure used in the first experiment was followed. Thus, 40 plants of each variety were placed in each house.

In this experiment the temperature in the low temperature house was kept at approximately 75°F during the day and 65°F at night. The temperature in the hot house, on the other hand, fluctuated with daily variations in outside temperature. This was the reverse of the situation in the first experiment. Temperatures were recorded in the high temperature house in the early morning and in mid-afternoon. Mean maximum, mean minimum, and mean daily temperatures were determined for both houses in both experiments.

Ten to 12 days after inoculation of the tomato seedlings, the virus titre was determined following the procedure described previously. The concentration 1:1,000 was found to be the best for local lesion assay in this experiment.

Once the virus titre was determined, assaying of the individual plants followed. One Nectrotic Turk tobacco leaf was used for each tomato plant. The same methods of preparing the inoculum discussed before were used in this test. Each tobacco leaf was tagged with the name of the variety, treatment, temperature, and number of the plant. The fresh weights in grams were recorded.

RESULTS

Experiment 1

Local Lesions

Table 1 shows the means of ten plants which constituted the replications of each treatment. The statistical analysis is presented in Appendix Table 1. The data show that under low temperature conditions zinc tended to increase the number of local lesions compared to the plants receiving no zinc treatments. On the other hand, at the high temperature zinc treatments tended to reduce the number of local lesions; however in both cases, the differences were not statistically significant.

The table clearly shows differences due to variety. Although the varieties Hawaii 6832, California 62 PM 22, and OSU-8 were reported as resistant to TMV, the data show clearly an erratic behavior in resistance of the variety California 62 PM 22. Inoculum from the varieties Hawaii 6832 and OSU-8 produced no local lesions when inoculated to Necrotic Turk tobacco leaves, indicating that these varieties have a high degree of resistance under the conditions of this experiment. The variety differences were highly significant at both one and five percent levels. Temperature differences were not significant.

Table 1. Effect of temperature and zinc on multiplication of TMV in tomato plants, measured by development of local lesions in Necrotic Turk tobacco. Experiment 1. (1)

Temperature	Treatments	Varieties							
		OSU-435-4		Hawaii 6832		California 62 PM 22		OSU-8	
		Check	TMV	Check	TMV	Check	TMV	Check	TMV
Low*	Zinc	0	58.8	0	0	0	48.4	0	0
	Without Zinc	0	47.7	0	0	0	9.3	0	0
High**	Zinc	0	50.9	0	0	0	25.5	0	0
	Without Zinc	0	66.8	0	0	0	30.0	0	0

L. S. D. for variety differences at : 0.01 20.19
0.05 15.08

Temperature and treatment differences were not significant

(1) Means of ten plants

* Mean maximum: 68.5°F, mean minimum: 63.9°F, mean daily: 66.2°F

** Mean maximum: 75 ° F, mean minimum: 65 ° F, mean daily: 70°F

Fresh Weight

Table 2 indicates the means of ten plants which constituted the replications of each treatment. The statistical analysis is presented in Appendix Table 2. The fresh weights were affected by both zinc and temperature as well as by TMV. In general, applications of zinc tended to increase fresh weights under both temperatures. The trend was somewhat greater in non-inoculated plants but the differences were not significant.

In general, fresh weights were higher under low temperatures than under high temperature conditions. The differences, however, were not significant.

Variety differences in fresh weight, although not significant, were interesting; there is the possibility that the trends would have been significant under conditions of better replication. The OSU-435-4 non-inoculated plants with and without zinc, and at both temperatures, showed higher mean values than the inoculated plants of the same variety grown under the same conditions. On the other hand, Hawaii 6832 inoculated plants, with and without zinc and at both temperatures had higher mean values than the plants without zinc grown under similar conditions. The variety OSU-8, although it is resistant, behaved similarly to OSU-435-4, since inoculated plants with and without zinc had lower mean values for fresh weight, under both temperatures, than non-inoculated plants. The variety California 62 PM 22 again showed very erratic behavior. Under low temperature the non-inoculated plants with or without zinc had higher mean values for fresh weight than inoculated plants, but under high temperature conditions the reverse occurred. Here the inoculated plants with or without zinc showed higher mean values than non-inoculated plants.

Table 2. Effect of temperature and zinc on the fresh weight in TMV-inoculated and non-inoculated resistant and susceptible tomatoes. Experiment 1. (1) (Grams)

Temperature	Treatments	Varieties							
		OSU-435-4		Hawaii 6832		California 62 PM 22		OSU-8	
		Check	TMV	Check	TMV	Check	TMV	Check	TMV
Low	Zinc	43.50	32.63	33.48	39.90	34.57	29.14	47.12	29.47
	Without Zinc	40.71	35.01	37.05	46.30	33.31	33.10	45.03	31.69
High	Zinc	32.10	26.24	34.89	35.27	37.38	38.55	44.99	28.66
	Without Zinc	32.06	25.72	39.51	44.23	25.99	27.78	34.99	34.40

Variety differences were not significant.

Temperature differences were not significant.

Treatment differences were not significant.

(1) Means of ten plants.

Experiment 2

Local Lesions

Table 3 shows the means of ten plants for each treatment. The statistical analysis is presented in Appendix Table 3.

Analysis of variance shows that treatments with zinc resulted in consistent, but not significant, increases in number of local lesions produced by inoculum from plants grown under both temperatures. One exception was the Hawaii 6832 line in which no local lesions were produced by inoculum prepared from plants grown at high temperature.

Variety differences for local lesions were highly significant. Because of the extremely erratic behavior for resistance to TMV, the variety California 62 PM 22 was omitted from the statistical analysis of variance. Individual plants obviously varied genetically for resistance and this source of error was thereby removed. Although Hawaii 6832 normally is highly resistant, in this experiment the inoculum prepared from this variety, with and without zinc, produced some local lesions when plants were grown under the low temperature but none when they were grown under the high temperature conditions. The number of local lesions was greater with the inoculum prepared from zinc-treated plants than from plants without zinc; but in both cases the number of local lesions was consistently less than in the other varieties.

Table 3. Effect of temperature and zinc on multiplication of TMV in tomato plants, measured by development of local lesions in Necrotic Turk tobacco. Experiment 2. (1)

Temperature	Treatments	OSU 435-4		Hawaii 6832		California 62 PM 22	
		Check	TMV	Check	TMV	Check	TMV
Low*	Zinc	0	168.6	0	6.7	0	107.8
	Without Zinc	0	149.4	0	2.3	0	15.1
High**	Zinc	0	268.8	0	0	0	92.6
	Without Zinc	0	124.0	0	0	0	67.3

(Varieties: OSU 435-4, Hawaii 6832, California 62 PM 22)

L. S. D. for variety differences at: 0.01 47.42
0.05 35.66

Temperature differences were not significant.

Treatment differences were not significant.

(1) Means of ten plants

* Mean maximum: 75°F. mean minimum: 65°F. mean daily: 70°F

** Mean maximum: 81.1°F. mean minimum: 61.7°F. mean daily: 71.1°F

Fresh Weight

The statistical analysis of variance for fresh weights is presented in Appendix Table 4.

In general, low temperature conditions increased the fresh weight means significantly at one and five percent levels.

Zinc treatments increased the fresh weight means, but differences were not significant.

The data show highly significant differences for the varieties at one and five percent levels. The variety OSU-435-4 had higher fresh weights in the non-inoculated plants, with and without zinc, than in the inoculated plants, with the exception of the zinc-treated plants in the low temperature. The California 62 PM 22 zinc-treated and TMV-inoculated plants under high temperature had greater mean values than the non-inoculated plants with zinc; however, under low temperature the reverse occurred. In general, the fresh weights of the Hawaii 6832 line were somewhat similar in all treatments and temperatures. However, under the low temperature the zinc-treated plants inoculated and non-inoculated had greater fresh weights than the plants without zinc, inoculated and non-inoculated, under the same temperature.

The greatest differences appeared to be between the California 62 PM 22 and the other two lines. The fresh weights of this variety were consistently lower than those of the varieties Hawaii 6832 and OSU 435-4. The differences between these latter two varieties were not significant; but the variety OSU 435-4 generally had higher mean values than Hawaii 6832.

Table 4. Effect of temperature and zinc on fresh weight in TMV-inoculated and non-inoculated resistant and susceptible tomatoes. Experiment 2. (1) (Grams)

Temperature	Treatments	Varieties: OSU 435-4		Hawaii 6832		California 62 PM 22	
		Check	TMV	Check	TMV	Check	TMV
Low	Zinc	194.93	200.12	180.54	176.53	153.68	141.87
	Without Zinc	198.52	175.75	180.44	175.04	156.57	164.44
	Zinc	184.47	167.33	170.50	175.52	150.06	154.73
	Without Zinc	187.75	168.90	183.48	181.44	137.63	125.53

L. S. D.	for variety differences at:	0.01	8.08
		0.05	6.12
	for temperature differences	0.01	16.19
		0.05	12.27

Treatment differences were not significant.

(1) Means of ten plants.

DISCUSSION

The data presented indicate that, in general, high temperature increased the development of TMV in tomato plants as shown by the increased number of local lesions produced on Necrotic Turk tobacco plants. In both experiments, however, the differences were not significant. In the first experiment a 1:10 dilution was shown to be appropriate for the local lesion assays; in the second experiment the virus concentration was higher and it was necessary to use a dilution of 1:1,000.

The first experiment was run in the late winter and early spring when short, cool, and cloudy days prevailed; the second test was run in late spring and summer when days were longer, rather sunny, and warmer. It seems likely that these environmental factors affected virus concentration and multiplication. Vaughan (37, vol. XX(3), p. 389-398) reported that the most rapid multiplication of TMV occurred at temperatures of 25° and 30°C. Similarly, Bancroft and Pound (1, vol. 44, p. 481-482), and (2, vol. 2, p. 29-43) found that virus concentration increased with increase in temperature. Pound and Bancroft (27, vol. 2, p. 44-56) showed that photoperiods of 12 and 16 hours increased concentration of TMV in inoculated tobacco plants. They also found that, in general, high light intensity favored virus multiplication in the same plants. It appears that in the present experiments increased temperature and day-length tended to increase the virus concentration and multiplication. Temperature and photoperiod affect the rate of growth as well as the general physiologic and metabolic processes of the host plant. As the temperature is increased, within limits, with an increase in day-length, the rate of growth is favored and, indirectly, virus multiplication may be increased. Bawden and Kassanis (3, vol. 37, p. 46-57), and (4, vol. 37, p. 215-228) in two experiments reported that virus multiplication was correlated with host plant growth.

Fresh weights were increased slightly with increased temperature in the first experiment; however, the differences were not significant. On the other hand, in the second experiment the trend was towards increase in fresh weight under the low temperature and the differences were significant. It seems that the tomato plants grow better at temperatures from 65°F to 75°F rather than at lower or higher temperatures. It is interesting to note that the temperature in the high temperature house in the first experiment was the same as that for the low temperature house of the second experiment.

Zinc increased virus concentration under the low temperature in the first experiment, and under both temperatures in the second. These findings are in accordance with those of Helms and Pound

(11, vol. 1, p. 408-423) who found that the virus concentrations increased with increasing levels of zinc up to the optimum level for growth. Zinc is well known as one of the essential elements for enzyme activation. Thus it may be that this element plays an important role in the enzymatic system responsible for virus synthesis and multiplication.

In the first experiment zinc increased the fresh weights of the varieties OSU-435-4, California 62 PM 22, and OSU-8 when applied to non-inoculated plants under both temperatures, but the zinc-treated and TMV-inoculated plants of the same varieties showed a decrease in fresh weights. These differences, however, were not significant.

In the second experiment zinc-treated plants showed some differences in fresh weights as compared with the plants without zinc; however, the differences were not significant. The variety OSU-435-4, zinc-treated, and TMV-inoculated plants under low temperature showed greater fresh weights than the non-inoculated plants, whereas similarly treated plants of the same variety under high temperature had lower fresh weights than the non-inoculated plants. The opposite occurred in the California 62 PM 22 line. The zinc-treated and TMV-inoculated plants under the low temperature had lower fresh weights than the non-inoculated plants, but under the high temperature similarly treated plants of the same line had greater fresh weights. With the Hawaii 6832 line at low temperature both with and without zinc the non-inoculated plants had greater fresh weights than the inoculated plants similarly treated. Under high temperature conditions, the without-zinc, inoculated, and non-inoculated plants had greater fresh weights than the zinc-treated plants. These results indicate that zinc effects vary with temperature but especially with the varieties, as would be expected. The general trend, however, was towards a decrease in fresh weight with TMV-inoculated plants. Apparently either zinc is utilized in the virus synthesis or its effects on plant growth are inhibited by the impact of the virus.

The data show variety differences in local lesions in both experiments. Of the varieties used in the first experiment, three were reported as resistant and one susceptible to TMV. However, in the first experiment a high degree of heterozygosity for resistance of the line California 62 PM 22 was noticed. This breeding line is thus highly heterozygous for resistance to the common strain of TMV. Some plants showed a very high degree of resistance; others were intermediate; and still others were highly susceptible. The number of local lesions produced on inoculated tobacco leaves by inoculum prepared from TMV-infected plants of this variety was used as the criterion. The variety OSU-435-4 behaved as expected, indicating its susceptibility by the high number of local lesions produced on tobacco leaves by inoculum prepared from TMV-infected plants of this variety. Inoculum prepared from TMV-infected plants of the varieties Hawaii 6832 and OSU-8 produced no lesions in inoculated tobacco leaves, indicating their high degree of resistance to the common strain of TMV under the conditions of this study.

In the second experiment the varieties behaved much the same as in the first experiment. Inoculum prepared from TMV-infected Hawaii 6832 plants produced local lesions in tobacco plants in this test, but they were reduced in number as compared with the other two varieties. It is interesting to note that the inoculum was prepared from Hawaii 6832 plants grown under the low temperatures conditions under which, in general, better growth took place. These findings coincide with studies conducted with tobacco plants by different workers (3, vol. 37, p. 46-57), (4, vol. 37, p. 215-223), and (31, vol. 50, p. 26-30).

In the first experiment differences for fresh weight between varieties existed but they were not significant; however, in the second experiment the differences were highly significant. It is worthwhile to note that in the first test five plants were grown per can whereas in the second individual plants grew in each can. There was competition for food, air, and light in the first experiment which affected the general growth of the plants. Besides, the first experiment was run in late winter and early spring with rather cool, cloudy, and short days whereas the second test was run in late spring and summer with rather longer, sunny and warmer days more favorable for general growth.

CONCLUSIONS

The effects of temperature and zinc on development of TMV in resistant and susceptible varieties of tomato were studied in two experiments conducted under greenhouse conditions. The data of this study show that:

1. Increase in temperature tended to increase virus multiplication.
2. Zinc applied as foliar sprays appeared to be involved in virus synthesis and multiplication, with higher virus concentrations associated with addition of zinc.
3. The varieties Hawaii 6832 and OSU-8 were highly resistant; the variety California 62 PM-22 was heterozygous for resistance; and the variety OSU-435-4 was highly susceptible to the common strain of TMV under the conditions of these experiments.

BIBLIOGRAPHY

1. Bancroft, J. B. and G. S. Pound. Effect of air temperature on multiplication of tobacco mosaic virus in tobacco. Phytopathology 44:481-482. 1954.

2. Bancroft, J. B. and G. S. Pound. Cumulative concentrations of tobacco mosaic virus in tobacco and tomato at different temperatures. Virology 2:29-43. 1956.

3. Bawden, F. C. and B. Kassanis. Some effects of host nutrition on the susceptibility of plants to infection by certain viruses. Annals of Applied Biology 37:46-57. 1950.

4. Bawden, F. C. and B. Kassanis. Some effects of host nutrition on the multiplication of plant viruses. Annals of Applied Biology 37:215-228. 1950.

5. Beijerinck, M. W. Veber ein contagium virum fluidum als Vrsache der Fleckenkrankheit der Tabaksblatter. Verhandelingen der Koninklyke akademie van Wettenschappen te Amsterdam. 65:(2) 3-21. 1899.

6. Bennett, C. W. Relation of food translocation to movement of tobacco mosaic. Journal of Agricultural Research 60:361-390. 1940.

7. Bergman, E. L. and J. S. Boyle. Effect of tobacco mosaic virus on mineral content of tomato leaves. Phytopathology 52:956-957. 1962.

8. Cartwright, T. E. and M. A. Gauffer. Some kinetic studies on the reaction of tobacco mosaic virus with formaldehyde. Phytopathology 44:484. 1954.

9. Ford, R. E. and A. F. Ross. Effect of temperature on the interaction of potato viruses X and Y in inoculated tobacco leaves. Phytopathology 52:71-77. 1962.

10. Frazier, W. A., and R. K. Dennett. Tomato lines of _Lycopersicon esculentum_ type resistant to tobacco mosaic virus. Proceedings of the American Society for Horticultural Science. 54:265-271. 1949.

11. Helms, K., and G. S. Pound. Zinc nutrition of Nicotiana tabacum in relation to multiplication of tobacco mosaic virus. Virology 1:408-423. 1955.

12. Hildebrandt, A. C., A. J. Riker, and J. L. Watertor. Virus infectivity host tissue growth on synthetic media at different temperatures and acidities. Phytopathology 44:492. 1954.

13. Holmes, F. O. The filterable viruses. Supplement No. 2 Bergey's Manual of Determinative Bacteriology. Edition 6. p. 1164-1166. 1948.

14. Holmes, F. O. A tendency to escape tobacco mosaic virus disease in derivatives from a hybrid tomato. Phytopathology 33:691-697. 1943.

15. Holmes, F. O. Inheritance of resistance to tobacco mosaic virus in tomato. Phytopathology 42:467. 1952.

16. Iwanowski, D. Veber die Mosaikkrankheit der Tabakspflanze. St Petersb. Acad. Imp. Sci. Bul. 35:67-70. Sept. 1892.

17. Kikuta, K. and W. A. Frazier. Preliminary report on breeding tomatoes for resistance to tobacco mosaic virus. Proceedings of the American Society for Horticultural Science. 49:256-261. 1947.

18. Lingle, J. C., D. M. Holmberg, and M. P. Zobel. Zinc deficiency of tomatoes. California Report of Progress in Research. California Agricultural Experiment Station 11(9): 10. Sept., 1957.

19. Lingle, J. C., D. M. Holmberg, and M. P. Zobel. The correction of zinc deficiency of tomatoes in California. Proceedings of the American Society for Horticultural Science. 72:397-402. 1958.

20. Link, K. C. and G. S. Pound. Sulfur nutrition of Nicotiana tabacum in relation to multiplication of tobacco mosaic virus. Phytopathology 52:155-158. 1962.

21. Loring, H. S. and R. S. Waritz. Occurrence of iron, copper, calcium, and magnesium in tobacco mosaic virus. Science 25:646-648. 1957.

22. Mayer, A. Ueber die Mosaikkrankheit des Tabacks. Die Landwirtschaftliehen Versuchs-Stationen 32: 451-476. 1886.

23. Murakishi, H. H. Virus multiplication and the synergistic response in tomato as modified by host resistance and temperature. Phytopathology 52:22. 1962.

24. Nichols, C. W. and G. S. Pound. The retarding effect of certain hormones on tobacco mosaic symptoms. Phytopathology 42:579-580. 1952.

25. Pirone, T. P. and G. S. Pound. Molybdenum nutrition of Nicotiana tabacum in relation to multiplication of tobacco mosaic virus. Phytopathology 52:822-827. 1962.

26. Pound, G. S. The effect of air temperature on virus concentration and leaf morphology of mosaic-infected horse-radish. Journal of Agricultural Research 78:161-170. 1949.

27. Pound, G. S. and J. B. Bancroft. Cumulative concentrations of tobacco mosaic virus at different photoperiods and light intensities. Virology 2:44-56. 1956.

28. Pound, G. S. and G. W. Welkie. Iron nutrition of Nicotiana tabacum L. in relation to multiplication of tobacco mosaic virus. Virology 5:371-381. 1958.

29. Resconovitch, E. C. Heat-induced susceptibility to tobacco mosaic virus and thermal injury in bean. Virology 13:338-347. 1961.

30. Samuel, G. Some experiments on inoculating methods with plant viruses and local lesions. Annals of Applied Biology 18:494-507. 1931.

31. Shepherd, R. J. and G. S. Pound. Influence of boron nutrition on Nicotiana tabacum on the multiplication of tobacco mosaic virus. Phytopathology 50:26-30. 1960.

32. Shepherd, R. J. and G. S. Pound. Magnesium nutrition of Nicotiana tabacum in relation to multiplication of tobacco mosaic virus. Phytopathology 50:195-198. 1960.

33. Skoog, F. Relations between zinc and auxin in the growth of higher plants. American Journal of Botany 27:939-951. 1940.

34. Smith, C. B. The nutrient-element balance of the tomato and its susceptibility to Phytophthora infestans as affected by two levels of zinc. Plant Physiology 26:737-749. 1951.

35. Smith, K. M. Plant Viruses. Methuen's Monographs on Biological Subjects. Third Edition, 1960. p. 4-5, 19-24, 95-96, 98-100, 127-129, and 150-153. 1960.

36. Spencer, E. L. The effect of host nutrition in concentration of tobacco mosaic virus. Plant Physiology 14:769-782. 1937.

37. Vaughan, E. K. The influence of temperature on multiplication of tobacco mosaic virus. Mededelingen Van De Landbouwhogeschool En De Opzoekingsstations Van De Staat Te Gent. Deel XX(3):389-398. 1955.

38. Watson, R. D. and E. E. Heinrich. The inheritance to tobacco mosaic virus in interspecific tomato crosses. Phytopathology 44:944. 1951.

39. Weathers, L. G., and G. S. Pound. Host nutrition in relation to multiplication of tobacco mosaic virus in tobacco. Phytopathology 44:74-80. 1954.

40. Weintraub, M., J. D. Gilpatrick, and R. S. Willison. The effect of certain water soluble compounds in virus infection. Phytopathology 42:417. 1952.

41. Welkie, G. W. and G. S. Pound. Manganese nutrition of Nicotiana tabacum in relation to multiplication of tobacco mosaic virus. Virology 5:92-109. 1958.

42. Yarwood, C. E. Zinc increases susceptibility of bean leaves to tobacco mosaic virus. Phytopathology 44:230-233. 1954.

APPENDIX

Appendix Table 1. Analysis of variance on the effect of temperature and zinc on multiplication of TMV in tomato plants, measured by development of local lesions in Necrotic Turk tobacco. Experiment 1.

Source of variation	Degrees of freedom DF	Mean square M. S.	F
Replication	9	1, 483. 46	1. 2748
Treatment	1	555. 03	0. 4769
Variety	3	28, 850. 58	24. 7969**
Temperature	1	50. 63	0. 0044
Treatment x variety	3	831. 83	0. 7148
Vareity x temperature	3	91. 69	0. 0788
Treatment x temperature	1	3, 115. 23	2. 6769
Treatment x temperature x variety	3	1, 153. 23	0. 9909
Error	116	1, 163. 71	
Total	140	1, 748. 88	

** Significant at . 01 level.

Appendix Table 2. Analysis of variance on the effects of tobacco mosaic virus, zinc and temperature on the fresh weight of resistant and susceptible tomatoes. Experiment 1.

Source of variation	Degrees of freedom DF	Mean square M. S.	F
Replication	9	1, 263. 11	2. 2329
Treatments	3	506. 13	0. 8947
Varieties	3	709. 55	1. 2543
Temperature	1	757. 99	1. 3399
Treatment x variety	9	528. 85	0. 9349
Variety x temperature	3	326. 62	0. 5774
Treatment x temperature	3	98. 29	0. 1737
Treatment x variety x temperature	9	139. 33	0. 2463
Error	249	565. 68	
Total	289	567. 18	

Appendix Table 3. Analysis of variance on the effect of temperature and zinc on multiplication of TMV in tomato plants, measured by development of local lesions in Necrotic Turk tobacco. Experiment 2.

Source of variation	Degrees of freedom DF	Mean square M. S.	F
Replication	9	3, 649. 52	0. 5740
Treatment	1	21, 125. 00	3. 3226
Variety	1	615, 654. 05	96. 8326**
Temperature	1	5, 412. 05	0. 8512
Treatment x variety	1	18, 361. 80	2. 8880
Vareity x temperature	1	8, 778. 05	1. 3806
Treatment x temperature	1	31, 840. 20	5. 0079*
Treatment x temperature x variety	1	35, 448. 20	5. 5754*
Error	63	6, 357. 92	
Total	79	14, 810. 30	

* Significant at . 05 level.
** Significant at . 01 level.

Appendix Table 4. Analysis of variance on the effects of Tobacco Mosaic Virus, zinc, and temperature on the fresh weight of resistant and susceptible tomatoes. Experiment 2.

Source of variation	Degrees of freedom DF	Mean square M. S.	F
Replication	9	787. 56	2. 0301*
Treatments	3	910. 02	2, 3457
Varieties	2	30, 428. 99	78. 4373**
Temperature	1	5, 142. 08	13. 2548**
Treatment x variety	6	548. 20	1. 4131
Variety x temperature	2	1, 223. 13	3. 1528
Treatment x temperature	3	108. 61	0. 2799
Treatment x variety x temperature	6	1, 639. 91	4. 2272**
Error	207	387. 94	
Total	239	719. 76	

* Significant at . 05 level.
** Significant at . 01 level.