THE JOURNAL OF AGRICULTURE
OF THE UNIVERSITY OF PUERTO RICO

Issued quarterly by the Agricultural Experiment Station of the University of Puerto Rico, for the publication of articles by members of its personnel, or others, dealing with any of the more technical aspects of scientific agriculture in Puerto Rico or the Caribbean Area.

Vol. XXVII October, 1943 No. 4

STUDIES ON TOMATO MOSAIC IN PUERTO RICO

A NEW MOSAIC DISEASE OF TOMATO

By L. A. ALVAREZ GARCÍA

Phytopathologist

AND

JOSÉ ADSUAR

Associate Phytopathologist

INTRODUCTION

Tomatoes are extensively grown in Puerto Rico for home consumption and for the export trade. A fair idea of their importance is shown by the fact that 1500 acres of tomatoes were planted in the vicinity of Villalba and Jayuya during the four-month crop of 1941–42.

Tomato mosaic is a serious and widespread disease and is responsible, in many cases, for great losses. The nature of the causal element or elements of tomato mosaic in Puerto Rico has not been ascertained so far. A survey and study of the virus or viruses causing tomato mosaic; conditions under which it becomes serious, methods of transmission, host range, and the reaction of different commercial and non-commercial varieties of tomatoes which are mosaic resistant or tolerant to the virus or viruses encountered, are necessary before attempting to produce commercial varieties of tomatoes adapted to local conditions.

During the summer and fall of 1942, the Agronomy Department of the Agricultural Experiment Station of the University of Puerto Rico was confronted with a mosaic-like disease which rendered useless the tomato seedlings propagated for distribution among farmers. Some of these seedlings planted in the Station propagating garden were severely affected with the disease. The tomato plants found affected were of the varieties Marglobe, King, and Newark.

SYMPTOMS

Under field conditions, during the hot and humid summer and fall months of 1942, the affected tomato plants showed, in their late stages of growth, a characteristic faint yellowish mottling of the leaves with little or no leaf

distortion. Often the mottling was barely perceptible, or it might altogether disappear. Such affected tomato plants apparently grew normal and produced fairly good crops.

Tomato plants found affected early in their development, showed a pronounced retardation in upward growth, with a condensation of the axis, a progressive decrease in leaf size and various forms of leaf deformation. Necrosis of the growing tips developed frequently. Necrosis also occurred in leaves showing marked reduction in size and deformation. The midribs and lateral veins of affected leaves showed, especially on the underside, a peculiar purplish coloration followed by necrosis, the latter extending throughout the leaf forming large, irregular blotches. Eventually when the whole lamina was affected and had disintegrated, the midrib was left bare. The stems of such severely affected plants became heavily streaked with broad, black and short, or narrow, longitudinal streaks of varying lengths. New shoots were produced below the affected parts of the axis and also frequently became equally affected. On new shoots, formation of leaves was almost always reduced to malformed, purplish outgrowths which finally dried up. The flowers of such severely affected plants were commonly malformed and abortive and, if any fruits were produced, they were small and streaked.

Under greenhouse conditions, young, artificially inoculated tomato plants showed the mottling symptoms described above with some slight inward curling of the margins and tips of the leaves. Other tomato plants showed tip blight, bushy growth, and some of them succumbed to infection. Under such conditions young tomato plants produced small, narrow or filiform leathery leaves, with a peculiar bronzing previous to blighting.

MATERIALS AND METHODS

Tomato leaves (1) with mottle symptoms, (2) without mottle symptoms and (3) necrotic leave tissue, free from extraneous material, were taken from naturally infected tomato plants and macerated respectively in sterilized mortars. The individual macerates were immediately expressed through sterile cheesecloth. Each inoculum was prepared by diluting the filtrated juices in nine parts of distilled, sterile water, and used immediately. These macerates will be referred to as extracts (1), (2) and (3) in the course of this paper.

The virus once isolated and identified was kept in pure culture in tomato (*Lycopersicum esculentum* var. Marglobe), tobacco (*Nicotiana tabacum* var. Virginia), and pepper (*Capsicum frutescens* var. California Wonder). The extracts prepared with leaves of the infected plants were identified by the name of the source plant, i.e., tomato extract, tobacco extract and pepper extract.

All inoculations were performed by rubbing gently the upper side of the leaves of the differential host plants with Mikado No. 5 brushes with bristles cut to ½ inch, dipped, in every instance, in the respective inoculum. The inoculated plants were kept in an insect-free greenhouse. Temperature inside the green house fluctuated from 70°F. during the night to 90°F. at daytime. Relative humidity fluctuated from 30 to 80%.

Differential and tomato host plants used throughout the work were grown in sterilized compost soil in 5-inch pots in insect-free insectaries inside greenhouses. Inoculations were performed when the young plants had developed 3 or 4 pairs of leaves. Inoculated plants were kept growing until flowering to observe their reaction throughout their whole life-span.

EXPERIMENTAL RESULTS

The Causal Agent

Young plants of tomato (*Lycopersicum esculentum* var. Marglobe), tobacco (*Nicotiana tabacum* var. Virginia), and *N. glutinosa* were respectively inoculated with each of the three virus extracts.

Tomato plants reacted to inoculation with extracts (1) and (2) with systemic mottling and inward curling of the margins and tips of the leaves. The same extracts produced systemic vein clearing, vein banding, and a chlorotic mottling in tobacco. *N. glutinosa* reacted with vein clearing, systemic mottling and chlorosis.

Percent of infection obtained by inoculating with extracts (1) and (2) was very low. None of the host plants utilized reacted to inoculation with extract (3) (Table I).

Absence of local necrotic spots on *N. glutinosa* when inoculated with extracts (1) and (2) discarded the possibility that the mosaic of tomato might be due to *Tobacco Virus 1*, Johnson.

Young cucumber (*Cucumis sativus*) and black-seeded cowpea (*Vigna sinensis*) plants failed to react when inoculated with any of the virus extracts.

In order to determine the possible relation of the virus or virus entities attacking tomatoes to that recently reported in peppers by Roque and Adsuar (6), pepper plants (*Capsicum frutescens* var. Large Bell Hot) were respectively inoculated with tomato, tobacco and *N. glutinosa* virus extracts. Within five to six days the inoculated pepper plants showed the characteristic symptoms described on this host when infected with the pepper virus as reported by these workers, i.e. systemic vein necrosis, stem streak, and finally defoliation and death. *C. frutescens* var. California Wonder, reacted with vein clearing, mottling, and stunted growth.

The physical properties of the tomato virus under study in relation to

mechanical and insect transmission, longevity in vitro, thermal inactivation, and dilution end point, were identical to those already reported for the pepper virus found by Roque and Adsuar (6), demonstrating that the virus found in tomato is identical to their pepper virus.

TABLE I

Reaction of Host Plants to Virus Extracts from Various Parts of Affected Tomato Plants. Plants Inoculated in August 1942 and Kept at All Times Inside the Greenhouse in Insect-Free, Screened Rooms

Host Plant	Virus Source	No. Inoculated	Diseased	First Symptoms	Reaction
				days	
Tomato v. Marglobe	Mottled leaves	20	7	15–20	Faint mottled leaves
	Un-mottled leaves	20	5	15–20	Faint mottled leaves
	Necrotic leaf tissue	20	0	0	Negative
Tobacco v. Virginia	Mottled leaves	20	5	7–8	Vein clearing and faint mottle
	Un-mottled leaves	20	9	7–8	Vein clearing and faint mottle
	Necrotic leaf tissue	20	0	0	Negative
Nicotiana glutinosa	Mottled leaves	20	4	25–30	Faint mottle leaves
	Un-mottled leaves	20	2	25–30	Faint mottle leaves
	Necrotic leaf tissue	20	0	0	Negative
Pepper v. Large Bell Hot	Mottled leaves	20	11	5–6	Vein clearing and necrosis, defoliation and death
	Un-mottled leaves	20	9	5–6	Vein clearing and necrosis, defoliation and death
	Necrotic leaf tissue	20	0	0	Negative
Pepper v. California Wonder	Mottled leaves	20	4	10–12	Systemic leaf chlorosis and deformation
	Un-mottled leaves	20	6	10–12	Systemic leaf chlorosis and deformation
	Necrotic leaf tissue	20	0	0	Negative

Virus Source and Virulence

Infection of tomato and tobacco with virus extracts from affected tomato plants is not so readily obtained as when the virus is taken from *N. tabacum* var. Virginia or from *N. glutinosa*. An increase in virulence seems to result when the virus is passed through these species of tobacco. De-

creased virulence was noticed when the virus was obtained from tomatoes (Table II).

While infection of *N. glutinosa* by inoculation with tomato mottle-leaf extracts is difficult, however, a high percentage of infection was obtained by inoculating *N. glutinosa* with extracts of either affected *N. tabacum* or *N. glutinosa* plants.

The symptoms in *N. tabacum* are more obvious and more severe. Generally *N. tabacum* var. Virginia, showed vein clearing, faint mottling and vein banding without deformation. When inoculated with a virulent tobacco-virus extract the leaves showed deformation and peculiar dark

TABLE II

Virus Source and Virulence. Young Host Plants Kept Inside Insect-Free Greenhouse

Host	Virus Source	Plants Inoculated	Plants Diseased	Symptoms	Reaction
				days	
Tobacco v. Virginia	Tomato	20	8	7–8	Vein clearing, mottle
	Tobacco v. Virginia	20	17	5–7	Vein clearing, mottle
	N. glutinosa	20	15	5–7	Vein clearing, mottle
N. glutinosa	Tomato	20	0	0	Negative
	Tobacco v. Virginia	20	13	7–8	Faint mottle, chlorosis and death
	N. glutinosa	20	15	7–8	Faint mottle, chlorosis and death
Pepper v. Large Bell Hot	Tomato	20	7	5–6	Necrosis, death
	Tobacco v. Virginia	20	20	5–6	Necrosis, death
	N. glutinosa	20	20	5–6	Necrosis, death
Cucumber	Tobacco v. Virginia	20	0	0	Negative
	N. glutinosa	20	0	0	Negative
		20	0	0	

green markings. *N. glutinosa* inoculated with virulent tobacco-virus extracts reacted within five to six days in contrast with twenty or more days when inoculated with attenuated tomato virus extracts, a high percentage of plants becoming infected. Young *N. glutinosa* infected with the virulent virus showed marked systemic chlorosis, blistering and leaf mottling and finally wilting. This wilting reaction is not produced when inoculation is performed with apparently attenuated virus extracts from tomato.

Tomato plants inoculated with tobacco and *N. glutinosa* virus extracts, responded readily with characteristic leaf mottling, puckering and some-

times with leaf deformation and tip blight. The virus entity on tomatoes inoculated with these two virus extracts is consistently recovered by inoculating expressed sap of infected tomatoes on tobacco and *N. glutinosa*. When a more sensitive test is to be performed there is no better indicator than young, actively growing Large Bell Hot pepper plants. This variety of pepper is so sensitive that it has shown the presence of the virus entity in infected old pepper plants, and in *N. tabacum* v. Virginia and *N. glutinosa* which had been previously inoculated with attenuated virus extracts from tomato and old pepper plants. These tobacco plants had shown a mild expression of the disease becoming later symptomless carriers.

DISCUSSION AND CONCLUSIONS

The present studies demonstrate the presence of the pepper virus (Roque and Adsuar) (6) in tomato plants affected with mosaic. The virus causes a characteristic faint, yellowish mottling and downward puckering of tomato leaves. This has been repeatedly verified by inoculating tomato plants with (1) pepper virus obtained from tomato mosaic affected plants, (2) tomato mosaic virus passed through tobacco and *N. glutinosa* and (3) with pure cultures of the pepper virus thru commercial peppers. The identity of the virus has been substantiated in all cases by the severe vein-necrosis reaction it produces in the Large Bell Hot pepper variety.

Necrotic symptoms, in the form of tip blight and streak, were found to be sometimes associated with the faint mottling, yellowing and puckering so characteristic of the pepper virus in tomato. In that respect it can be stated that it has not been possible to reproduce consistently tip blight necrosis when healthy tomato plants were inoculated either with pepper virus extracts obtained directly from tomato or passed through tobacco. Faint mottling, yellowing and puckering were invariably obtained when such inoculations were performed. In a few cases tip blight symptoms appeared in tomato plants inoculated with virus extracts from tomatoes showing tip blight. This reaction was lost in subsequent transfers, only faint mottling, yellowing and puckering of leaves being observed.

It seems likely therefore, that the blight and necrosis sometimes found associated with tomato mosaic is due, either to a single entity, or to its interaction with the pepper mosaic virus in the tomato plant. In any case the failure to artificially reproduce consistently the tip blight symptoms in plants grown in greenhouses might be attributed, among other causes either to ignorance as to its method of transmission or to a very rapid inactivation of the responsible agent "in vitro."

The fact remains however, that tomato tip-blight like viruses very similar to the one described here have been encountered and reported by other investigators. Among those so far described, that responsible for the

Oregon tip blight of tomatoes (4–5) is very similar, at least as to clinical syndrom. Until more is known about the tip blight virus found in Puerto Rico in respect to transmission and physical properties, its nature will remain a mooted question.

The apparent loss of virulence resulting when the tomato mosaic virus is consecutively passed through tomato or submitted to a long sojourn in either tomato or old pepper plants, as well as its increased infectivity when transferred to tobacco, are phenomena already well established for other viruses. Its importance in connection with this or similar work is that it serves to explain the low percentages of infection, or even at times, the failure to transmit the disease when the virus is passed through different hosts.

Failure to recover the virus entity from dried leaves of affected tobacco plants and from necrotic tomato leaves suggest the possibility of control by cutting affected plants and letting them dry "in situ." As the virus is transmitted mechanically, care should be taken not to carry it from affected tomato, tobacco or pepper plants while working in seed beds. Pepper is extensively grown in Puerto Rico and is an important virus source.

RESUMEN EN ESPAÑOL

Se confirma la existencia en Puerto Rico de un mosaico del tomato causado por el virus del pimiento tal como ha sido informado por Roque y Adsuar (6).

La enfermedad se caracteriza por un matizado del follaje acompañado frecuentemente por una necrosis de las hojas y tallos jóvenes. Esta suele extenderse en estrías negruzcas al largo de los tallos.

Se ha demostrado que el virus pierde su poder infeccioso en tejidos necróticos.

El hecho que los síntomas necróticos no acompañen necesaria y consistentemente al matizado de las hojas, hace pensar que haya mas de un virus envuelto en la compleja manifestación de la enfermedad.

Todo parece indicar que la necrosis que acompaña al matizado de las hojas se deba o bien a una o varias entidades distintas al virus del pimiento, o a la interacción de estas con el virus del pimiento.

Se ha observado una decidida atenuación del virus del pimiento al recuperarse del tomato. Por otro lado se ha comprobado que éste puede recobrar su virulencia al cultivarse en tabaco.

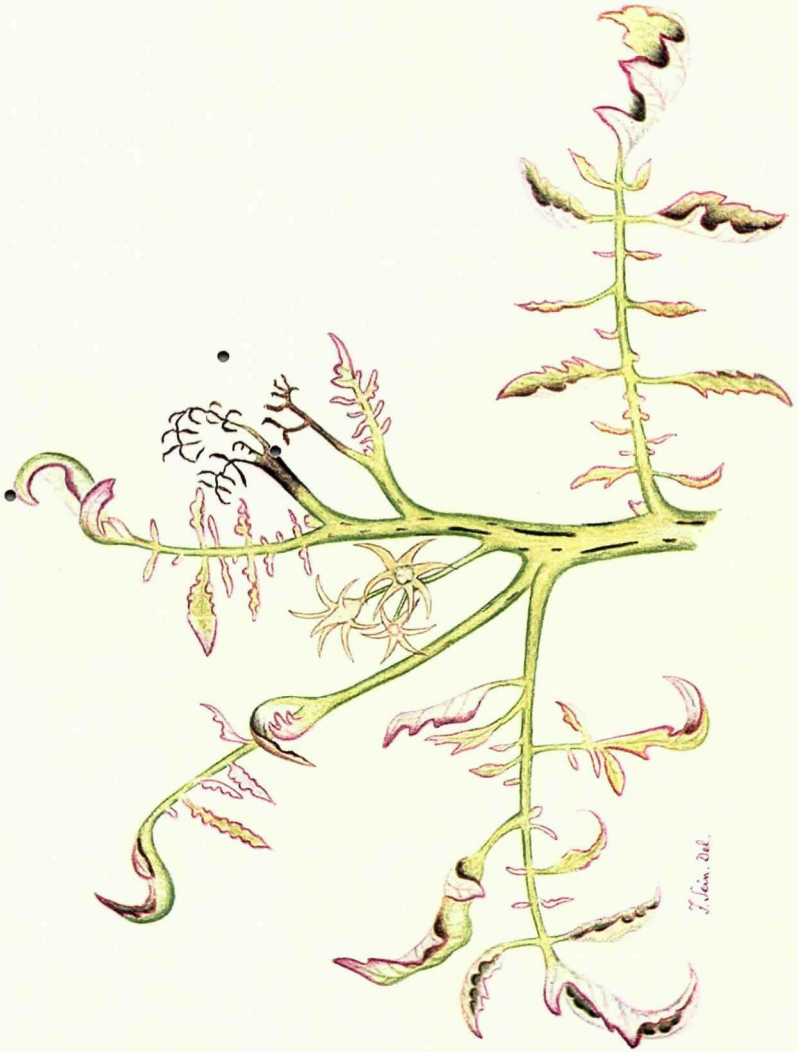
REFERENCES

1. Blood, H. L., An unusual occurrence of "spotted wilt" of tomato in Utah. *U. S. Dept. Agr. Plant. Dis. Rep.*, **20**, 143–144. 1936.
2. Doolittle, S. P., and C. B. Summer, The occurrence of the Australian spotted wilt of tomatoes in Wisconsin. (Abstract.) *Phytopath.*, **21** (1), 106. 1931.

3. Imle, E. P., and R. W. Samson, Studies on a ring-spot type of virus of tomato. (Abstract.) *Phytopath.*, **27** (2), 143. 1937.
4. McWhorter, F. P., and J. A. Milbrath, The interpretation of Oregon tip blight on a basis of causal viruses. (Abstract.) *Phytopath.*, **25** (9), 897–898. 1935.
5. Milbrath, J. A., Tomato tip blight virus. *Phytopath.*, **29** (2), 156–168. 1939.
6. Roque, A., and Adsuar, J., Studies on the mosaic of peppers (*Capsicum frutescens*) in Puerto Rico. *Jour. Agric. of U. of P. R.*, **25** (4), 40–50. 1941.
7. Samuel, Geof., J. G. Bald, and H. A. Pittman, Investigations on "spotted wilt" of tomatoes. *Austral. Counc. Sci. Indust. Res. Bul.*, **44**, 1–64. 1930.
8. Samuel, Geof., J. G. Bald, and H. A. Pittman, Some experiments on inoculating methods with plant viruses, and on local lesions. *Ann. Appl. Biol.*, **18** (4), 494–507. 1931.
9. Shapovalov, M., The die-back form of tomato streak. *Phytopath.*, **23** (11), 928. 1933.
10. Shapovalov, M., Some host responses in graft transmissions of die-back streak of tomatoes. *Phytopath.*, **24** (10), 1149. 1943.
11. Shapovalov, M., Effect of certain chemicals on the "combination streak" virus of tomatoes. *Phytopath.*, **25** (9), 864–874. 1935.
12. Smith, Kenneth M., Studies of plant virus diseases. XI. Further experiments with a ring-spot virus: Its identification with spotted wilt of tomato. *Ann. Appl. Biol.*, **19** (3), 305–330. 1932.
13. Smith, Kenneth M., *Recent Advances in Plant Viruses*. 423 pp. P. Blakiston's Son & Co., Inc., (Philadelphia). 1934.
14. Smith, Kenneth M., A new virus disease of tomato. *Ann. Appl. Biol.*, **22** (4), 731–741.
15. Smith, Kenneth M., *Plant Virus Diseases*. 615 pp. P. Blakiston's Son & Co., Inc., (Philadelphia). 1937.
16. Young, P. A., G. E. Altstatt, and A. L. Harrison, Plant disease survey in southeast Texas. *U. S. Dept. Agr. Plant Dis. Rep.*, **22**, 6–7. 1938.

PLATE A

FIG. 1. Terminal shoot of naturally infected tomato plant showing symptoms of mosaic in the form of leaf deformation, a peculiar purplish coloration of growing tips followed by necrosis and broad, black, short or narrow, longitudinal streaks on stem and branches.



(García and Adsuar: Studies on Tomato Mosaic in Puerto Rico)