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SYMPTOM DEVELOPMENT AND THE CHLOROTIC RESPONSE OF LYCOPERSICON ESCULENTUM MILL. CV. RUTGERS INFECTED WITH POTATO SPINDLE TUBER VIROID

presented by

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has been accepted towards fulfillment
of the requirements for

M.S. degree in Dept. of Botany and Plant Pathology

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Date Feb. 20, 1980

SYMPTOM DEVELOPMENT AND THE CHLOROTIC RESPONSE OF *LYCOPERSICON ESCULENTUM* MILL. CV. RUTGERS INFECTED WITH POTATO SPINDLE TUBER VIROID

by

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Botany and Plant Pathology

1980

ABSTRACT

SYMPTOM DEVELOPMENT AND THE CHLOROTIC
RESPONSE OF LYCOPERSICON ESCULENTUM MILL. CV.
RUTGERS INFECTED WITH POTATO SPINDLE TUBER VIROID

By

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Tomato seedlings (Lycopersicon esculentum Mill. vs. Rutgers) were grown in various environments in an attempt to define the conditions which best promote potato spindle tuber viroid (PSTV) symptoms. Under continuous illumination, stunting of tomatoes infected with PSTV was promoted at high temperatures (26°-30°C) while other PSTV symptoms (veinal necrosis and leaf rugosity) became most apparent at lower temperatures (22°-24°C). Since chlorosis and albinism appeared on non-infected and PSTV infected plants in these experiments, this type of leaf injury is not believed to be a diagnostic symptom for PSTV.

Plastids in leaf palisade cells from PSTV infected and non-infected tomatoes were studied using the transmission electron microscope. Similar plastid aberrations were observed in infected and non-infected tissues from constant light environments. The extremely aberrant plastids which contained vesicles and no

functional thylakoids have been termed albidoplasts. Albidoplasts are probably the product of an injurious photoperiod, rather than the result of activity by an infectious agent.

ACKNOWLEDGEMENTS

To acknowledge all those individuals whose guidance and expertise played a role in completing this work would be a voluminous task, as I have learned from many. The following persons, however, were particularly influential in this project, for it was their love, support and patience which served as a constant source of inspiration: my parents, Mr. and Mrs. C. A. Beaubien, my friend Mr. Ronald Riley and Michigan State University faculty members Drs. Gary R. Hooper and Karen L. Baker.

TABLE OF CONTENTS

LIST OF FIGURES

FIGURE PAGE

LIST OF TABLES

LIST OF ABBREVIATIONS

CE	chloroplast envelope
CR	chloroplast ribosomes
CW	cell wall
CYT	cytoplasm
D	dictyosome
GL	grana lamella
GS	grana stack
IS	intercellular space
IT	intergrana thylakoids
LB	lipid body
M	mitochondria
N	nucleus
NM	nuclear membrane
OB	osmiophillic body
OIS	osmiophillic interthylakoid spaces
P	plastid
PC	palisade cell
PE	plastid envelope
PM	plasma membrane
PS	plastid stroma
PV	plastid vesicles

LIST OF ABBREVIATIONS, Continued

PVM	plastid vesicle membrane
R	ribosomes
RMF	reticulate membrane formation
S-PSTV	severe strain of potato spindle tuber viroid
SG	starch grain
T	tonoplast
TS	thylakoid segments
V	vacuole
VN	veinal necrosis

INTRODUCTION

Potato spindle tuber disease is caused by a low molecular weight, single stranded RNA (Diener and Raymer, 1969b; Diener, 1971b; Singh and Clark, 1971). Recent work on the structure of the infectious agent has revealed a closed circle of 359 ribonucleotides which contains base-paired segments, giving the molecule a rod-like shape (Gross et al., 1978). This type of incitant pathogenic nucleic acid has been termed viroid (Diener, 1971b). The potato spindle tuber viroid (PSTV) exists as at least two strains which incite different degrees of foliage symptoms and yield loss on potato (Fernow, 1967; Singh et al., 1970; Singh et al., 1974). The foliage symptoms, however, are not striking (even with infection by the most severe PSTV strain) and field infections often go undetected until poor tuber yields are realized in the form of fewer tubers per plant, and small misshapen tubers (Folsom, 1923; Hunter and Rich, 1964).

Due to the ambiguity of PSTV symptoms on potato (the host on which most economic loss occurs) and the fact that PSTV is carried in the seed pieces and true seed of potato (Hunter et al., 1969; Singh, 1970; Fernow et al., 1970) much effort has been extended toward developing a satisfactory method of detecting the presence of this pathogen in potato propagating materials (Morris and Wright, 1975; Morris and Smith, 1977). Polyacrylamide gel electrophoresis (PAGE)

of host RNA has provided a means of isolating and visualizing the viroid in the form of a band in the gel column (Diener and Smith, 1971; Diener and Smith, 1973; Morris and Wright, 1975). The required procedure for successful detection of PSTV RNA using PAGE is very time-consuming and must be exercised with great care in order to see the minute amounts of pathogenic nucleic acid present in infected tissues (Morris and Wright, 1975; Morris and Smith, 1977). This renders the use of PAGE impractical for routine indexing purposes. PAGE is, however, useful for indexing certain potato breeding stock when an absolute check of PSTV incidence may be desirable (Morris and Wright, 1975). Other detection procedures used for conventional viruses, such as serological reactions and negative stained leaf dip preparations for transmission electron microscopy (TEM), have not been successfully employed with PSTV.

The major efforts toward a suitable PSTV screening method have been in developing a reliable bioassay procedure (Raymer and O'Brien, 1962; Whitney and Peterson, 1963; Raymer et al., 1964; Fernow, 1967; Singh and Bagnall, 1968; Fernow et al., 1969; Singh, 1971). A local lesion host for PSTV, Scopolia sinesis, has been reported and utilized in some indexing experiments (Singh, 1971). Although a local lesion host would be highly desirable, this plant species yields inconsistent results from one seed lot to another (Singh, 1973) and the response varies greatly under different environments. Attempts at estimating viroid concentration from local lesion counts on S. sinensis must therefore be given dubious consideration.

Of the several systemic hosts for PSTV which yield more acute symptoms than on potato, the tomato plant has been most extensively used as an indicator host for PSTV (Raymer and O'Brien, 1962; Whitney and Peterson, 1963; Raymer et al., 1964; Fernow, 1967). Symptom development in tomato is consistent among inoculated individuals of the same variety. The symptom response of groups of tomatoes inoculated with different concentrations of PSTV inoculum has been used to estimate relative viroid concentrations in the host (Diener and Raymer, 1969). Fernow (1967) used tomato seedlings in a "challenge inoculation" technique to index potatoes for both mild and severe strains of PSTV in the field. It was later suggested that this technique be used in conjunction with field roguing to detect and control infections in the potato field (Fernow, et al., 1969).

The drawbacks associated with using tomato as a bioassay host are twofold: 1) the lengthy time period between inoculation and symptom expression and, 2) distinguishing PSTV symptoms (which may be subtle in early stages of development) from the effects of various environmental growth factors (Morriss and Smith, 1923, Goss and Peltier, 1925). As regards the first drawback, and ideal systemic assay host for many applications would be one which yields a rapid hypersensitive reaction to only one specific pathogen, even in the presence of low inoculum levels. A lethal reaction by a host in a short period of time (as observed with certain apple seedling varieties inoculated with Erwinia amylovora the fire blight pathogen, Ritchie and Klos, 1974) would facilitate rapid, reliable

indexing of great numbers of potato lots suspected of carrying PSTV. The second drawback, that of interpreting early systemic symptoms, becomes very significant when screening for mild strains of PSTV which usually induce inconspicuous symptoms on tomato, and are more prevalent in nature than the severe strains (Singh et al., 1970; Singh et al., 1971). In addition, environmental factors affecting the growth and development of tomato are known to interfere with and alter the symptom expression of other virus diseases on tomato (Went, 1945).

It has been observed that PSTV symptoms on tomatoes in the greenhouse develop faster and more severely during high temperatures and long daylengths of the summer season, as opposed to the cooler, shorter days during the winter (Raymer et al., 1964). Yang and Hooker (1977) described an albino response on the new growth of tomato seedlings inoculated with PSTV and grown in controlled-environment cabinets under continuous illumination and temperatures from 16°-30°C. They reported that the albino growth developed most rapidly on inoculated plants, while non-inoculated plants (grown in these same conditions) produced only sporadic chlorotic patches on some leaves. The discovery of the albino response to PSTV in continuous light greatly facilitates the use of tomato for PSTV bioassays, especially for detecting mild strains of the viroid which normally promote weak symptom development.

This research was an attempt to find the environmental conditions most conducive to the rapid development of albino tissue on PSTV infected tomatoes, while allowing non-infected tomatoes to remain green, thereby easily resolving diseased from healthy individuals. In order to gain an understanding into the subcellular mechanisms mediating the production of albino tomato tissue, chloroplasts of tomato palisade mesophyll were studied using the transmission electron microscope (TEM). In a broader sense, these experiments explored the host response to a pathogen as expressed in different environments, a concept often graphically presented as a 'host-pathogen-environment interaction triangle'.

LITERATURE REVIEW

The Nature of PSTV, the Causal Organism

The nature of the pathogenic agent for potato spindle tuber disease was thought to be a virus (Benson, et al., 1965). MacLachlan (1960) concluded from symptomology and transmission characteristics that potato spindle tuber disease was caused by a yellows virus. PSTV was later distinguished from the yellows virus group because it was easily transmitted mechanically (Raymer and O'Brien, 1962). It has been shown that PSTV has a smaller sedimentation rate than conventional viruses, can withstand treatment with phenol, and will precipitate in ethanol (Diener and Raymer, 1969b; Diener, 1971a). These observations are consistent with the concept that PSTV is a free nucleic acid. Sogo et al., (1973) characterized the nucleic acid as RNA using TEM preparations (with RNA standards), and its molecular weight was later estimated to be 7-9 X 10^4 daltons (Diener and Smith, 1973). Diener (1971b) used the term viroid to describe PSTV as well as the causal agents of several other diseases which have physical properties and molecular weights similar to PSTV (Semancik and Weathers, 1972; Diener and Lawson, 1973; Van-Dorst and Peters, 1974; Romaine and Horst, 1975; Randles, 1975; Sasaki and Shikata, 1977). Viroids are the smallest known agents of infectious diseases.

Gross et al., (1978) sequenced the PSTV RNA and found it to be composed of 101 guanines, 108 cytosines, 73 adenines and 77 uracils, giving PSTV a molecular weight 118,701 daltons. Extensive base-pairing gives it a rod-like secondary structure (Sanger et al., 1976). The length of the viroid molecule as visualized by electron microscopy of purified PSTV RNA preparations is about 500 Å (Sogo et al., 1973). Diener and Stollar (1971) determined the dilution end points of concentrated PSTV preparations to be in the range of 10^{-6} to 10^{-7}, which means that solutions containing only 1.4×10^{-8} µg RNA/ml are still infectious (calculated from the initial RNA concentrations of the preparations as determined by U.V. absorbance). PSTV's high specific infectivity has led to speculation that entry of a single PSTV particle into a host cell will facilitate infection of the entire plant (Diener, 1972). The small size and the fact that PSTV is present in such low quantities in infected tissue, has made imaging the pathogen in situ with the electron microscope an improbability (Diener, 1971a; Sogo et al., 1973).

Tomato as an Indicator Host for PSTV

The first reported PSTV infection in tomato was probably McClean (1932) in South Africa, who described a disease of tomato he called "bunchy top". Although a description of the disease-causing entity was not given, the disease syndrome on tomato and the host range closely correlate with those now known for the severe strain of PSTV (McClean, 1932; McClean, 1948). The author did not discuss

the possibility that bunchy top disease of tomato was caused by the same pathogenic agent as that of the spindle tuber disease of potato which was first reported by Martin (1922) and later by others (Goss, 1925; Morris and Smith, 1977).

Plant species representing ten families are susceptible to infection by PSTV (Singh, 1973). Most of the susceptible plant species, however, are symptomless carriers of the viroid and are useless for spindle tuber disease indexing purposes (O'Brien and Raymer, 1964; O'Brien, 1972; Singh, 1973). The family Solanaceae contains several species which produce symptoms in response to inoculation with PSTV (Singh, 1973; O'Brien, 1972). With some solanaceous species, i.e., Petunia spp., only floral or subtle foliar symptoms are produced which are not distinct and could be confused with other disorders of the plant such as nutrient deficiencies, genetic disorders or other virus diseases (Raymer et al., 1964; Fernow, 1967). Of the susceptible plants which do produce diagnostic symptoms, the tomato (Lycopersicon esculentum) produces outstanding foliar symptoms in a relatively short time, when inoculated with the severe strain of PSTV (Fernow, 1967).

Tomato was first used as an indicator host for PSTV in an effort to reliably diagnose potato plants and seed pieces thought to be carrying spindle tuber disease (Raymer and O'Brien, 1962). This initial assay description reports that PSTV symptom expression takes twenty days with graft inoculations (potato to tomato) and thirty days for tomato seedlings mechanically inoculated with potato sap.

Since that time, the tomato has been most commonly used to check the infectivity of treated PSTV extracts (Singh and Bagnall, 1968; Diener, 1971a; Diener and Smith, 1971; Diener et al., 1972; Diener et al., 1974), and extracts from symptomless hosts (O'Brien and Raymer, 1964). Tomato has become widely used as an indicator host for PSTV for the following reasons: 1) ease in producing large numbers of viable seed, 2) tomatoes are easily grown, and have a fairly rapid growth rate, 3) the seedlings can be inoculated by simple mechanical methods and, 4) inoculated tomato plants produce distinct systemic symptoms.

Various procedures to decrease the time for PSTV symptom development in tomato have been employed. Whitney and Peterson (1963) decapitated inoculated seedlings with subsequent symptom development on auxillary shoots becoming apparent within fourteen days. The time for symptom development was reduced somewhat by growing the inoculated seedlings at temperatures for 24° to 29°C, and keeping the plants in brightly illuminated portions of the greenhouse (rather than shaded portions) with twelve to fifteen hour daylengths. Under these conditions PSTV symptoms were often expressed in ten days post-inoculation.

Lee and Singh (1972) altered soil nutrient levels to achieve "enhancement" of PSTV symptoms. Their experiments showed that when Mn:Fe ratios exceed 12 in the soil, PSTV symptoms on tomato were pronounced, however, the time for symptom development was not necessarily reduced.

Studies on the effects of temperature on symptom development have demonstrated that symptoms appear faster at warmer temperatures increasing from 13° to 30°C (O'Brien and Raymer, 1964; Raymer et al., 1964; Singh and O'Brien, 1970). Yang and Hooker (1977) studied in detail the effects of light and temperature on PSTV symptom development by growing the plants in environmental growth cabinets. Under certain experimental conditions (continuous light and temperatures from 24° to 30°C), they reported the production of extensive chlorotic (albino) leaf tissue on PSTV inoculated seedlings within ten days. The albino response was exclusive to PSTV infected tomatoes and rapid in appearing, thereby providing a clear-cut distinction between healthy and diseased individuals. The quality of tomato as an indicator host for PSTV was improved by using the albino symptom criteria.

Albinism on Tomato

Leaf injury on tomatoes grown in unfavorable photoperiods and temperature regimes has long been known (Guthrie, 1929; Went, 1944; Withrow and Withrow, 1949; Highkin and Hanson, 1954). The observation by these investigators that long photoperiods (which were often lethal to tomato) incited the production of small yellow leaves has been substantiated by others studying optimal growth conditions for tomato (Arthur et al., 1930; Kristoffersen, 1963). Their experimental results implicate an upset in the plant's endogenous growth rhythms with the chlorotic response. The general phenomenon is that tomato seedlings grown under continuous light develop various degrees of

chlorosis on the foliage after a period of at least one week in these conditions. Higher temperatures (26° to 30°C) combined with constant illumination induce more chlorosis than lower temperatures and constant light (Hillman, 1956). Leaf injury is most pronounced on young leaves, whose complete development occurred under conditions of constant light, and not apparent on older leaves whose expansion was completed before the seedling was given the continuous light treatment. Partially expanded leaves develop an intermediate degree of injury, usually in the form of light yellow streaks radiating outward from the leaflet midribs toward the leaf margin (Withrow and Withrow, 1949; Hillman, 1956).

More detailed studies of the factors affecting leaf injury in tomato revealed that the extent of injury is also dependent on the amount of soil nutrients available to the plant and moisture stress of the leaves in continuous light (Kristoffersen, 1963). Vigorously growing, well fertilized plants developed more injury than non-fertilized plants grown in continuous light. Also, plants grown in constant illumination and 75% relative humidity developed more injury than those in constant light and 100% relative humidity (Kristoffersen, 1963). These observations suggest that the injury may be an effect of dehydration of the leaf tissues under conditions conducive to a high transpiration rate (Kristoffersen, 1963).

Genetic studies of the tomato leaf injury phenomenon have revealed that the sensitivity to long photoperiods is under the regulation of recessive alleles which are not expressed in normal twenty four hour thermo- and photoperiodic cycles. Daskaloff and Ognajanova (1964) used three Lycopersicon spp. (L. esculentum, L. hirsutum, and L. racemigerum) to show a differential reaction to continuous light. L. esculentum reacted most adversely to this condition, while L. hirsutum was somewhat affected and L. racemigerum showed no adverse response to continuous illumination. The F progeny of a cross between L. esculentum and L. racemigerum showed a proportion of 2.7 to 1 tolerant to sensitive response to continuous light. This suggests that the response in controlled by a single allele which is dominant in L. racemigerum. The gene expression is environmentally controlled and is only observed when the plants are grown in continuous light.

The "albinism" on tomato plants infected with PSTV described by Yong and Hooker (1977) which developed in conditions of continuous light and continuous temperatures is similar to the leaf injury on non-infected tomatoes reported by others (Highkin and Hanson, 1954; Hillman, 1956). PSTV infected plants grown in continuous light developed symptoms of rugosity, eipnasty, veinal necrosis and reduced growth rate (which are also seen in intermittant light cycles, but appear earlier in constant light) along with the albino response. Yang and Hooker (1977) did recognize that some of the non-inoculated

seedlings in their experiments developed chlorotic leaf areas, however, they prevented this injury by maintaining the seedlings in the greenhouse for 10 to 14 days before beginning the constant light treatment. Hillman (1956) reported that healthy seedlings grown in the greenhouse before the continuous light treatment are more resistant to leaf injury than those raised from seed in constant light. The terminal growth on his non-inoculated tomatoes developed "small stiff yellow leaves" similar to the observation by Went (1945) who described the symptoms as "complete bleaching of large areas of the younger [tomato] leaves" in conditions of continuous light. Yang and Hooker (1977) reported that albino leaves on PSTV infected plants did not green when placed in non-continuous light conditions, although subsequent growth was green in intermittant light. Hillman (1956) made this observation with the non-infected plants used in his experiments. He also made the intriguing discovery that a period of reduced temperature, during each 24 hours of continuous light, prevented the chlorotic leaf injury promoted by continuous light and constant temperature.

PART I

LIGHT AND TEMPERATURE EXPERIMENTS

MATERIALS AND METHODS

Light and Temperature Experiments

In preliminary experiments with tomatoes grown in various light-temperature regimes, non-inoculated and PSTV inoculated plants consistently developed similar degrees of chlorosis when grown in constant light. The experiments that follow are an attempt to find conditions which best promote a distinction between healthy and PSTV infected plants within a 14 day period. The experiments utilize Hillman's observation (1956) that fluctuating temperatures prevented leaf injury in constant light, in an effort to show conditions which prevent leaf injury in non-inoculated plants yet promote the chlorotic response by PSTV inoculated tomatoes reported by Yang and Hooker (1977).

The tomato variety "Rutgers" was used for all experiments. Seeds were germinated in potting soil/perlite mix (1:1) and grown until their cotyledons were fully expanded (7-10 days) at which time individual seedlings were transplanted into 4" plastic pots. Additional soil nutrients in the form of 2 gm of 14-14-14 granulated time-release plant food (Osmocote, Sierra Chemical Company, Milpitas, California) was sprinkled on the soil mix in each pot at the time of transplanting. The soil was kept moist throughout the experiment.

The seedlings were maintained in the greenhouse and inoculated when they had reached the 2-4 leaf stage (6-7 cm tall) which occurred about 10 days after transplanting. Plants of similar size and vigor were mechanically inoculated by dusting the plants with 400 mesh carborundum and rubbing the leaves with a sterile foam rubber test tube stopper soaked in either distilled water or PSTV inoculum. The inoculum was made by macerating 1 gm of rugose leaf tissue from a severely infected Rutgers tomato plant in 4 ml of distilled water with a sterile mortar and pestle. Only severe PSTV inoculum was used in these experiments in an effort to reduce the variety of symptom expressions. [At various times during the course of these experiments, healthy tomato tissue was used as the source of inoculum and rubbed on seedlings as described. In all of these cases no symptoms developed, and the growth of the plants was indistinguishable from H_2O inoculated plants]. Inoculated seedlings were grown in the greenhouse for 7 days before beginning the environmental experiments.

Each experiment consisted of randomly placing 24 inoculated plants (12 water inoculated and 12 PSTV inoculated) in each of two controlled environment cabinets. The cabinet shelves were adjusted throughout the experiment to keep the leaf canopy about 12 inches below the light. Continuous illumination was provided by five four-foot cool-white fluorescent tubes and four 25w incandescent bulbs in each cabinet. The light intensity provided at the leaf canopy was about 700 f.c. One of the two cabinets (cabinet #1) was kept at a

constant temperature, and growth cabinet #2 was maintained at the same temperature as cabinet #1 for twenty hours, followed by a four hour period at 8°C less, thus constituting each 24 hour thermoperiod. The temperature regimes tested are listed in Table 1. Each experimental run in the growth cabinets was accompanied by 24 similarly inoculated plants in the greenhouse. The greenhouse was illuminated by incident solar radiation alone. The temperature in the greenhouse varied from 24° to 27°C from night to day.

Table 1: Experimental temperatures (°C) of two growth cabinets kept continuously illuminated. Each run lasted 2 weeks.

Run #	Cabinet #1	Cabinet #2*
1	30	30/22
2	28	28/20
3	26	26/18
4	24	24/16
5	22	22/14

* 20 hour temperature/4 hour temperature, for every 24 hour period.

After two weeks in the experimental conditions, the three groups of plants (cabinet #1, cabinet #2, greenhouse) were harvested. Each group consisted of two sub-groups: water inoculated and PSTV inoculated. The plants in each sub-group and their respective data were labeled CK (water inoculated), or PSTV (PSTV inoculated) followed by the temperature condition of the growth cabinet in which they were grown or GH (greenhouse). For example, the notation PSTV 24/16 refers to plants inoculated with PSTV and grown in cabinet #2 under

continuous light for two weeks during the fourth experimental run. Before the plants were harvested for weighing, they were measured and visual estimates of chlorosis, leaf rugosity, epinasty, leaf veinal necrosis, and stem necrosis were made. Leaf area was measured by defoliating the plants and running the leaves through a LI-COR Model LI-3000 leaf area meter from Lambda Instruments Corporation. The soil was carefully washed from the roots and they were dried separately for weight determination. The data from each sub-group was summed and averaged for presentation in tabular form.

RESULTS

Light and Temperature Experiments

The experimental data reflecting PSTV symptom expression in various environments is listed in Tables 2a through 6b (pages 19, 20, 22, 23, 27, 28, 31, 32, 36, 37). Tables with like numbers (2a and 2b, 3a and 3b, etc.) contain the data for one particular growth or symptom parameter. Numbered tables designated with an "a" list symptom data for constant light and constant tmeperature. The tables with a "b" subscript, list data for the same symptom as "a" tables, but in a fluctuating thermal condition and constant light. For purposes of interpreting these results, the optimal temperature regime (that which these experiments sought) is the one which promotes the greatest difference in CK and PSTV plants. This can be expressed as an absolute or relative difference, however, the significance of any non-uniformity in the development of CK and PSTV plants must be reviewed in terms of its usefulness as a diagnostic symptom in PSTV bioassays.

The greatest relative and absolute height difference between CK and PSTV plants was recorded at 30°C constant temperatures (Tables 2a and 2b, pages 19 and 20). The CK seedlings, averaging 23 cm in height were 53% taller than PSTV seedlings which averaged 15 cm in height. At 28°C and 26°C the CK seedlings were also distinctly larger

TABLE 2a: Height of Rutgers tomato plants mechanically inoculated with either distilled H_2O (CK) or PSTV inoculum (PSTV) and grown in continuous light at the listed temperatures for 14 days. Results from plants similarly inoculated and grown in the greenhouse for 14 days are also included (GH).

Temp. (C)	Height (cm)*	
	CK	PSTV
30	23	15
28	21	16
26	20	15
24	16	13
22	13	11
GH		

* Average height for 12 plants

TABLE 2b: Height of plants grown in the same conditions as for Table 2a, except the temperature was decreased 8°C for 4 hours each 24 hour period.

Temp. (C)**	Height (cm)*	
	CK	PSTV
30/22	18	15
28/20	16	14
26/18	15	13
24/16	14	12
22/14	12	9

* Average height for 12 plants

** Temperature for 20 hours/Temperature for 4 hours, each 24 hour period.

than the PSTV seedlings (31% and 33% respectively). For the constant temperature runs, the height difference between CK and PSTV seedlings decreased at lower temperatures. The height difference in fluctuating thermal conditions shows a similar trend (Table 2b, page 20) with the greatest distinction being observed at higher temperature regimes. In the greenhouse conditions (GH) CK plants grew only marginally taller than PSTV plants during the 14 day experimental runs.

The temperature regime which promoted the greatest leaf area difference was 26/18, where CK seedlings averaged 139% more leaf area than PSTV seedlings (Table 3b, page 23). When representative plants from this experimental run are observed together, the height and leaf area differences between CK and PSTV plants can readily be seen (Figure 1, page 25). Large differences were also noted at 30°, 28° and 28°/20° conditions, with CK plants having at least twice the leaf area as PSTV plants. Overall, the leaf area of CK plants in any of the experimental conditions was considerably greater than that of PSTV plants, even in the greenhouse. CK plants from this environment had, on the average, 68% greater leaf area than PSTV plants. From these results a generalization can be made similar to that from the height measurements, that is, higher temperatures promote greater size differences in CK and PSTV plants than lower temperatures.

TABLE 3a: Leaf area of Rutgers tomato plants mechanically inoculated with either distilled H_2O (CK) or PSTV inoculum (PSTV) and grown in continuous light at the listed temperatures for 14 days. Results from plants similarly inoculated and grown in the greenhouse for 14 days are also included.

Temp. (C)	Leaf Area (cm^2)*	
	CK	PSTV
30	397	180
28	430	201
26	416	205
24	400	215
22	320	226
GH	360	214

* Leaf area was determined using a LI-COR, Model LI-3000, Leaf Area Meter from Lambda Instruments Corporation. Each listed leaf area is an average for 12 plants.

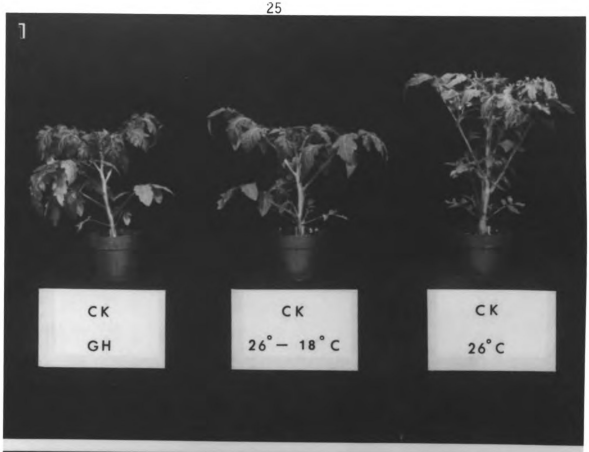
TABLE 3b: Leaf area of plants grown in the same conditions as for Table 3a, except the temperature was decreased 8°C for 4 hours each 24 hour period.

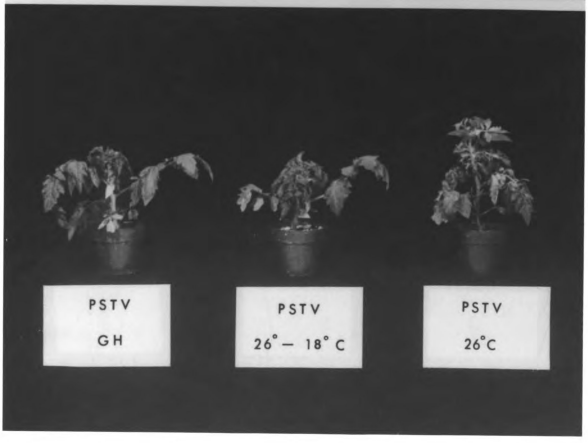
Temp. (C)**	Leaf Area (cm^2)*	
	CK	PSTV
30/22	418	217
28/20	411	198
26/18	334	140
24/16	301	190
22/14	261	182

* Leaf area was determined using a LI-COR, Model LI-3000 Leaf Area Meter from Lambda Instruments Corporation. Each listed leaf area is an average for 12 plants.

**Temperature for 20 hours/Temperature for 4 hours each 24 hour period.

Figure 1: Comparison between non-infected Lycopersicon esculentum Mill. cv. Rutgers tomato plants (CK) and S-PSTV infected plants (PSTV) grown in different light-temperature regimes. GH = Greenhouse conditions of intermittent solar light at 24°-27°C. 26°- 18°C = 20 hours at 26°C and 4 hours at 18°C for each 24 hour period, and constant illumination. 26°C = continuous 26°C temperature and constant illumination. (See text for a further explanation of the experimental conditions).


1
CK
GH
CK
26° – 18° C
CK
26° C


PSTV
GH
PSTV
26° – 18° C
PSTV
26° C

Rugosity, as a symptom of PSTV in tomato, is a severe upward puckering of the interveinal leaf tissue. First seen on young leaves, the puckering persists on older leaves appearing very much like numerous small blisters on the adaxial leaf surface (causing extreme distortion in the shape and size of mature leaves, as seen in Figures 2 and 3, page 30). Leaf rugosity was initially seen on PSTV plants after 10-12 days in the experimental conditions, and could be distinguished from the natural rough texture of young healthy tomato leaves by its severity and persistance (Figure 4, page 34). The experimental results indicate that rugosity was most completely expressed in PSTV plants at the lower temperatures tested and in the greenhouse (Tables 4a and 4b, pages 27 and 28).

An estimate of veinal necrosis was the fourth PSTV symptom parameter recorded (Tables 5a and 5b, pages 31 and 32). Veinal necrosis usually is evident in PSTV infected plants as dark brown to black segments of the veinal tissue on the abaxial surface of mature leaves (Figure 5, page 34). The necrosis often first appears distal to the leaf petiole and spreads along conductive tissues, towards it. Eventually, the leaf mid-vein and petiole can become extremely necrotic leading to death of the entire leaf. The necrosis may also spread into the main stem of the plant causing dark streaks to appear on the internodes. Of the various symptoms known to make up the PSTV disease syndrome on tomato, veinal necrosis is one of the last to appear. The expression of this symptom on PSTV plants in the experimental conditions was quiet variable in time of expression,

TABLE 4a: Leaf rugosity appearing on Rutgers tomato plants mechanically inoculated with distilled H_2O (CK) or PSTV inoculum (PSTV) and grown in continuous light at the listed temperature for 14 days. Results from plants similarly inoculated and grown in the greenhouse for 14 days are also included (GH).

Temp. (C)	Leaf Rugosity*	
	CK	PSTV
30	0/12	0/12
28	0/12	3/12
26	0/12	12/12
24	1/12	11/12
22	0/12	12/12
GH	0/12	12/12

* Leaf rugosity appears as severe upward puckering of the interveinal leaf tissue. The data is recorded as the number of plants exhibiting rugosity over the number inoculated.

TABLE 4b: Leaf rugosity on plants grown in the same conditions as for Table 4a except the temperature was decreased 8°C for 4 hours each 24 hour period.

Temp. (C)**	Leaf Rugosity*	
	CK	PSTV
30/22	0/12	0/12
28/20	0/12	2/12
26/18	0/12	8/12
24/16	0/12	12/12
22/14	0/12	12/12

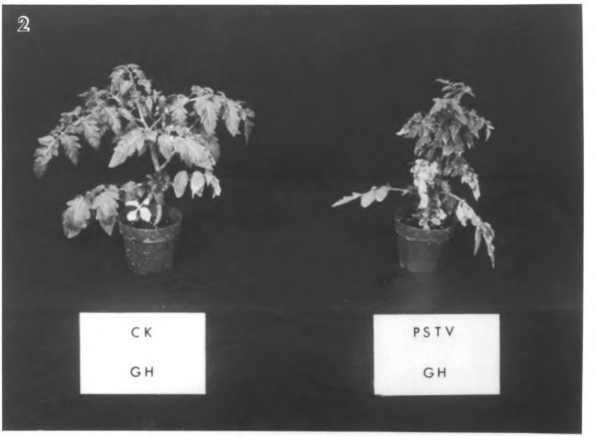
* Leaf rugosity appears as severe upward puckering of the interveinal leaf tissue. The data is recorded as the number of plants exhibiting rugosity over the number inoculated.

**Temperature for 20 hours per 24 hour period/temperature for 4 hours per 24 hour period.

Figures 2 and 3: Symptoms of PSTV on Lycopersicon esculentum Mill. cv. Rutgers tomato plant.

Figure 2: Symptoms of S-PSTV appearing on a 7 week old tomato plant grown under natural light conditions in the greenhouse. Left: Healthy plant. Right: Plant inoculated with S-PSTV 5 weeks prior to this photo.

Figure 3: S-PSTV symptoms on a 7 week old tomato plant. Left: Healthy plant. Right: S-PSTV infected plant. (Note severe rugosity and bunching of upper leaves on S-PSTV infected plant).


2
CK
GH
PSTV
GH


3

TABLE 5a: Veinal necrosis appearing on Rutgers tomato plants mechanically inoculated with distilled H_2O (CK) or PSTV inoculum (PSTV) and grown in continuous light at the listed temperature for 14 days. Results from plants similarly inoculated and grown in the greenhouse for 14 days are also included (GH).

Temp. (C)	Veinal Necrosis*	
	CK	PSTV
30	3/12	6/12
28	1/12	9/12
26	4/12	7/12
24	0/12	11/12
22	4/12	6/12
GH	0/12	7/12

* Veinal necrosis is evident as conspicuous dark brown to black veinal tissue, usually seen first on the undersides of leaves. The data is recorded as the number of plants with the symptom, over the number inoculated.

**Temperature for 20 hours per 24 hour period/Temperature for 4 hours per 24 hour period.

TABLE 5b: Veinal necrosis on plants grown in the same conditions as for Table 5a except the temperature was decreased 8°C for 4 hours each 24 hour period.

Temp. (C)**	Veinal Necrosis*	
	CK	PSTV
30/22	4/12	2/12
28/20	1/12	3/12
26/18	1/12	2/12
24/16	4/12	12/12
22/14	1/12	6/12

* Veinal necrosis is evident as conspicuous dark brown to black veinal tissue, usually seen first on the undersides of leaves. The data is recorded as the number of plants with the symptom, over the number inoculated.

**Temperature for 20 hours per 24 hour period/Temperature for 4 hours per 24 hour period.

Figure 4 and 5: Symptoms of PSTV on Lycopersicon esculentum Mill. cv. Rutgers tomato leaflets.

Figure 4: S-PSTV symptoms on adaxial surface of tomato leaflets. Left: Leaflet from healthy plant. Right: Leaflet from plant infected with S-PSTV. (Note severe rugosity and veinal necrosis of S-PSTV infected leaflet).

Figure 5: S-PSTV symptoms appearing on abaxial surface of young tomato leaf. (Note veinal necrosis indicated by arrows, and curling of leaf margins).

4

CK PSTV

5

location on the leaf, color and extent of necrosis. Some CK plants grown in the controlled environment cabinets also displayed an adaxial necrosis on the leaf veinal tissue. Both PSTV and CK plants frequently developed necrotic areas in the leaf lamina adjacent to veinal tissue. None of the CK plants grown in the greenhouse displayed this type of necrosis, which may suggest that the environment cabinets promoted the non-specific leaf lamina necrosis. These observations led to some ambiguity in evaluating the quality of using this particular symptom as a diagnostic tool. In certain experimental conditions, such as 24°C, nearly all of the PSTV infected plants displayed obvious veinal necrosis while none of the CK plants were necrotic. Similarly, in the greenhouse 7 of 12 PSTV plants showed distinct veinal necrosis while the CK plants were symptom-free.

The last symptom to be evaluated was the chlorotic response of CK and PSTV plants to the different experimental conditions (Tables 6a and 6b, pages 36 and 37). A chlorosis indexing method was devised as an aid in quantifying the results (described below Table 6b, page 37). At most of the temperature regimes tested CK and PSTV infected plants developed similar degrees of chlorotic/albino tissue. In some conditions, i.e., 24°C, 22°C, 28°/20°C, CK plants were more chlorotic than PSTV infected plants. Chlorosis was induced to a greater extent at higher temperatures in both CK and PSTV seedlings. No chlorosis or albinism was observed on any of the plants grown at 24°/16°C, 22°/14°C or in the greenhouse (GH).

TABLE 6a: Chlorosis indices of Rutgers tomato plants mechanically inoculated with distilled H_2O (CK) or PSTV inoculum (PSTV) and grown in continuous light at the listed temperatures for 14 days. Results from plants similarly inoculated and grown in the greenhouse for 14 days are also included (GH).

Temp. (C)	Chlorosis Index (0 to 3)*	
	CK	PSTV
30	2.25	2.42
28	1.17	1.25
26	1.17	0.58
24	0.33	0.33
22	0.25	0.08
GH	0.00	0.00

* A chlorosis index was assigned to each plant by the following criteria: 0 = 0-5% of the leaflets on the plant with $\geq$ 50% chlorotic tissue, 1 = 6-33% of the leaflets on the plant with $\geq$ 50% chlorotic tissue, 2 = 34-66% of the leaflets on the plant with $\geq$ 50% chlorotic tissue, 3 = > 66% of the leaflets on the plant with $\geq$ 50% chlorotic tissue. Each listed index is an average for 12 plants.

TABLE 6b: Chlorosis indices of plants grown in the same conditions as for Table 6a except the temperature was decreased 8°C for 4 hours each 24 hour period.

Temp. (C)**	Chlorosis Index (0 to 3)*	
	CK	PSTV
30/22	0.75	1.75
28/20	0.50	0.25
26/18	0.00	0.33
24/16	0.00	0.00
22/14	0.00	0.00

* A chlorosis index was assigned to each plant by the following criteria: 0 = 0-5% of the leaflets on the plant with $\geq$ 50% chlorotic tissue, 1 = 6-33% of the leaflets on the plant with $\geq$ 50% chlorotic tissue, 2 = 34-66% of the leaflets on the plant with $\geq$ 50% chlorotic tissue, 3 = > 66% of the leaflets on the plant with $\geq$ 50% chlorotic tissue. Each listed index is an average for 12 plants.

PART II

ELECTRON MICROSCOPY

MATERIALS AND METHODS

Electron Microscopy

The transmission electron microscope (TEM) has the magnification and resolution capabilities necessary for observing the ultrastructure of tomato cell chloroplasts. These organelles, which contain the plant pigments and supportive membranes (thylakoids) follow a well characterized developmental sequence as the cell matures (Kirk and Tilney-Basset, 1967; Bradbeer et al., 1977). In chlorotic tissue, some (perhaps all) of the cells' plastid component has deviated from the normal developmental sequence. The purpose of these observations was to gather information in the following four areas: 1) healthy tomato chloroplast ultrastructure, 2) differences between plastids in non-infected and PSTV infected tissue, 3) developmental stages of plastids from chlorotic leaf tissue and, 4) cytoplasmic differences between non-infected and PSTV infected leaf tissue.

Rutgers tomato seedlings were grown and inoculated as for the light and temperature experiments. One group of plants (water inoculated and PSTV inoculated plants) was placed in a growth cabinet under continuous light and constant 28°C. Another group of plants was grown in the greenhouse as previously described. After four weeks, many of the plants in the growth cabinets had developed various degrees of chlorosis and albinism. Tissue was removed from the second or third

leaf from the apex of healthy plants and ones showing various degrees of leaf injury (albinism, yellowing, mosaic) and fixed with cold 5% glutaraldehyde in .1 M phosphate buffer (pH 7.2). Similar age leaf tissue was sampled and fixed from the greenhouse-grown plants (none of these plants showed chlorosis). The fixation lasted four hours, after which the tissue was washed twice in .1 M phosphate buffer (pH 7.2) and post-fixed for eight hours in cold 2% osmium tetroxide (in .1 M phosphate, Ph 7.2). The tissue pieces were again rinsed in .1 M phosphate buffer (pH 7.2) before dehydrating in a graded ethanol series and embedding in ERL epoxy resin (Spurr, 1969). Ultrathin sections were prepared on a Sorvall MT-2 ultramicrotome and mounted on 400 mesh copper grids. The sections were stained in .5-1% uranyl acetate and 1% lead citrate prior to observation. Sections were examined with either a Philips 300 or Philips 201 transmission electron microscope.

Freeze fracture tissue preparation consisted of immersing fresh tissue pieces (1 mm^2) in liquid freon. The frozen tissue pieces were then transferred to a cryo stage in a Balzers freeze etch unti, where fracturing and etching were carried out. After fracturing, the frozen specimens were carbon coated and shadowed with platinum in the Balzers unit. The specimen replica was then cleared in concentrated chromic acid, mounted on a 400 mesh copper grid and viewed in one of the aforementioned TEMs.

RESULTS

Electron Microscopy (CK Plants)

Palisade mesophyll cells of healthy green tomato leaves contained one large central vacuole around which the chloroplasts are situated along the periphery of the cell is a thin layer of cytoplasm (Figures 6 and 8, page 48). Normal tomato chloroplasts are lenticular, 4-6 μm long and limited by a double membrane (chloroplast envelope). An extensive system of parallel membranes forming grana and intergrana thylakoids, is found within this bi-membrane envelope (Figures 6 and 7, page 48).. The chloroplasts also contained numerous ribosomes dispersed throughout the stroma. All of the healthy chloroplasts examined had at least one osmiophyllic body 0.05-0.1 μm in diameter, which exhibited a granular texture in this section. This structure was usually seen in close association with one or more thylakoid membranes. Most of the chloroplasts from these young palisade cells did not contain starch, but when starch was observed it was a single grain in the chloroplast (Figure 8, page 48).

A few of the palisade cells contained an abnormal type of chloroplast (Figure 9, page 48). Cells containing such plastids had all of the chloroplasts in this abnormal condition, while neighboring palisade cells contained exclusively "normal" chloroplasts.

Distinguishing features of these abnormal chloroplasts are a reticulate membrane formation at one end of the plastid stroma, vesicles within the stroma and unusual staining properties of the thylakoids. Unlike thylakoid membranes in the "normal" cholorplasts, thylakoids from these chloroplasts are very electron opaque while the inter-thylakoid spaces are osmiophyllic. The abnormal chloroplasts always contained at least one large starch grain, and sometimes two or more. In cells with abnormal chloroplasts, other cytoplasmic organelles such as ribosomes and mitochondria appeared in a "normal" condition, as did the vacuole.

Palisade chloroplasts from green leaves on CK plants grown under continuous light in the controlled environment cabinets, had the "normal" lamella arrangement of grana and intergrana thylakoids. The lamella was appressed in a compact configuration (often against the chloroplast envelope) due to large accumulations of starch within the chloroplast. Three to five starch grains ranging in sizes up to 1μ in length altered the shape of the chloroplasts causing them to be short and semi-circular in shape (Figure 10, page 50), when compared to "normal" lenticular chloroplasts (Figures 6 and 8, page 48). In an attempt to better observe the disposition of the thylakoids and their relationship to these large starch grains, some of this tissue was prepared by freeze-fracture. This technique did not lend itself to ease in preparing many samples, and the samples prepared contained only small areas of well preserved tissue. Starch grain/thylakoid interfaces were usually damaged by this preparation technique

due to the difficulty of fracturing through the starch grains. The freeze fracture technique did afford views of thylakoid membrane fracture faces, and chloroplast cross sections (Figure 11, page 52), which revealed segments of the intergrana lamella, and confirmed the membrane arrangement as seen in this section.

The ultrastructure of chloroplasts from chlorotic (yellow) leaf tissue on CK plants grown in continuous light deviated in several ways from that of normal chloroplasts. They were smaller, 2-3 μm in length, and circular to ellipsoid in shape. The stroma within these plastids was usually very electron dense and contained many vesicles. Thylakoids were very sparse, existing as 1-2 μm segments, and never forming lamella. Sometimes larger thylakoid segments were arranged parallel (Figure 12, page 50). A spiral configuration of the thaylakoid segments was observed in one plastid (Figure 13, page 50). Other cellular features of this tissue (nuclei, mitochondria, vacuole, etc.) appeared "normal" as in green tissue of CK plants.

The most dramatic alteration of plastid ultrastructure in CK plants was observed in white (albino) tissue from plants grown in continuous light. Plastids from this tissue had a very electron dense stroma (similar to those in chlorotic tissue) and appearing in clusters within the palisade cells rather than being dispersed evenly along the cell wall/plasma membrane, as found in green and chlorotic tissue. These plastid clusters were often seen in close association with the nucleus. This culstering divided the vacuole into two or more units in some palisade cells (Figure 14, page 52). The individual plastids were oval in shape and 2-3 μm long.

Conspicuous plastid vesicles were usually present as one to ten large membrane-bound vesicles (0.25-1.0 μm) and ten to forty small ones (<0.25μm) dispersed within the stroma of each plastid. About 50% of the plastids observed in this tissue also contained a reticulate membrane formation (Figure 15, page 52), similar to that seen in the "abnormal" chloroplast in Figure 9 (page 48). Thylakoid segments were very small (or non-existent) in plastids from white tissue. The segments were never found in any ordered arrangement, parallel or otherwise (Figure 16, page 52). Osmiophyllic bodies with a granular texture, as described in "normal" chloroplasts, were also usually present in these plastids (Figures 16 and 17, page 52).

The plastids in white CK tissue were often clustered near the nucleus, however, physical contact between the plastid envelope and nuclear membrane was not observed. A thin band of cytoplasm, containing ribosomes, always separated the two structures. Although the ultrastructure and position of the plastids within these cells was aberrant, other ultrastructural features were normal (Figure 17, page 52).

Electron Microscopy (PSTV Plants)

Chloroplasts from green leaves of PSTV infected tomato seedlings grown in the greenhouse (Figure 18, page 52) contained all the structures found in healthy tomato chloroplasts. An extensive thylakoid system of grana and intergrana lamella was seen

in all these chloroplasts. One subtle difference in the ultrastructure of CK and PSTV chloroplasts was that PSTV chloroplasts had larger stacks of grana lamella than seen in CK chloroplasts. As with greenhouse-grown CK plants, these chloroplasts rarely contained starch. No "abnormal" chloroplasts of the type in Figure 9 (page 48) were encountered in PSTV infected plants. Chloroplasts from green tissue of PSTV infected plants grown in constant light universally contained starch. The starch grains in these plants (Figure 19, page 54), were not as large or numerous as those found in CK plants grown in similar conditions. As with the greenhouse grown plants, chloroplasts from green tissue of PSTV infected seedlings in 24 hour light had larger grana stacks that CK chloroplasts grown in 24 hour light.

Yellow (chlorotic) leaf tissue, which was usually observed in abundance on CK plants grown in continuous light, was rarely seen on PSTV-infected tomato seedlings in constant light. The production of chlorotic tissue on CK plants always appeared as a transitional stage between green and white growth. On PSTV infected seedlings white tissue was most often seen directly adjacent to green tissue without any intervening yellow leaf areas. When plastids from the white tissue of PSTV infected plants were examined, they appeared very similar to those from white CK tomato tissue. These PSTV plastids were clustered in groups (often near the nucleus) in palisade cells and they contained numerous vesicles (Figures 20 and 21, page 54). Usually these plastids were circular in their circumference outline and were 2-3 μm in diameter.

Other features similar to CK and PSTV plastids in white tissue were reticulate membrane formations and small thylakoid segments (Figure 22, page 56). Sometimes these aberrant PSTV plastids contained several large vesicles and had a conspicuous lack of small plastid vesicles (Figure 23, page 56). Plastids of this type, with only large vesicles, were often found in cells which also displayed aberrant mitochondria and a very electron dense cytoplasm.

In an attempt to further examine the arrangement and nature of the membranous vesicles in white tissue plastids, both CK and PSTV leaf tissues were prepared by freeze fracture (Figures 24, 25, 26 and 27, page 58). The plastids' intra-vesicle regions appeared as a very smooth granular matrix in PSTV tissue. The plastid vesicles displayed a circular outline bounded by a single membrane, as seen in this sections. Freeze fracture revealed a rough granular disposition of a plastid stroma which was primarily due to the presence of plastid ribosomes. Freeze-fracture preparations of plastid vesicle membranes (Figure 26, page 58), revealed different particle size distributions of the EF and PF membrane leaves (using terminology of Staehelin et al., 1976), and no orderly arrangement of these vesicle membrane particles was observed. Freeze fracturing did not reveal any consistent distinction between plastids in white tissue from CK plants (Figure 27, page 58) and PSTV plants (Figures 24 and 26, page 58).

Signs of the viroid were not evident in any of the thin sections or freeze-fracture preparations. Although empahsis was

placed on the plastids, the cytoplasm, nuclei, and mitochondria of infected tissue was carefully examined for manifestations of the viroid (inclusions, crystals, x-bodies, etc. as described by Esau, 1968) with none being found. Of the aberrations described in the infected plastids none could be ascribed to the viroid particles themselves. It is possible, however, that vesicles, thylakoid segments and osmiophyllic bodies in these plastids were in direct association with viroid particles, but these entities could not be resolved due to their small size and/or low concentration.

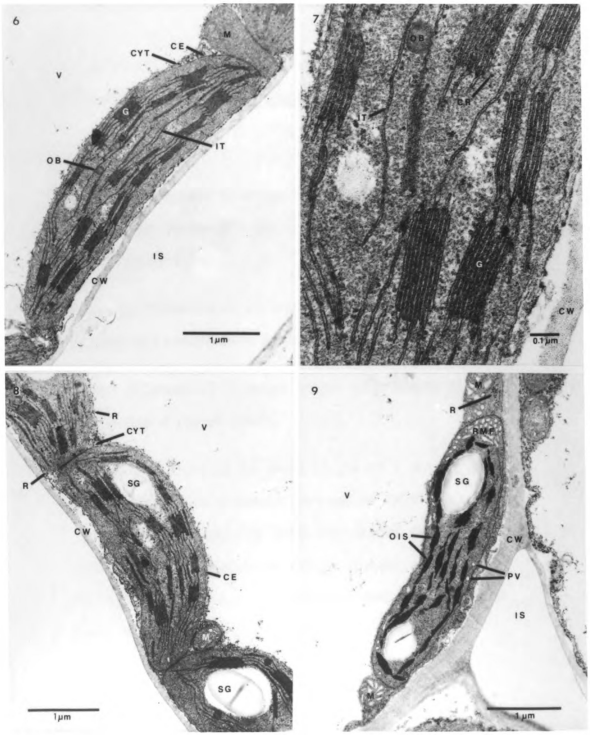
Figures 6-9: Ultrastructure of chloroplasts in palisade mesophyll cells of Lycopersicon esculentum Mill. cv. Rutgers.

Figure 6: Normal chloroplast in green tissue from a healthy tomato plant grown in the greenhouse. (Note the apparent lack of starch deposition).

Figure 7: High magnification of chloroplast in Figure 6. (Note the bi-membrane arrangement of the chloroplast lamella).

Figure 8: Normal chloroplast in green tissue of a tomato plant, containing a starch grain.

Figure 9: Abnormal chloroplast in green tissue of a healthy tomato plant. (Note the abnormal features of this chloroplast: 1) swollen osmiophillic [electron dense] inter-thylakoid spaces, 2) osmiophobic [electron opaque] property of thylakoid membranes, 3) reticulate membrane formation seen in the upper end of this chloroplast).


6
M
CYT
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IT
OB
IS
CW
1μm
7
Oh
CP
G
CW
0.1μm
8
R
CYT
V
R
SG
CW
CE
M
SG
1μm
9
M
R
RMC
SG
V
OIS
CW
PV
IS
M
1μm

Figures 10-13: Ultrastructure of chloroplasts from Lycopersicon esculentum Mill. cv. Rutgers seedlings grown under continuous light.

Figure 10: Chloroplast from a non-inoculated tomato plant grown under continuous light. This plastid was from a green leaf. (Note the presence of several large starch grains).

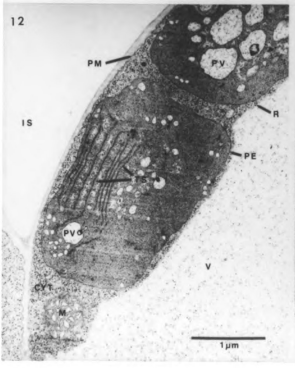
Figure 11: Freeze fraction preparation of chloroplast in green leaf tissue from a tomato plant grown under continuous light. (Note the presence of an extensive thylakoid system. Compare this plastid to those in Figures 24, 25 and 27, page 56).

Figure 12: Chloroplast in yellow (chlorotic) tissue from a tomato plant grown under continuous light. (Note the long, parallel, unstacked thylakoids and numerous chloroplast vesicles).

Figure 13: Chloroplast from the same tissue as Figure 12. (Note the spiraled configuration of the thylakoids).


10
CE
SG
SG
V
M
1 µm


11
1 µm
CE
IT
CW


12
PM
PV
IS
R
PE
PVC
V
CYT
M
1 µm


13
R
M
TS
PE
1 µm

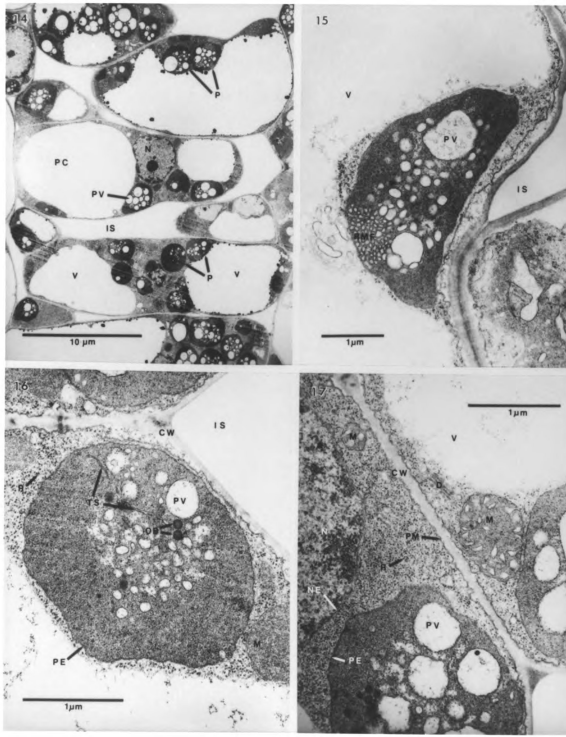
Figures 14-17: Ultrastructure of palisade mesophyll cells of Lycopersicon esculentum Mill. cv. Rutgers seedlings grown under continuous light.

Figure 14: Palisade mesophyll in white tissue from a non-inoculated tomato plant grown under continuous light. (Note the electron dense stroma within the plastids and the numerous plastid vesicles).

Figure 15: Single plastid from tissue described in Figure 14. (Note the dense plastid stroma, numerous plastid vesicles and the reticulate membrane area).

Figure 16: Single plastid from tissue described in Figure 14. (Note the presence of thylakoid segments and the lack of a reticulate membrane formation as seen in Figure 15).

Figure 17: Portions of two cells from white tissue described in Figure 14. (Note the apparent normal conditions of the nucleus, mitochondria, ribosomes and dictyosome. The cells containing these normal organelles also have the aberrant plastids).


14
P
PC
N
PV
IS
V
P
V
10 µm
15
V
PV
IS
RMF
1µm
16
CW
IS
PV
TS
PE
1µm
17
1µm
V
CW
PM
PV
PE

Figures 18-21: Ultrastructure of chloroplasts and aberrant plastids in palisade mesophyll cells of PSTV infected Lycopersicon esculentum Mill. cv. Rutgers seedlings.

Figure 18: Chloroplast in green tissue from a S-PSTV infected tomato plant, grown in the greenhouse under natural intermittent light conditions. (Note the extensive thylakoid system and large grana stacks. Compare this chloroplast with that seen in Figure 6, page 46).

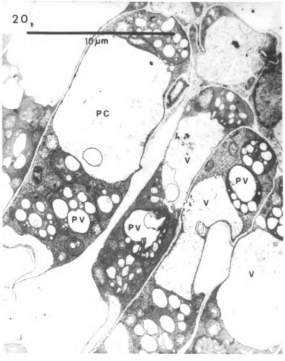
Figure 19: Chloroplast in green tissue from a S-PSTV infected tomato plant grown under continuous light. (Note the numerous lipid droplets and starch grain. Compare this chloroplast with that in Figure 8, page 46).

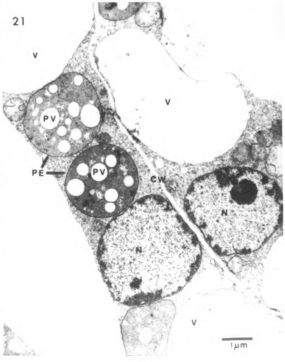
Figure 20: Palisade mesophyll in white tissue from a S-PSTV infected tomato plant grown under continuous light. (Note the large vesicles within some of the plastids. Compare this figure with Figure 14, page 50).

Figure 21: Palisade mesophyll tissue as in Figure 20. (Note the normal appearance of the nuclei, and the nearly circular outline of the plastids).


18
V
CYT
OB
G
IS
1 µm


19
V
OB
SG
G
1 µm

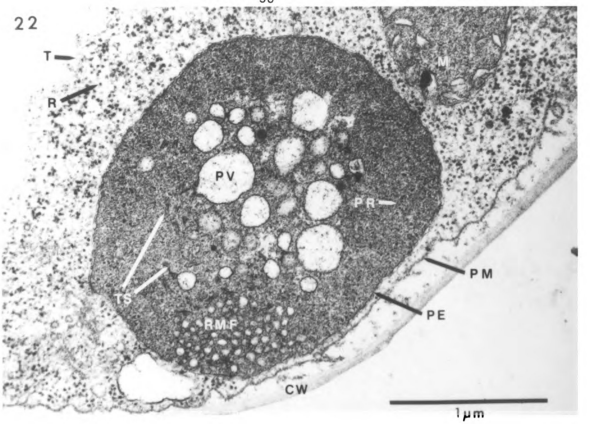

20
10 µm
PC
V
PV
PV
PV
V
V
V

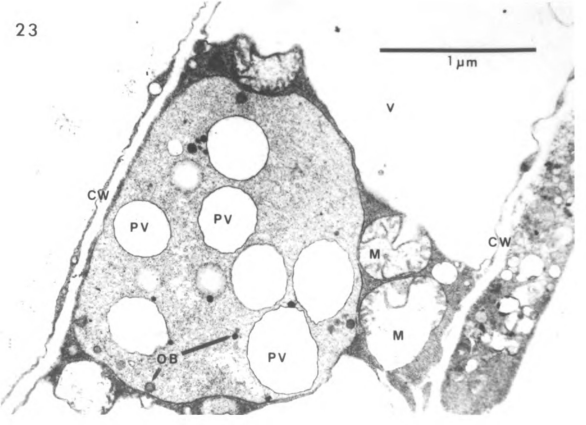

21
V
V
PV
PE
PV
CW
N
N
V
1 µm

Figures 22 and 23: Ultrastructure of aberrant plastids in PSTV infected Lycopersicon esculentum Mill. cv. Rutgers seedlings.

Figure 22: Plastid in white tissue from a tomato plant inoculated with S-PSTV and grown under continuous light. (Note the reticulate membrane formation and plastid vesicles. Compare this plastid with that seen in Figure 15, page 50).

Figure 23: Plastid from the same tissue as described in Figure 22. (Note the lack of a reticulate membrane formation and small plastid vesicles).


22
T
R
M
PV
PR
PM
PE
TS
RM-F
CW
1 µm


23
1 µm
V
CW
PV
PV
M
CW
M
OB
PV

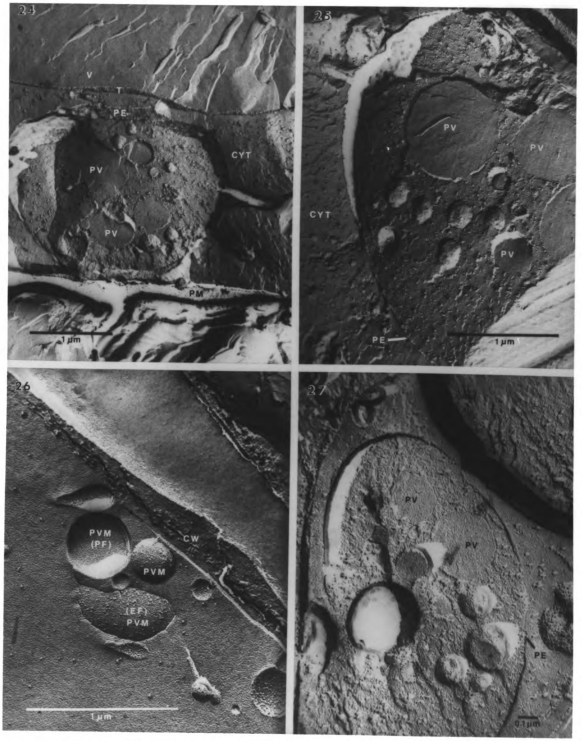
Figures 24-27: Freeze-fracture preparations of aberrant plastids from PSTV-infected and non-infected seedlings of Lycopersicon esculentum Mill. cv. Rutgers seedlings.

Figure 24: Freeze-fractured plastid from white tissue of a tomato plant infected with S-PSTV and grown under continuous light.

Figure 25: Freeze-fractured plastid from tissue described in Figure 24, showing fine granular matrix within the large plastid vesicles.

Figure 26: Freeze-etch preparation showing membrane surfaces of plastid vesicles in tissue described for Figure 24. (Note the size and distribution differences of particles on the membrane surfaces).

Figure 27: Freeze-fractured plastid from white tissue of a non-infected tomato plant grown under continuous light. (Note the similarities of this plastid and those seen in Figures 24 and 25).


24
V
T
PE
CYT
PV
PV
PM
1 µm
25
PV
PV
CYT
PV
PE
1 µm
26
PVM
(PF)
CW
PVM
(EF)
PVM
1 µm
27
PV
PV
PE
0.1 µm

DISCUSSION

The timing and severity of the symptoms which make up the PSTV disease syndrome have been shown to be highly dependent upon the environment. In continuous light, stunting of Rutgers tomato seedlings caused by PSTV infection was most severe at high, constant temperatures (28° and 30°C). The detailed experiments by Kristoffersen (1963) have shown that "healthy" tomatoes achieve a maximum growth rate in continuous light and at temperatures of 26°C or greater (depending on the tomato variety tested). Stunting, as measured by reduced stem elongation and leaf area in PSTV plants, was caused by the physiological inability of infected plants to capitalize on an optimal growth envirnoment.

During the 14 day test period in these experiments, reduced growth was the most obvious symptoms of the disease. Other PSTV symptoms, i.e., leaf rugosity and leaf veinal necrosis, were less pronounced and probably reached only initial stages of development. Stunting was most severe in conditions which masked the expression of other PSTV symptoms. At high temperatures leaf rugosity was often not apparent on PSTV plants, yet was expressed on almost every PSTV individual at low temperatures (22° and 24°C). Leaf veinal necrosis was also most distinctive at low temperatures. The diagnostic quality of this particular symptom in these conditions

is questionable due to the necrosis appearing on leaves of non-infected plants. Hillman (1956) described a similar necrosis in "Healthy" tomatoes grown in continuous light and a wide temperature range (17°-30°C). The veinal necrosis attributed to PSTV can be confused with this non-specific necrosis apparently caused by artificial illumination. In a general sense the diagnostic symptoms of PSTV on tomato seedlings grown in constant light for 14 days can be separated into two categories where they are most clearly expressed: 1) high temperature dependent - reduced stem elongation and leaf area and, 2) low temperature dependent - leaf rugosity and leaf veinal necrosis. Since reduced growth could conceivably be caused by numerous other infectious agents, the complete disease syndrome must be present for absolute certainty of infection by PSTV. The optimal diagnostic conditions as shown by these experiments is continuous light and 24°-26°C constant temperature. In these conditions PSTV symptoms of stunting, leaf rugosity, and veinal necrosis are usually apparent within 10 days, and well developed at 14 days.

The chlorotic response of CK and PSTV plants to the various environments tested are similar. Conditions promoting chlorosis in PSTV plants also produced chlorotic CK plants. And in environments where CK plants remained green, chlorosis was not seen on PSTV plants. Qualitatively, the form and appearance of the chlorotic tissue on CK and PSTV plants varied. Chlorosis on PSTV plants usually appeared as completely white (albino) leaves as opposed

to yellowish-white on CK plant leaves. Also, chlorotic CK leaves often achieved normal size while white leaves on PSTV plants remained small and mis-shapened. Quantitatively, however, the chlorosis on CK and PSTV plants was very similar as demonstrated by like chlorosis indices for the experimental runs.

The differential chlorotic response to continuous illumination by CK and PSTV plants reported by Yang and Hooker (1977) did not manifest itself in these experiments. This may have been caused by a difference in the growing environments of their plants and the experiments described here. Leaf injury in the form of chlorosis on tomato leaves is a very complex physiological response. Some of the factors affecting this response are the diurnal period (Ketellapper, 1959), light intensity (Arthur et al., 1930), temperature (Hillman, 1956), thermoperiod (Went, 1944), nutrient availability (Kristoffersen, 1963), humidity (Kristoffersen, 1963), genetics (Daskaloff and Ognajanova, 1965), plant age (Went, 1945) and an upset in the plant's endogenous rhythm (Highkin and Hanson, 1954). Although these experiments were designed closely around those of Yang and Hooker (1977) it is possible that one or more of these variables was different in the two studies. The fluctuating thermoperiod experimental runs did reduce chlorosis in CK and PSTV plants in comparison to the constant temperature runs. This result is consistent with the notion that chlorotic leaf injury on tomato is not a diagnostic symptom for PSTV, but rather a response by tomato to an unfavorable photoperiod as influenced by the aforementioned factors.

Further evidence supporting the similarity of PSTV-infected and non-infected tomatoes' chlorotic response in continuous light has been shown by the electron micrographs of tomato plastids. Chloroplasts from CK and PSTV plants grown in the greenhouse had similar ultrastructures. The only difference being noted was larger grana stacks (more thylakoid units) in PSTV chloroplasts. The similarity also existed when CK and PSTV chloroplasts from a constant light environment were compared. In constant light both types of chloroplasts assimilated large starch grains, which demonstrates (at least in one respect) a "normal" physiology in PSTV chloroplasts. No signs of the viroid were observed in chloroplasts from infected plants, concurring with the work of others' showing the subcellular location and cite of viroid synthesis to be the nucleus (Diener, 1971a; Takahashi and Diener, 1975). [This does not, however, preclude the possibility that viroid symptoms could be expressed in the chloroplasts. It is known that polypeptides and M-RNA coded by nuclear DNA are required for complete chloroplast development (Bradbeer et al., 1977). Interference in these nuclear/cytoplasmic activities by the viroid could conceivably become apparent as aberrations in the plastids]. Chloroplast aberrations caused by conventional viruses have been studied (tobacco mosaic virus - Arnott et al., 1969; beet yellow virus - Esau, 1968; barley stripe mosaic virus - McMullen et al., 1978; beet western yellow virus - Tomlinson and Webb, 1978) and none of

the plastid deformities described were seen in PSTV plastids. PSTV did not manifest itself or induce any ultrastructural deformities to chloroplasts in infected tomato cells.

In chlorotic tomato leaf tissue, plastids from infected and non-infected plants appeared similar. Descomps and Deroche (1973) studied chlorotic "healthy" tomato tissue grown in continuous light, and their micrographs show the same aberrations (reticulate membrane formations, vesicles, etc.) as shown for CK and PSTV plastids in this study. The clustering of plastids in white tissue "at the upper and lower ends of palisade cells" was reported by Yang (1975) in infected leaves. Similar aggregations of plastids (often near the nucleus) have been shown by this study in both CK and PSTV white tissue. I have applied the term albidoplast (albido = white) to these plastids which are found in white tissue of tomato plants grown in long photoperiods, displaying the discussed aberrations (as shown in Figures 15, 22, 23, pages 50 and 54) and containing no functional thylakoids. Albidoplasts from non-infected plants were indistinguishable from those in PSTV-infected plants.

Albidoplasts may be the product of an altered developmental morphology or the result of a breakdown of normal chloroplasts in cells exposed to long daylengths. Because green tomato tissue does not turn chlorotic when healthy plants are placed in continuous light (Hillman, 1956) the albidoplast is probably the product of an aberrant ontogeny from proplastids in the young cells, exposed to damaging light periods. The developmental alterations must be

universal to all plastids in affected tissues and irreversible, since white tissue does not green when placed in a favorable environment (Yang and Hooker, 1977). An explanation for this failure of white tissue to green may be that plastids do not arise de novo in the cells. Rather, a mature cell's entire plastid population is derived (through division) from a single or several proplastids in the young cell (Kirk and Tilney-Bassett, 1967). An irreversible aberration in the young cell's plastid initial(s) may take place in continuous light, leading to an adult cell with its entire plastid complement in an aberrant condition. New growth, developing in a non-damaging daylength from white tissue, is green because the proplastids in young cells pass through critical developmental stages in a favorable photoperiod.

The observation that PSTV-infected tomatoes grown in constant light displayed white growth as opposed to the yellowish-white, chlorotic growth on CK plants may be explained by the reduced growth rate of PSTV plants. Some critical stage (which is intolerant to long exposures to light) in the ontogeny of proplastids into chloroplasts is perhaps prolonged in viroid-infected cells. CK plants may pass through this sequence faster, with some plastids retaining a limited capacity to form thylakoids and synthesize pigments. The qualitative color difference is a direct effect of the rate of plastid development in infected plants, while the phenomenon of the albidoplast is common to any Rutgers tomato seedling subjected to long photoperiods. It is believed that chlorotic/albino tissue caused by the presence of albidplastids in tomato cells, is not diagnostic for any biological agent.

SUMMARY

1. PSTV symptom expression on Rutgers tomato seedlings in continuous light is variable depending on the temperature at which the plants are grown. High temperatures promote stunting, while at low temperatures symptoms of veinal necrosis and leaf rugosity are strongly expressed.
2. Chlorosis and/or albinism appearing on Rutgers tomato plants grown in continuous light is not a diagnostic symptom of PSTV.
3. Chloroplasts and other cytoplasmic organelles in PSTV-infected, greenhouse-grown Rutgers tomatoes were free of aberrations and no signs of the viroid (inclusion material, x-bodies, crystals etc) were observed in TEM thin sections.
4. Similar aberrant plastids were found in chlorotic tissue from non-infected and PSTV-infected Rutgers tomatoes maintained in continuous light. The extremely aberrant plastids which contained vesicles and no functional thylakoids have been termed albidoplasts.
5. Albidoplasts are believed to be the product of an injurous photoperiod, and not the result of infection with a biotic agent.

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