POTATO SPINDLE TUBER VIRUS IN CERTAIN SOLANUM SPECIES

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ABSTRACT

POTATO SPINDLE TUBER VIRUS IN CERTAIN SOLANUM SPECIES

By

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Isolates of the several strains of potato spindle tuber virus (PSTV) were obtained from the United States and Canada and maintained in Rutgers tomato (Lycopersicon esculentum Mill. cv. Rutgers). Crude sap of the infected plants was used for inoculum.

Solanum acaule Bitt., PI 230-554, developed severe symptoms of chlorosis, leaf lesions, veinal necrosis, rugosity and rosetting approximately one month after inoculation when young plants at 2-3 leaf stage were incubated under continuous light of approximately 1,000 ft-c.

PSTV survived over winter in Solanum dulcamara L., a common perennial weed of Michigan. In this plant the virus is symptomless and is seed transmitted.

Young portions of PSTV-infected Rutgers tomato plants developed albinism under continuous light of approximately 1,000 ft-c. New growth of infected plants was green or white depending on day length. Virus infectivity was higher in extracts of white leaf tissue than that of green leaf tissue.

PSTV infected Rutgers tomato, D. tatula, potato, and S. acaule plants were intensively studied for cytological and histological changes after infection. Necrosis was examined in the parenchyma tissue of tomato, S. acaule and potato plants. Virus inclusion bodies were not consistently present in infected plants examined with phase contrast microscopy or when stained with acridine orange under ultraviolet illumination or with certain conventional stain techniques.

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By

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To

My Parents, Wife,

Brothers and Sisters

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INTRODUCTION AND LITERATURE REVIEW

A disorder of potato characterized by development of abnormally elongated tubers was first described by Martin in Irish Cobbler potato, Solanum tuberosum L., in 1922 (Martin, 1922). The disease was soon reported in other potato varieties (Folsom, 1923; Werner, 1926). This disorder is now commonly known as the potato spindle tuber virus (PSTV) disease.

The disease was widely spread and caused heavy loss of the potato crop. Martin (1924) reported in the 1921 New Jersey crop of the late planted non-certified Irish Cobbler, 16% of primes were harvested from diseased plants in comparison with 74% from healthy plants. In Mississippi, Wedgworth (1928) reported loss caused by the disease was nearly 50% in Irish potato. In northern Minnesota, the spindle tuber disease caused up to 40% loss of the crop among Early Ohio and Irish Cobbler potatoes. Loss of the crop was reported mainly due to the small size, rather than reduction in number of tubers (Hunter and Rich, 1964 a).

Since the early history of the disease, the causal agent had been thought to be a virus although no virus has ever been isolated. The similarity of PSTV and the causal agent of tomato bunchy top virus disease was first suggested by Raymer and O'Brien (1962). Later the two viruses were proven identical (O'Brien and Raymer, 1964; Benson et al., 1965).

Diener (1971 a) reported that PSTV is a replicating, non-protein coated ribonucleic acid (RNA) of low molecular weight. The virus was associated with the nuclei fraction of the infected cells. Other fractions containing soluble constituents, ribosomes, mitochondria or chloroplasts were practically non-infectious (Diener, 1971 b). Because of these distinct characteristics, he proposed the term 'viroid' to the causal agent.

Singh, Clark, and Weathers (1972) studied the similarity of host symptoms induced by citrus exocortis and PSTV and found that both viruses induced the same symptoms on the same group of plants tested.

Mechanical transmission of PSTV between potato tubers and plants was reported by a number of workers. Macleod (1927) reported that the disease was transmissible by rubbing the juice of diseased plants into abrasions on healthy leaves and by juice injections into the petioles and leaves. He found that the vine symptoms were more pronounced in Irish Cobbler than in Green Mountain and that yield reduction of second year infected plants was higher than in the first year of infection. Goss (1926) obtained 47% transmission by rubbing cut surfaces of healthy and diseased tubers and 32% transmission by cutting knives. Kotila (1927) in Michigan and Tisdale (1929) also reported transmission of the disease by cutting knives. Transmission of the disease by contaminated tractor wheels and by foliage contact with diseased plants was reported by Merriam and Bonde (1954).

This is especially important in the tall varieties as Katadin and Kennebec (Manzer and Merriam,1961).

Raymer and O'Brien (1962) reported successful transmission of the virus from Saco potato to Rutgers tomato (Lycopersicon esculentum Mill.) plants by both grafting and mechanical inoculation methods. This was the first reported artificial transmission of the virus to a non-potato host. PSTV inoculated Rutgers tomato plants showed distinct systemic symptoms, including rugosity of the leaves, epinasty of the petioles, and necrosis of the veins, etc., 2 to 5 weeks after mechanical inoculation. Rutgers tomatoes have since become a generally used indexing plants for this virus. Another tomato cultivar, 'Sheyenne', was later reported by Singh, Benson and Salama (1964) to be quite susceptible to PSTV. It developed necrotic symptoms 2 to 3 weeks after inoculation. They suggested that this cultivar also could be used as an indicator plant.

Studying the host range of the virus, O'Brien and Raymer (1963) found that artificially inoculated, Petunia hybrida var. Flaming Velvet, Nicotiana rustica, N. debneyi, and Physalis peruviana became infected but carried the virus symptomlessly. Later (1964) they reported Capsicum, Datura, and Nicandra as symptomless hosts. They found that the virus caused a color break in flowers of the inoculated N. glutinosa plants.

In other research, several investigators have attempted to improve techniques of inoculation and determine

conditions of incubation which enhance symptoms of the disease on indicator plants. Whitney and Peterson (1963) showed that new growth of Rutgers tomato developed twisted, rugose, and stunted symptoms following PSTV inoculation when plants were decapitated approximately 2 weeks after inoculation. They observed that response was better at 75°F than 65°F. Hunter and Rich (1964 b) used liquid nitrogen as an aid in studying PSTV. They ground infected tomato leaves with liquid nitrogen into powder which was then added to a small amount of phosphate buffer at pH 8. This preparation when used for inoculum possessed higher infectivity than that prepared by grinding diseased leaves in buffer directly. Hunter (1965) found that the maximum concentration of the virus developed 2 to 3 days after symptom appearance.

Lee and Singh (1972) reported correlation of intensive virus-induced vein necrosis with Mn concentration and Mn/Fe ratio of the inoculated tomato plants. They employed sand culture and found that veinal necrosis of plants was severe when the inoculated tomato plants had a Mn content of 1,500 μg/g fresh tissue or above and when the Mn, Fe concentration ratio was 12 to 1 or higher.

The first local lesion host of PSTV, Scopolia sinensis Hemsl. (Atropanthe sinensis Pascher) was reported by Singh (1971). True leaves of S. sinensis developed dark brown, necrotic and roughly circular local lesions in 7 to 10 days after inoculation with crude sap of PSTV-infected tomato

plants. Shortly after, veinal necrosis and necrotic spots appeared on new leaves of inoculated plants. Other Scopolia species were found to react to PSTV similarly (Singh, 1973).

Benson and Singh (1964) found the virus was transmitted in tomato by both seed and pollen. They observed that the fruits and the seeds of the diseased plants were smaller than those of the healthy ones. Germinability of seeds from the diseased plants was also reduced. Pollen and seed transmission of the virus was also reported by other investigators in independent research conducted later (Fernow et al., 1970;Hunter et al., 1969). Nimnoi (unpublished data) indicated that germination of the pollen grains from the diseased plants was slower, the germ tubes appeared irregular, and grew slower than the healthy ones.

This study was directed toward identification of internal cell modification following PSTV-infections and environmental factors influencing symptom expression in various hosts.

MATERIALS AND METHODS

Four isolates of PSTV, referred to as Canadian, Wisconsin, Schultz, and Diener, respectively were obtained from: 1) Dr. N. S. Wright, Canada Agriculture Research Station, Vancouver, British Columbia; 2) Dr. H. M. Darling, University of Wisconsin, Madison; 3) Miss M. J. O'Brien (isolate #48 of E. S. Schultz collection), Potato Investigation Laboratory, ARS, USDA, Beltsville, Md.; 4) Dr. T. O. Diener, Crops Research Div., ARS, USDA, Beltsville, Md. The virus isolates were maintained on Rutgers tomato plants in the greenhouse.

Seed of Rutgers tomato were sown in greenhouse potting soil, composed of equal parts of sand, peat, and loam. Seedlings were transplanted into 4-in. or occasionally larger clay pots about 10 days later. Inoculations were made when plants had recovered from transplanting, ordinarily in 1 or 2 days. For inoculation, 3 or 4 relatively young leaflets and young shoots were removed from infected plants with a dry heat sterilized razor blade, and ground in a mortar and pestle in approximately 1-10 dilution with distilled water with a small amount of 400-mesh silicon carbide. In some inoculations, 0.05 M phosphate buffer at pH 7-8 was used for preparing inoculum. The upper leaf surfaces of tested plants were gently rubbed with a cotton swab in a

wooden stick (Q-tip) dipped in the inoculum, while the leaf was supported with a few layers of paper towel. Sap of healthy tomato plants was used for rubbing control plants. After inoculation, plants were kept under various temperature and light conditions for symptom development.

Once a week plants were given a nutrient solution (Plant Marvel, Plant Marvel Laboratories, Chicago 28, Ill., containing 12, 31, and 14% of N, P, and K, respectively) at the concentration of 5 g per 4 liters of water (0.125%). Observations were made daily and photographs were taken when distinct symptoms appeared. Wooden growth chambers, 122 x 76 x 56 cm, with automatic day length adjustment and temperature control were used. Light intensity of approximately 1,000 ft-c was provided by 12 40-watt cool white fluorescent tubes and 6 25-watt incandescent light bulbs. Lights were situated 50 cm above the plant level at the cotyledon stage. A walk-in growth chamber, Sherer Model Cel 512-37 was also used in some experiments. A light meter manufactured by Weston Electrical Instrument Corp., Newark, N.J. was used for measuring light intensity.

The degree of chlorosis was expressed either by the percent chlorotic leaflets per plant or by actual chlorophyll content of the leaf tissue. For determining the chlorophyll content, 0.1 g of leaflet tissue samples from various parts of the plant was ground by a mortar and pestle with 9.9 ml of a mixture of 9 parts of acetone and

1 part of 0.1 M $MgCo_3$ in water. The homogenate was filtered through Whatman No. 2 filter paper and the filtrate absorbance was read with a Spectronic 20 spectrophotometer, Bausch and Lomb Inc., at 645 and 633 mμ. The chlorophyll concentration of the tissue extract (μg/ml) was calculated as $20.2 \times A_{645} + 8.02 \times A_{633}$ (Arnon, 1949). The resulting number was multiplied by 100 for the chlorophyll concentration of the original sap of the leaf tissue.

For comparing virus concentration, a 10-fold serial dilution method was followed. In the absence of an easily grown local lesion host, infectivity was determined on Rutgers tomato, a systemic host plant. Relative virus concentration was expressed as the number of plants infected per number of plants inoculated at different dilutions of the virus preparations.

For cytological studies, leaves, or leaflets of the test plants were infiltrated with water as described by McWhorter (1951) to remove air existing in the intercellular spaces. Cross sections of leaves were made with a Lab-line/ Hooker plant microtome (Hooker, 1967). A 3-mm wide leaf strip was cut on a piece of supporting carrot root into sections of approximately 25μ thickness. The upper epidermis was removed by free hand sectioning from water infiltrated leaves placed on a wedge-shaped paraffin block on the stage of a binocular microscope. The epidermis was removed with a razor blade in a holder. Sections were examined either

unstained with phase contrast or stained with fluorescent dye and examined under ultraviolet light. Certain other staining methods were used with the conventional light microscope.

For acridine orange staining, sections of epidermal slices were fixed and stained with the method described by Hooker and Summanwar (1963). Shorter fixation time was used in some stainings. A modified fixation procedure of fixing the specimens in 80% ethyl alcohol (ETOH) for 20 min following 10 min of fixation in 50% ETOH was also used. Observation were made with an American Optical microscope illuminated with ultraviolet light generated by A-H6 high mercury pressure bulb and filtered through a blue filter (Corning 5113, 4.05 mm thick) which transmitted wave lengths between 350 and 480 mμ with the peak transmission between 390 and 420 mμ. A yellow barrier filter (Kodak Wratten K2 No. 8) consisting of two layers of gelatine was placed between the microscope objective and the prism. Photographs were made using Ektachrome X daylight film.

EXPERIMENTAL RESULTS

Solanum acaule Bitt.(PI 230-554), its symptoms and use in isolating PSTV from plants doubly infected with PSTV and PVX (potato virus X)

PSTV in naturally infected potato is frequently accompanied by PVX and possibly other potato viruses. A host for PSTV also immune to PVX is highly desirable. Plants grown from true seed, as is possible with *S. acaule*, should be free from many tuber borne potato viruses. Among the large number of reported PSTV-susceptible plants, only a few species develop visible symptoms and many others carry the virus as a latent infection (Singh, 1973). Those reported to produce symptoms are: *Gynura aurantica* DC, *Petunia hybrida* Vilm. cv. 'Burpee Blue', *Scopolia* spp. *Solanum avicular* Forst, *S. depilatum* Kitag., *S. tuberosum* L., *S. rostratum* Dunal and *Lycopersicon esculentum* Mill. Furthermore, many of the plants reported susceptible to PSTV especially among the Solanaceae are also susceptible to potato virus X (PVX).

Certain clones of *S. acaule* were first shown susceptible to PSTV by Bagnall (1972). However, no detailed description of symptoms was presented. Certain clones and seedling populations of *S. acaule* have been known for some time to be immune to PVX (Ross and Baerecke, 1950; Wetter, 1961).

This portion of the thesis describes symptoms of PSTV on seedling plants of S. acaule, PI 230-554, and describes isolation of PSTV from plants doubly inoculated with PSTV and PVX.

True seed of Solanum acaule, PI 230-554 (Plant Introduction Accession Number) was originally obtained from the Potato Introduction Station, Sturgeon Bay, Wisconsin. Young seedlings were transplanted at the 1-2 leaf stage individually into 4-in. clay pots. Plants were dusted with 400-mesh silicon carbide and mechanically inoculated with crude sap from PSTV-infected tomato plants. Proof of immunity to PVX was established by inoculation with crude sap of PVX infected Datura tatula plants. PVX_5, (Timian's isolate 5) (Timian et al., 1955), was selected because it consistently produces strong symptoms on D. tatula. After inoculation, plants were either kept on the greenhouse bench under natural light or in the growth chamber under artificial light of approximately 1,000 ft-c.

Isolation of spindle tuber virus from inoculated S. acaule was done either by mechanical transfer or by grafting methods. Rutgers tomato plants were used for indexing the PSTV, and D. tatula for indexing PVX, since symptoms of both viruses are distinct and clearly evident.

Seedlings of S. acaule were inoculated at the very early stage when 3-4 leaves first became sufficiently large. Symptoms of PSTV infection were consistently obtained when S. acaule was inoculated with any of the isolates tested in

at least 10 distinct inoculation trials. Symptoms first developed within a month to 6 weeks after inoculation. By this time, inoculated leaves had become senescent and may have dropped. New apical growth was characterized by rugosity of leaves and distinct backward and downward rolling of the young leaves (Fig. 1). In general, symptoms were suggestive of those in the young growth of tomato. Older leaves of the plant which had developed soon after inoculation were often chlorotic at least in certain areas. Frequently necrosis followed. Necrosis involved the leaf lamina but was most severe in the mid-vein and other major leaf veins. Symptoms were most severe in continuous light of 1,000 ft-c at approximately 24°C. On the greenhouse bench in the winter, symptoms were not distinct and were delayed in development.

S. acaule developed essentially the same kind and severity of symptoms when inoculated with the 4 different isolates of PSTV. Crude sap of the S. acaule plants each infected with a different isolate of PSTV was respectively inoculated to young Rutgers tomato plants and incubated under continuous light. The tomato plants developed similar degrees of white symptoms. Virus concentration in infected S. acaule plants appeared to be high.

Figure 1.--Rosetting and dwarfing symptoms of PSTV infection on S. acaule, PI 230-554.

A) Right, distinct rosetting symptom following inoculation with Schultz isolate of PSTV and incubated for one month in continuous light at 1,000 ft-c. Left, non-inoculated control.

B) Right, plant inoculated with the Canadian isolate of PSTV from which a few leaflets have dried and dropped. One tip leaflet shows chlorosis and some other leaflets have necrotic lesions on them. The upper leaves show the rosette symptom. Left, non-inoculated control.

Figure 1 A

Figure 1 B

Immunity of this S. acaule seedling population to PVX was demonstrated by grafting it as a scion to D. tatula stocks which were infected previously with PVX and had been showing characteristic symptoms of PVX. Six D. tatula plants growing in 4-in. pots were inoculated with PVX one month before the grafting. After grafting, plants were kept on the greenhouse bench. The S. acaule scions later grew quite vigorously, gave rise to some branches, and bore some fruit. PVX was not recovered from leaves of the scion S. acaule by mechanical inoculation to young D. tatula plants.

In other trials, tomato plants were inoculated with PVX, and one month after inoculation, S. acaule was grafted to the top of these tomato plants. One month after the grafting, the scion S. acaule and the tomato stock were tested on seedling D. tatula by mechanical sap inoculation. PVX was recovered from all of the stock tomato plants but none of the S. acaule scions carried the virus.

Tests to determine if this selection of S. acaule could serve to separate PSTV from PVX were made. Crude sap from PVX infected D. tatula plants and PSTV infected tomato plants were ground respectively in distilled water and phosphate buffer, 0.05 M, pH 7-8. Young seedlings of S. acaule were inoculated mechanically with either or both of the virus preparations. The two virus preparations were either mixed just before inoculation or plants were inoculated with one virus first and with another 5 days

later. One month after inoculation, visible symptoms of PSTV on S. acaule were recorded and PVX was indexed by grinding diseased tissue and transferring to indicator D. tatula plants. D. tatula was used as an indicator since it develops distinct systemic mottle with PVX and is a symptomless carrier of PSTV.

PVX was not recovered on D. tatula from any S. acaule plants mechanically inoculated with PVX or with PVX and PSTV. PSTV was consistently recovered from S. acaule inoculated with PSTV or with or without PVX (Table 1).

Table 1.--Reisolation of PSTV from S. acaule plants mechanically inoculated with PSTV or PSTV and PVX.

Viruses inoculated to S. acaule	Virus symptoms on indicator plants	
	PVX on D. tatula	PSTV on tomato
PVX	-	
PSTV		+
PVX + PSTV	-	+
PVX followed by PSTV	-	+
PSTV followed by PVX	-	+

\- No virus symptoms on inoculated plants.
\+ Virus symptoms on inoculated plants.

Attempts were also made to isolate PSTV without PVX using S. acaule grafts from doubly infected tomato. Young Rutgers tomato plants were inoculated with PVX and 5 days later were inoculated with PSTV. One month after the PSTV inoculation, they were top grafted with S. acaule. Later, attempts were made to reisolate each of the viruses from leaves of the tomato stock and from the S. acaule scion.

In the first experiment, when relatively young S. acaule was grafted, characteristic symptoms of PSTV developed on the scion S. acaule. The symptoms consisted of chlorosis and rosetting of the leaves (Figure 2). No PVX could be reisolated by indexing leaves of S. acaule on D. tatula seedlings. In the second experiment, when slightly older S. acaule plants were used for top grafting, PSTV symptoms did not show as distinctly as when the younger S. acaule plants had been used. PSTV was consistently reisolated from the scion S. acaule and from the stock tomato, while PVX was not reisolated from the scion S. acaule but was obtained from the stock tomato.

Figure 2.--Virus free scions of S. acaule top-grafted onto tomato previously inoculated with PVX (left) and with PVX and PSTV (right). Left, scions did not accept PVX, exhibited no PVX symptoms, and the virus could not be recovered from them. PVX was recovered from the previously infected tomato stocks; Right, S. acaule scions with chlorotic symptoms of PSTV infection. PSTV was recovered from both S. acaule scions and the tomato stocks. PVX, however, was recovered from the stock tomato only.

Overwintering of potato spindle tuber virus in Solanum dulcamara L.

Potato spindle tuber virus (PSTV) is generally believed disseminated by infected seed tubers (Goss, 1926; Merriam and Bonde, 1954; and Werner, 1926). PSTV has been transmitted to a number of annual plants and to a few perennials by various investigators. However, the ability of the virus to survive over winter outside in a perennial host has not been demonstrated. Attempts were made to determine if Solanum dulcamara L. is capable of carrying PSTV over winter under natural conditions in Michigan.

In a search of suitable plants for cytological studies of PSTV, I demonstrated S. dulcamara L. (bitter night shade) in this laboratory in February, 1972 to be a symptomless host. Since S. dulcamara is a perennial weed which grows commonly throughout Michigan its importance as a weed host deserves study. Singh has recently confirmed this earlier observation and has listed S. dulcamara as a symptomless host (Singh, 1973).

Plants grown from seed of S. dulcamara L. collected from the campus of Michigan State University, were identified by Dr. J. H. Beaman of the Beal-Darlington Herbarium at Michigan State University. S. dulcamara plants at the 1-2 leaf stage were dusted with 400-mesh silicon carbide and mechanically inoculated with the crude

sap of PSTV infected tomato plants (Canadian isolate of PSTV). After inoculation, plants were kept in 8-in. pots outside of the greenhouse under natural conditions. Demonstration of PSTV in S. dulcamara was attempted after inoculation to Rutgers tomato plants using both mechanical and graft methods.

After inoculation, no visible differences either in growth rate or in flowering and fruit bearing characteristics were observed between inoculated and non-inoculated S. dulcamara plants, although there was inconsistently some chlorosis in young parts of the inoculated plants. No virus inclusion bodies were present within the leaf cells of inoculated plants when observed either by phase contrast microscopy or by acridine orange fluorescence microscopy.

The presence of PSTV in S. dulcamara was demonstrated by grafts to Rutgers tomato. PSTV was demonstrated from all of the Rutgers tomato plants graft-inoculated at 3 different times in 1972 (Table 2). Grafting S. dulcamara scions onto tomato plants was accomplished with ease as S. dulcamara grafts grew vigorously on tomato.

Table 2.--Infection following graft inoculation of Solanum dulcamara L. with PSTV.

Date of inoculation	Number of plants		
	Inoculated	With symptoms	From which PSTV was reisolated
2-20-72	5	0	5
4-19-72	4	0	4
7-17-72	3	0	3

Overwinter survival of PSTV was tested using seedling plants of S. dulcamara transplanted individually into 8-in. clay pots in the middle of July 1972. After inoculation, they were placed outside the greenhouse and subjected to natural conditions except for watering and fertilizing.

The first PSTV-reisolation test was done in late October of the same year (Table 3). A segment of young shoot was removed with heat-sterilized razor blades from each of the 3 inoculated plants, and top-grafted onto 6-week old Rutgers tomato plants. Characteristic PSTV symptoms developed later in the new growth of all grafted tomato plants. All control plants grafted with non-inoculated S. dulcamara remained healthy.

A second reisolation test was made from potted plants stored outside in the middle of January, 1973 (Table 3). The

plants were dormant and covered with snow at this time of the year. Leaves had frozen and dropped. Dry berries were hanging on the dormant branches. Twigs were removed from the plants outside and grafted to Rutgers tomato in the greenhouse. Within a week after grafting and being maintained in the warm greenhouse, buds on S. dulcamara had began to develop. Symptoms of PSTV developed in the new growth of all tomato plants grafted with infected S. dulcamara. The non-inoculated control plants were free from PSTV.

Leaves from the new buds developing on S. dulcamara scions were tested by mechanical inoculation to Rutgers tomato on February 12 and all indicator plants became infected.

PSTV was recovered in 3 later tests, late April, early June, and early July, 1973 by mechanical inoculation using the crude sap prepared from young S. dulcamara leaves removed directly from overwintered plants. PSTV was consistently recovered from the original inoculated S. dulcamara and control plants were consistently virus free (Table 3).

Table 3.--Overwinter survival of PSTV in *S. dulcamara* in Michigan.

Method of virus transfer	Date	PSTV infected tomato plants from: Non-inoculated (control) *S. dulcamara* plants #1	#2	#3	PSTV-inoculated *S. dulcamara* plants[1] #1	#2	#3
Graft	10-24-72	-	-	-[2]	+	+	+[3]
Graft	1-18-73	-	-	-	+	+	+
Sap	2-12-73	0/9	0/9	0/9	9/9	9/9	9/9[4]
Sap	4-23-73	0/9	0/9	0/9	9/9	9/9	9/9
Sap	6- 6-73	0/9	0/9	0/9	9/9	9/9	9/9
Sap	7- 7-73	0/9	0/9	0/9	9/9	9/9	9/9

1/ *S. dulcamara* plant, at cotyledon stage, mechanically inoculated on 7-17-72.

2/ - Negative reaction (no virus recovery).

3/ + New growth of the stock tomato plants showed PSTV symptoms.

4/ Number of plants with symptoms/number of plants inoculated.

Attempts were made to determine if PSTV was also seed transmissible in S. dulcamara plants. Bright red mature berries were harvested from inoculated S. dulcamara plants which had overwintered outside. They were individually crushed in a small amount of water in a 25-ml beaker. After standing for a few days under laboratory conditions seeds were cleaned and allowed to air-dry for a few days. A portion of the seeds obtained from each berry were sown in a pot in the greenhouse. When seedlings had grown to approximately 3 cm high, attempts were made to detect presence of the virus. The shoot carrying 2 young leaves of each seedling plant was removed and ground in small amount of distilled water in a mortar and pestle. The preparation was then used for index inoculation to 6 Rutgers tomato plants at the cotyledon stage.

Among the young seedlings of S. dulcamara originating from 6 berries, PSTV infected plants were grown from seeds of 4 berries. The virus apparently was carried in relatively low concentration as only a low percentage of the index tomato plants were infected. Furthermore, symptoms on tomato did not develop until approximately 2 months after inoculation. However, symptoms did develop in the new growth of decapitated tomato plants.

Albinism of PSTV-infected Rutgers tomato plants grown in continuous light

Symptoms on inoculated Rutgers tomato plants in 12-16 hr day length--Early symptoms, rugosity and slight downward rolling of the leaf blade, usually developed 2 weeks after plants had been inoculated in the cotyledon stage. These symptoms were essentially similar to those described elsewhere (Raymer and O'Brien, 1962). Leaf hairs of these diseased plants appeared more distinct and leaves appeared lighter greenish in color as compared with healthy ones. Epinasty of the petiole usually followed within a few days. In some cases, veinal necrosis developed. As infected plants grew older, the upper shoots became pointed; rugosity of the leaves became less distinct; leaves were smaller, somewhat thicker, and slightly grayer in color. Diseased plants produced some small fruits. These fruits became reddish orange color, similar to those of healthy ones, when they became matured. Only few seeds or no seeds at all were found in these fruits.

In some PSTV-inoculated plants the upward growth was remarkably reduced; the axillary buds developed and the plants were quite 'bunchy-topped'. These diseased plants generally had poor root systems. A high percentage of diseased plants fell down at the soil level or were bent at the lower stem. Growth of apical meristems of these diseased plants was either retarded or destroyed.

Symptoms of inoculated plants incubated in continuous light--Tomato plants in the cotyledon stage were mechanically inoculated, grown on the greenhouse bench under natural greenhouse light for 10 days to 2 weeks, and then moved to a growth chamber at 27°C with continuous light of approximately 1,000 ft-c. In a few days under continuous light, color of the young leaves gradually became deeper greenish and rugosity of the leaves was enhanced. Later differences in the growth rate between healthy and diseased plants became apparent. After approximately 10 days of continuous light, young leaves of diseased plants became off color and later chlorotic. New growth was at first chlorotic and it was white with light purple on the lower surface of the veins. Leaflets were small and rolled upward both crosswise and longitudinally.

Leaves already expanded at the time long day length incubation was started retained their normal green color and frequently developed veinal necrosis. No necrosis was observed in the white shoots of diseased plants.

Occasional albino axillary branches developed in continuous light from the lower green parts of plants with a white apex. Such albino plants or plant parts survived for approximately 3 months when maintained in continuous light conditions. Plants survived longer when moved away from the continuous light and kept under 12-16 hr day lengths so that new growth could develop green tissue.

When inoculated plants under continuous light were so trimmed that no new growth was allowed, petioles of cotyledons became extremely epinastic and inoculated cotyledons remained green.

Under certain conditions, not well understood, small non-inoculated tomato seedlings grown under continuous light of low intensity developed chlorosis often beginning at the center of the leaf while the margins remained green. These symptoms could be avoided by growing plants for 10-14 days on the greenhouse bench before incubation and exposure to continuous light.

Similarity of white symptoms on Rutgers tomato plants inoculated with 4 PSTV isolates--All 4 isolates of PSTV, i.e. Wisconsin, Schultz, Canadian, and Diener isolates, were compared for their ability to induce white symptoms on inoculated tomato plants. Tomato plants were mechanically inoculated at the cotyledon stage with the crude sap from PSTV-infected S. acaule plants. After inoculation, plants were kept in 12-hr day length for 10 days followed by incubation in continuous light for one month.

The average percentage of the white leaflets over the total leaflets of 15 plants each inoculated with Wisconsin, Schultz, Canadian, and Diener isolates of PSTV were, respectively, 81, 78, 74, and 79. The control

plants lacked white leaflets. Rutgers tomato plants inoculated with 4 different isolates of PSTV are shown with white albino shoots (Figure 3).

Figure 3.--Similarity of white symptoms of PSTV infection in Rutgers tomato plants induced by (left to right) Wisconsin, Schultz, Canadian, and Diener isolates of PSTV. (Extreme right) non-inoculated control. Plants were inoculated and grown in intermittent light for 10 days and then grown in continuous light for 30 days.

Influence of day length on symptom expression in PSTV-infected Rutgers tomato plants--This experiment was conducted in a walk-in growth chamber at 27°C with continuous light of 2,000 ft-c. Inoculated and control plants, growing in 4-in. pots were put in wooden frames with the sides covered with aluminum foil. The top of each frame was covered for 0, 6, 12, and 18 hours per day.

Plants exposed to 6 hr light period were slightly etiolated and the usual symptoms of rugosity did not develop within three weeks. Usual PSTV symptoms, rugosity etc., developed in approximately 2 weeks in inoculated plants held in 12 and 18 hrs of light per day. At these day lengths, there were no differences in symptom severity. In continuous light, green portions of inoculated plants were slightly gray. Both healthy and PSTV-infected leaves rolled upward slightly with some undetermined necrotic lesions on leaflets and stems. Under continuous light, upper portions of the inoculated plants later became white, characteristic of the albinism observed previously in PSTV-infected Rutgers tomato plants. No white symptoms were present in diseased plants held in shorter day-length regimes.

Influence of temperature on PSTV albinism in continuous light--Non-inoculated and PSTV-inoculated Rutgers tomato plants were grown in growth chamber at 30, 24, and 16°C with 24-hr illumination at approximately 1,000 ft-c. After 3 weeks, non-inoculated plants generally exhibited normal characteristics similar to those grown under natural light conditions on the greenhouse bench. At 30°C they had smaller, somewhat more chlorotic and thicker leaves than those grown in the 24°C chamber. At 16°C, leaves were dark, and purplish and growth was retarded.

No chlorotic or white tissue formed in continuous light at 16°C. New leaves and shoots of PSTV-inoculated plants at both 24 and 30°C were white. However, those at 30°C were more intensely white than those kept at 24°C which were slightly yellowish white. As in previous trials, no veinal necrosis was observed in the white leaves of the diseased plants incubated at any temperature. Veinal necrosis of inoculated plants was more severe in green leaves formed before exposure to continuous light at 24°C than at 30°C.

Influence of changing day length on symptom expression--When green, PSTV-infected tomato plants were transferred from 12-hr day light to continuous light, young parts of the plants became chlorotic and new growth forming later was white. This change first became evident in as short a time as five days. Plants with severe

rugosity but still green in color developed chlorotic albino new growth more rapidly than did the plants with mild symptoms. Healthy plants incubated under similar conditions remained green and growth was normal (Figure 4).

Conversely, when diseased plants with albino apical growth (those incubated under continuous light), were transferred to 12-hr day length, new young growth as it progressively developed was green. This new growth exhibited green color similar to that of diseased plants incubated under natural light with normal PSTV symptoms of green rugosity. The chlorophyll content of young leaves rapidly increased (Table 4). Frequently the lower white leaves became wilted and dried out after plants had been changed from continuous light to short-long day length. Similar results were obtained when plants were either transferred to the greenhouse bench, under natural light, or when they were covered for 12-hr per day in the growth chamber with continuous light. Leaf color of non-inoculated plants was not modified by exposure to continuous light (Figure 5). Expanded white leaves of PSTV inoculated plants which had developed under continuous light never became green later in 12 hr day length. PSTV-infected plants first incubated under 12-16 hr day length, then incubated in continuous light, and later returned to the intermittent light exhibited green leaves with typical PSTV-symptoms on the lower and upper parts of the plants with white symptoms in the middle portions (Figure 6).

Figure 4 A

Figure 4.--Albinism in PSTV-inoculated Rutgers tomato plants.

A) Right, plant with mild symptoms of PSTV infection (rugosity, rolling of the leaflets and stunting) following inoculation and incubation in 12 hr day length for 26 days. Left, non-inoculated control plant.

B) The same plants 20 days later after incubation under continuous light of 1,000 ft-c. The upper portion of the inoculated plant has become nearly white and remains stunted.

C) The same plants after 30 days under continuous light.

Figure 4 B

Figure 4 C

Table 4.--Chlorophyll content of young leaves of PSTV-infected plants during transition from white symptoms to green symptoms following change from continuous light to 12-hr day length.

Number of 12-hr days	Chlorophyll content (μg/ml)
0	50
2	443
4	2011
6	2346

Figure 5.--Symptom reversion from white to green leaves in PSTV-infected Rutgers tomato plants.

A) (Left) control and (right) white infected plants incubated under continuous light for over 30 days.

B) The same plants 30 days later after incubation under intermittent light. Some white leaves of the PSTV-inoculated plant (right) have dried out and the new growth exhibits green color with typical PSTV-symptoms.

Figure 5 A

Figure 5 B

Figure 6.--Symptom changes at different day lengths. Rutgers tomato inoculated with PSTV at intermittent day lengths for 30 days, continuous light for 25 days and intermittent light for 13 days. Central portion of the plant developing under continuous light is white.

Albinism in additional tomato varieties and other plants inoculated with PSTV--Several commercially available tomato varieties were inoculated with PSTV to determine varietal responses, particularly development of white symptoms, in continuous light. Seed of these tomato varieties were not all obtained at the same time, accordingly not all of the varieties were tested at the same time. However, the same growth chamber was used throughout with continuous light of approximately 1,000 ft-c and temperature of 27-30°C. Seedlings were transplanted, inoculated, and incubated as described previously. After 4 weeks of incubation, the number of plants showing white symptoms were recorded (Table 5).

All of the eight tomato varieties tested were susceptible to PSTV and all exhibited white symptoms under continuous light except that in Marglobe only 3 of 9 plants developed white symptoms. Among these varieties, Fantastic and Michigan-Ohio have been reported resistant to PSTV (Singh and O'Brien, 1970). My trials suggest no marked differences in resistance except for Marglobe. Resistance or escape of infection by PSTV in Marglobe was not explored further.

Development of the white symptoms seen on the PSTV-inoculated plants often followed usual symptoms such as rugosity etc. This however, was not always the case. Diseased plants occasionally developed white symptoms

Table 5.--Albinism of several tomato varieties inoculated with PSTV and incubated under continuous light.

Varieties tested	Plants tested		Plants with white symptoms	
	Control	PSTV-inoculated	Control	PSTV-inoculated
	No	No	No	No
Bonny Best	14	15	0	15
Michigan-Ohio	6	6	0	6
Fantastic	6	6	0	6
Beefsteak	3	9	0	9
Marglobe	3	9	0	3
Jubilee	3	9	0	9
Machine Harvest #1	3	9	0	9
Rutgers	9	9	0	9

without having first showed the usual rugosity symptoms. This was more often the case when older plants were inoculated or when a highly diluted virus preparation was used.

Attempts were made to determine if continuous light would induce white symptoms in certain other species of plant hosts. Inoculated and non-inoculated S. acaule plants were kept under continuous light. No white symptoms were obtained in 3 months. When S. acaule plants were top grafted to diseased Rutgers tomato plants and kept under continuous light conditions, the S. acaule scion often became chlorotic but no white symptoms were obtained (Figure 7). No white and only slight chlorotic symptoms were obtained when Onaway seedling potato plants were similarly treated.

Tests were also made to see if continuous light would stimulate symptom development of certain inoculated but otherwise symptomless host plants.

Datura tatula, D. metal, Solanum dulcamara, and Physalis floridana were inoculated with PSTV and incubated under continuous light conditions. No symptoms developed in any of the inoculated plants, while PSTV was consistantly recovered. In additional trials, no symptoms developed when these inoculated symptomless host plants were grafted to healthy tomato stocks and later incubated under continuous light.

Figure 7.--Right, chlorotic symptoms of S. acaule scion grafted onto PSTV-infected Rutgers tomato stock and incubated in continuous light. Note new side growth of infected tomato stock is white. Left, virus-free scion of S. acaule grafted on healthy tomato plant grew normally.

Virus infectivity in the green and white leaf tissue

Green and white leaflets were separately removed from infected tomato plants. One gram was ground in a 4-in. mortar with 9 ml of either distilled water or 0.05 M phosphate buffer at pH 7-8. This slurry which had been prepared at a 1 to 10 dilution was further diluted at 10 fold increments to provide 3 dilutions of 1-10, 1-100 and 1-1,000. These were then used for inoculating young Rutgers tomato plants. For each dilution, 30 plants, 3 per 4-in. pot, were mechanically inoculated. After inoculation, plants were kept on the greenhouse bench under natural light. Diseased plants were counted at intervals after inoculation.

Trial 1: In a virus-reisolation test, the shoots of one graft-inoculated Rutgers tomato plant incubated under continuous light became white. The shoot of another plant grafted with a different PSTV-infected scion remained green and showed the usual symptoms. Virus infectivity of the leaflets from these two shoots were compared.

Sap prepared from the white diseased leaflets was of higher infectivity at all dilutions than from green tissue as observed 20, 30, and 38 days after inoculation (Table 6).

Table 6.--Rutgers tomato plants with PSTV symptoms after inoculation with crude sap from green and white leaf tissue from two individual PSTV-infected tomato plants.

Inoculum and concentration	Plants with symptoms at intervals (days) after inoculation					
	20		30		38	
	no.[1/]	%	no.[1/]	%	no.[1/]	%
Control	0/30	0	0/30	0	0/30	0
Green 10^{-1}	5/30	17	10/30	33	19/30	63
Green 10^{-2}	1/30	3	6/30	20	13/30	43
Green 10^{-3}	0/30	0	0/30	0	3/30	10
White 10^{-1}	10/30	33	19/30	63	28/30	93
White 10^{-2}	6/30	20	13/30	43	20/30	67
White 10^{-3}	5/30	17	12/30	40	21/30	70

[1/] Number of plants with symptoms over the number of plants inoculated.

Trial 2: Two inoculated Rutgers tomato plants of comparable size and showing similar degrees of typical symptoms (green leaves, rugosity) were approach-grafted at their lower stems, and transplanted into a 6-in. clay pot. They were kept in a growth chamber with 24-hr illumination at approximate 1,000 ft-c. One of the shoots was covered with aluminum foil for 12 hrs each day and the other was exposed to the light for 24 hours. After 4 weeks of incubation, the shoot which has been covered for 12 hrs each day retained the usual diseased appearance, while new growth on the shoot which had been exposed for 24 hrs light became white. Virus infectivity of the leaflets from the white and the green shoots were compared as before in phosphate buffer solution.

The results (Table 7) were essentially similar to those previously obtained. Infectivity of sap from white leaves were higher than that of sap from green leaves at each corresponding dilution. However, differences between infectivities from green and white tissue were not as great as in the first trial.

Inoculum from green leaves was more infective at 10^{-2} concentration than at 10^{-1} as examined 20 days after inoculation. The reason for this is not apparent.

Table 7.--Rutgers tomato plants with PSTV symptoms after inoculation with crude sap of leaf tissue from the green or white shoots of grafted, diseased tomato plants.

Inoculum and concentration	Plants with symptoms at intervals (days) after inoculation			
	20		30	
	no.[1/]	%	no.[1/]	%
Control	0/30	0	0/30	0
Green 10^{-1}	4/30	13	28/30	93
Green 10^{-2}	7/30	23	26/30	89
Green 10^{-3}	6/30	20	22/30	73
White 10^{-1}	21/30	70	30/30	100
White 10^{-2}	14/30	47	29/30	97
White 10^{-3}	7/30	23	28/30	93

1/ Number of plants with symptoms over the number of plants inoculated.

Trial 3: Portions of a group of PSTV-infected Rutgers tomato plants incubated under continuous light and exhibiting white symptoms were covered for 12 hrs at night. Approximately 2 weeks later, new growth of upper leaves of these plants was green. The green leaflets were randomly removed from these covered plants and their virus infectivity was compared with those of the white leaflets from similar but uncovered plants. The original sap was diluted serially to 1-1,000 with phosphate buffer. Results obtained (Table 8) were similar to those of trials 1 and 2.

Table 8.--Rutgers tomato plants with PSTV symptoms after inoculation with crude sap of leaf tissue from the white shoots and green shoots of PSTV-infected tomato plants.

Inoculum and concentration	Plants with symptoms at intervals (days) after inoculation					
	15		25		35	
	no. 1/	%	no. 2/	%	no. 3/	%
Control	0/30	0	0/30	0	0/30	0
Green 10^{-1}	3/30	10	18/30	60	18/30	60
Green 10^{-2}	2/30	7	8/30	27	8/30	27
Green 10^{-3}	0/30	0	3/30	10	5/30	17
White 10^{-1}	16/30	53	25/30	83	29/30	97
White 10^{-2}	10/30	33	22/30	73	27/30	90
White 10^{-3}	7/30	23	16/30	53	22/30	73

1/ Number of plants with symptoms over the number of plants inoculated.

Trial 4: Green and white leaflets of comparable size were randomly collected from the upper portions of a group of 30 PSTV-infected tomato plants incubated in a growth chamber with continuous light. The leaflets were collected as 3 samples of green and 3 samples of white leaves. Sap of each green and white pair was prepared and infectivity tested at 1-1,000 dilution in water on cotyledons of 30 Rutgers tomato plants. The percentage of infected tomato plants was consistently higher in groups of plants inoculated with the sap prepared from white leaflets than sap from the green leaflets (Table 9).

Table 9.--Rutgers tomato plants with symptoms after inoculation with 1-1,000 dilution of leaf-sap prepared from green or white leaflets of PSTV-infected tomato plants.

Inoculum	Plants infected									
	30 days after inoculation					40 days after inoculation				
	Sample			Total		Sample			Total	
	1	2	3			1	2	3		
	no. 1/	no. 1/	no. 1/	no. 1/	%	no. 1/	no. 1/	no. 1/	no. 1/	%
Control	0/30	0/30	0/30	0/90	0	0/30	0/30	0/30	0/90	0
Green	0/30	1/30	4/30	5/90	6	1/30	4/30	8/30	13/90	14
White	4/30	22/30	5/30	31/90	34	8/30	25/30	10/30	43/90	48

1/ Number of plants with symptoms over the number of plants inoculated.

Cytological and histological studies

Attempts were made to obtain information on cytological and/or histological differences between healthy and PSTV-infected plants and possibly to use such differences for detecting early stages of virus infection. Potato, the plant from which this virus was first discovered; tomato, the first reported susceptible non-potato plant; Solanum acaule, another host plant responding to infection by definite symptoms as described earlier in this thesis; and Datura tatula, a symptomless host plant of PSTV were studied extensively.

Solanum tuberosum L.--In the first trial, two vigorously growing leaves of young Onaway potato seedlings (i.e. plants grown from selfed seed of the Onaway variety), approximately 10 cm high, were mechanically inoculated with PSTV-infected tomato sap and the upper portion above the inoculated leaves was removed. Plants were then grown in a growth chamber at 24°C and 14-hr day length at 2,000 ft-c light intensity. New growth of the plants was kept trimmed off for approximately the first 30 days and was later allowed to grow. At this stage, new growth of all inoculated plants developed severe symptoms of PSTV infection. Symptoms included dwarfing and chlorosis, upward rolling of leaves, and necrotic flecks which developed within petioles and stems. The angle between petioles and stems became acute. Some stems of diseased plants were killed. No such symptoms were present in control plants which grew normally after new growth was permitted.

Necrotic flecks were visible within petioles and stems. Sections of petioles and stems were examined microscopically. These necrotic areas were situated in the cortical parenchyma tissue of the veins, petioles and the stems and also in the pith parenchyma of the stems. A few starch grains were present in the necrotic cells. These grains were larger than normal ones and stained intensely dark blue to black with iodine. No such starch grains were evident in non-necrotic portions of the same

stem. Periderm formed in the cells adjacent to necrotic areas (Figure 8). No large starch grains were present in healthy tissues.

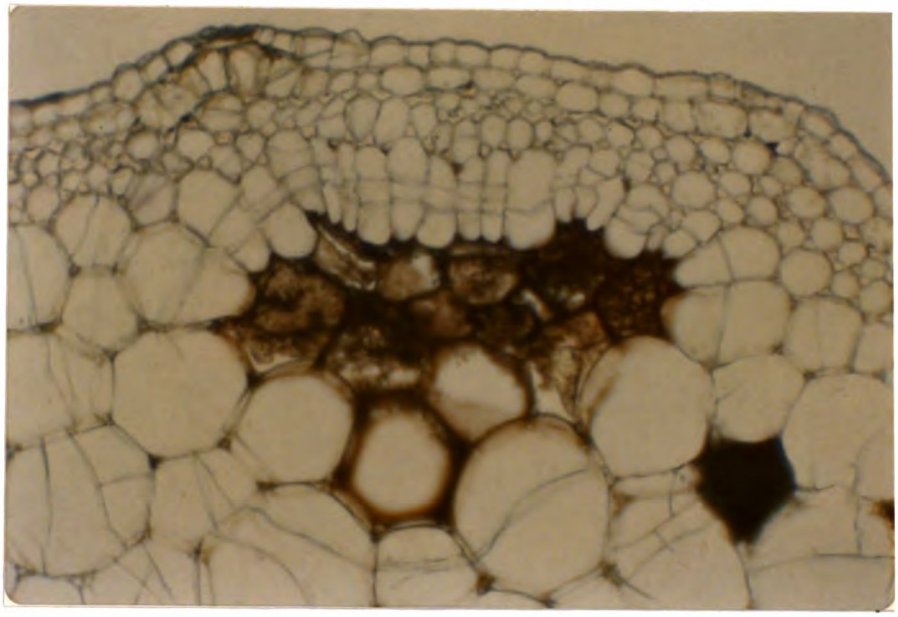
In another trial, young Onaway seedlings were grown for a month to approximately 10 cm high. Four leaves in each plant were mechanically inoculated and plants were kept in a growth chamber at 24°C and 14-hr day length at 1,000 ft-c. At intervals after inoculation, petioles of PSTV-inoculated and non-inoculated leaves were sectioned and examined microscopically. Internal parenchyma necrosis was not present in petioles 7 days after inoculation, but was evident 15 days after inoculation. In another trial, when 4 petioles were examined, 3 were found necrotic 10 days after inoculation. No internal necrosis was observed in the petioles of non-inoculated PSTV free plants.

Plants were grown from diseased tubers harvested from PSTV-inoculated Onaway seedlings. They were kept in a growth chamber at 24°C and 14-hr day length at 1,000 ft-c. Of 10 diseased tubers, 2 gave rise to stunted plants. A few upper leaves appeared chlorotic and showed upward rolling on the basal part of the leaflets. Necrotic flecks were visible from the lower abaxial surface of the petioles (Figure 9) and in the stems. These symptoms were not observed on other 8 plants grown from

Figure 8.--Necrosis within Onaway potato seedling 30 days after inoculation with the Canadian isolate of PSTV.

Top) Cross section of a petiole with internal parenchyma necrosis. Note the early stage of periderm formation in cells adjacent to the necrotic area.

Bottom) Stem cross section showing well developed periderm and necrosis in parenchyma cells of pith.



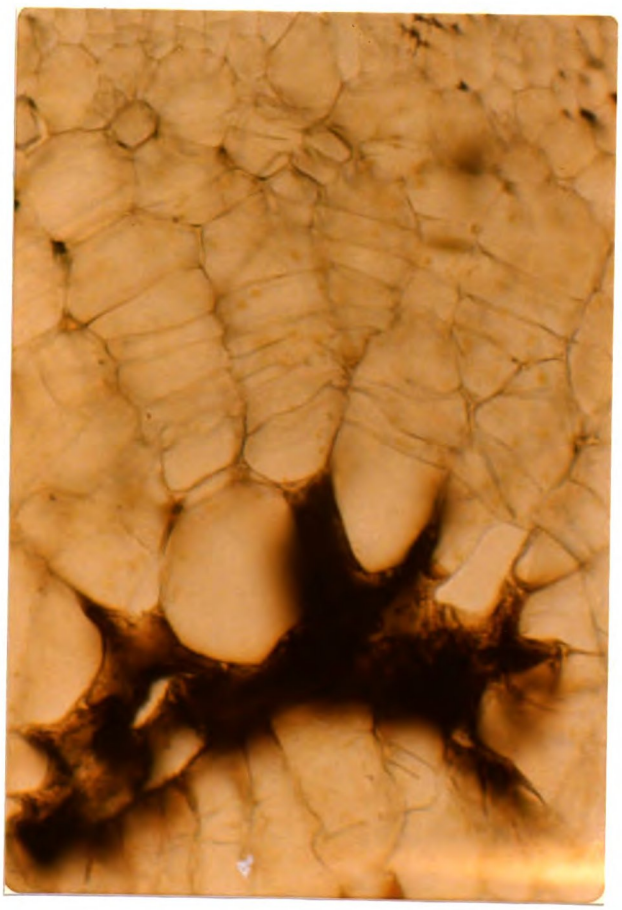


Figure 8

PSTV infected tubers. The upper portions of these 8 plants as well as 7 control plants grown from healthy tubers, were trimmed approximately one month after tubers were planted. After further growth, necrotic flecks were present in the newly developed petioles and in the stems of 4 out of 8 plants previously not showing such necrotic flecks. No necrotic flecks were visible from the petioles or stems of the other 4 inoculated plants grown from the diseased tubers. PSTV was present, however, as shown by grafting these plants onto Rutgers tomato plants. No necrosis was present in healthy controls.

Necrotic flecks were also present in PSTV-inoculated PVX-free Russet Burbank plants grown from tubers. Six plants were mechanically inoculated with PSTV and other 6 plants served as control. Approximately 2 months after inoculation, the petioles were examined. Necrotic flecks were present in 4 out of 6 inoculated plants, while no necrotic flecks were observed in the non-inoculated control plants.

Figure 9.--Necrotic flecks within the cortex of a petiole of PSTV-inoculated Onaway potato seedling.

Lycopersicon esculentum Mill. cv. Rutgers--Epidermal and cross sections of diseased leaves were examined with phase contrast microscopy of living cells and with acridine orange fluorescence of fixed tissue. At the early observations, particular attention was given to nuclei and the area in the cell surrounding the nuclei. Also cyclosis of cytoplasmic streaming was compared to that of the controls.

No consistent differences were identified except for plastids in epidermal cells of unstained diseased white leaves, diseased green leaves, and healthy leaves. Plastids often adhered to the nuclei in both healthy and diseased green cells. Sometimes, ellipsoid swollen portions of streaming cytoplasmic masses were observed in both healthy and diseased cells. In some PSTV-infected cells, a relatively large, actively moving cytoplasmic mass was present near the nuclei.

In acridine orange stained epidermal slices, differentiation of the DNA of nuclei, which fluoresced green, and RNA of nucleoli, which fluoresced red, was distinct. Cytoplasmic strands were not preserved by ethyl alcohol fixation in healthy or diseased tissues. No virus inclusion like materials were observed in diseased cells. In some relatively thick epidermal sections, small portions of the palisade cells stained intensely red. Although these were suspected of being inclusion bodies, evidence was inconclusive.

Cross sections of diseased white leaves were distinctly different from sections of green healthy leaves or green diseased leaves in the appearance of the plastids, particularly the chloroplasts. In healthy cells, the chloroplasts were larger, smooth surfaced and more turgid than in diseased white leaves and plastids in healthy cells were evenly distributed around the periphery. In cells of diseased white leaves, individual, very light green plastids were decidedly shrunken and were not evenly distributed around the cells. They frequently aggregated at the upper and lower ends of the palisade cells. In the spongy mesophyll cells, plastids were in groups but these groups distributed more or less at random.

Differences between diseased white leaves and healthy green leaves as described in the previous paragraph were also shown well by hematoxylin staining. Diseased green leaves and healthy leaves were essentially similar. When etiolated healthy leaves were cytologically compared with the diseased white leaves, differences were not evident.

Necrosis was frequently evident in diseased tomato plants. Necrotic lesions were frequent in parenchyma cells of veins, pith, and cortex essentially similar to that in potato. Necrosis originated in or near the cell

wall possibly within the middle lamella. Cell walls became discolored sooner than the other parts of the cells in necrotic areas. No virus inclusion body like materials, when stained by acridine orange, were present in cells in or surrounding necrotic lesions. When stained with iodine solution, only a few plastids stained blue indicating the presence of starch. In healthy cells, many blue stained plastids were present.

Solanum acaule Bitt.--Infected S. acaule plants were similar histologically to tomato and potato as described in the previous section except that veinal necrosis and necrosis in parenchyma of cortex and pith was more severe. Cross sections of the healthy leaves stained well with acridine orange. In these leaves, two layers of palisade cells were uniformly arranged on top of each other. Diseased green leaves and the healthy leaves were essentially similar except that in severely chlorotic diseased leaves palisade cells appeared to be somewhat disorganized.

Intensive internal necrosis was observed in veins, petioles and stems, particularly in parenchyma cells of diseased S. acaule (Figure 10). Attempts were made to stain and detect the early stage of necrotic activity in veins and petioles.

Virus inclusion-like materials were neither observed by examination of unstained material with phase contrast microscopy nor by AO staining with UV microscopy.

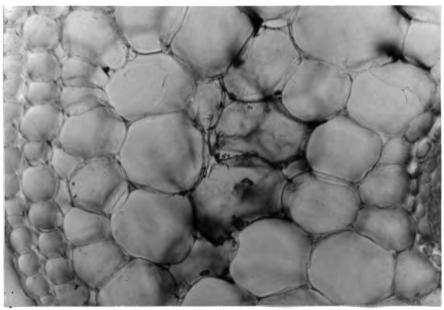


Figure 10.--Necrosis within S. acaule, PI 230-554, seedling one month after inoculation with the Wisconsin isolate of PSTV and grown under continuous light. The picture shows cross section of a petiole with necrosis in parenchyma cells.

Datura tatula L.--Inclusion-like materials (ILM) were inconsistently observed in upper epidermal cells of PSTV-infected cotyledons of D. tatula plants stained with acridine orange. A dense and actively moving mass of cytoplasm was also observed by phase contrast microscopy in unstained epidermis sectioned from the same material. No such structures were observed in epidermal cells of the non-inoculated but systemically invaded true leaves of the same plant.

The earliest ILM were observed 7 days after mechanical inoculation. Forty days after inoculation, ILM were still evident. However, they were most easily demonstrated approximately 30 days after inoculation.

The ILM were at first diffused and irregular in shap. Later they became more concentrated and solid in texture as viewed after AO staining under fluorescent microscopy. More often they were located at one side of the nucleus, but were not consistently situated close to the nucleolus. In some cases, nuclei were surrounded by the ILM. They stained brilliant reddish orange color, and often were more intensely stained than the nucleoli

When epidermal sections of healthy and PSTV-infected cotyledons were stained with iodine solution, there were no differences in shape and abundance of blue staining plastids. In cross sections of leaves, or in the stem pith, no necrosis was observed in any PSTV-infected D. tatula plants.

Epidermal sections of inoculated D. tatula plants were treated with pancreatic ribonuclease (RNAse) to determine the influence of the enzyme on the ILM. For this, 50 µg/ml RNAse solution was prepared by disolving RNAse (Worthington Biochemical Corp., Freehold, New Jersey) in 0.2 M phosphate buffer at pH 6.3. Epidermal sections were fixed in 50% ETOH for 20 min and rinsed with buffer solution for a few minutes. The sections were then immersed in enzyme solution for 1 hr at room temperature. After treating with enzyme solution, sections were rinsed with buffer solution again and stained with the regular AO staining procedure.

In RNAse treated sections, nucleoli were no longer stained reddish orange, only a faint red trace was seen under UV microscopy. Nuclei were stained yellowish green as were those in the untreated sections. ILM could not be found in RNAse treated sections, suggesting that the ILM contained at least some RNA.

In spite of these encouraging observations, the above responses were not sufficiently consistent to justify unqualified claims that these inclusions were, in fact, those of PSTV.

DISCUSSION

Potato spindle tuber virus was first shown to be capable of inducing diagnostic symptoms in Rutgers tomato plants in 1962 (Raymer and O'Brien, 1962). This was approximately 40 years after the disease was first reported on potato. Solanum acaule Bitt. PI 230-554, as reported here, produced severe symptoms under continuous light following inoculation with the crude sap from PSTV-infected plants. This is an additional host plant to the others already reported which produce distinct diagnostic symptoms of PSTV. This collection number of S. acaule was known to be immune to potato virus X. It is an ideal host for the study of PSTV since the seedling is readily obtained from true seed and it is free from the almost universally existing PVX and other tuber borne potato viruses. Among the host plants of PSTV known to exhibit definite responses of infection, selection PI 230-554 of S. acaule responds by distinct symptoms which include rosetting and intensive internal necrosis.

PSTV is generally disseminated by diseased potato seed tubers, and transmission within the field is mainly through foliage contact, chewing insects, and by machinery. Solanum dulcamara seedlings readily became infected with PSTV after inoculation when kept under greenhouse natural

light or kept out of doors under natural conditions. Perennial hosts serving as overwinter virus reservoirs and carrying the virus overwinter have not been identified in nature in Michigan. Inoculated S. dulcamara plants produced no visible symptoms under these incubation conditions or when the inoculated plants were incubated under continuous light.

In natural winter conditions of Michigan, PSTV survived and successfully overwintered out of doors in infected S. dulcamara plants. S. dulcamara plants grown from the seed harvested from PSTV-infected plants carried the virus.

S. dulcamara is a common perennial weed growing throughout Michigan and its neighbouring states. The plant is highly susceptible to PSTV. It is capable of carrying the virus overwinter under frozen conditions, since PSTV is seed transmitted and also pollen transmitted in S. dulcamara (Nimnoi, unpublished data); it is highly possible that naturally infected perennial weeds such as S. dulcamara exist. That naturally infected plants are actually carrying the virus and serve as primary inoculum has not yet been established.

Because of the perennial nature of S. dulcamara, the plant can be kept vegetative in the greenhouse for a long period of time. Young shoots and leaves are present

at any time of the year in the greenhouse. Because of this characteristic, the plant may be very useful for maintaining PSTV.

Although the albino white symptoms on PSTV-infected tomato developed more frequently after the plants had previously shown typical PSTV symptoms (rugosity of leaves and epinasty of the petioles), the albino white symptoms were observed in certain infected plants that had not previously shown typical symptoms. Mild symptoms may be overlooked, but the possibility is low of overlooking albino, white symptoms. Rutgers tomato is commonly used for indexing PSTV. Both satisfactory and unsatisfactory results have been reported with Rutgers tomato for PSTV-indexing (Fernow et al., 1969; Singh and Bagnall, 1968). Indistinct symptoms on Rutgers tomato plants following inoculation with PSTV were often obtained during October to December in Canada (Singh and Bagnall, 1968). Short day lengths during winter months was considered the influential factor for the unsatisfactory results. In this research, PSTV-inoculated Rutgers tomato plants developed distinct, typical symptoms under 12- and 16-hr day lengths. Inoculated plants at 6-hr day length developed very indistinct symptoms. Albinism developed under continuous light. These observations present additional evidence that long day lengths favor development of PSTV-symptoms. Albino white symptoms of PSTV-inoculated Rutgers

tomato plants, and certain other tomato varieties, grown under continuous light as described in this thesis is an unusual and possibly important diagnostic symptom of PSTV infection.

In Lee and Singh's work (1972), when PSTV-infected Rutgers tomato plants were grown in nutritional conditions so that the Mn content of plant tissue was 1,500 µg/g fresh tissue or above and when the Mn/Fe ratio was 12-1 or higher, veinal necrotic symptoms together with whitening symptoms (as shown in their photographs) were enhanced. The albino white symptoms which developed in PSTV-infected Rutgers tomato plants under continuous light as reported herein lacked the veinal necrosis. In this research, PSTV-inoculated Rutgers tomato plants when moved from 12-hr day length to continuous light developed veinal necrosis on green leaves only.

This research shows that internal necrosis of the parenchyma cells of cortex and pith is produced in PSTV-infected potato plants (grown either from true seed or those grown from tubers), in Rutgers tomato plants, and in Solanum acaule (PI 230-554). Among the plants tested, internal necrosis was most severe in S. acaule.

Information on cytological modifications of virus infected plants is important and useful at least for diagnosis of certain diseases. It was assumed that there

might be certain kinds of cytological modifications detectable by the light microscope since this virus is highly contagious and induced distinct external morphological modifications of infected plants. An intense effort was made to identify inclusion bodies in the infected cells. Acridine orange fluorescent staining was used for its sensitivity in staining RNA and DNA and other viruses (Hooker and Summanwar, 1963; Kauffman, 1967). It was also used as a main staining method in this research. Although no conclusive information was obtained, some observations indicated that cytological modifications were induced by PSTV infections. However, additional work is necessary before this can be established with certainty.

SUMMARY

Solanum acaule Bitt., PI 230-554, developed systemic chlorosis, rosetting and leaf lesions approximately 3 to 6 weeks following mechanical inoculation with PSTV and incubation in continuous light of approximate 1,000 ft-c. New growth became rosetted later. Severe internal necrosis developed in parenchyma cells of petioles and stems. *S. acaule* which is immune to PVX remained PVX-free following mechanical inoculation with sap, or grafting on PVX-infected *Datura tatula* or tomato plants. *S. acaule* served as a good differential host for isolating PSTV alone from plants doubly infected with PVX and PSTV.

Young *Solanum dulcamara* L. plants were readily infected with PSTV by artificial inoculation. Although no symptoms developed, the virus was consistently recovered from all inoculated plants. PSTV survived in infected plants overwintering outside in Michigan. All tested second year new growth of these plants carried PSTV. Some young seedlings grown from seeds obtained from diseased plants were demonstrated to carry virus.

Distinct PSTV symptoms developed on the inoculated Rutgers tomato plants incubated at 12 and 16-hr day lengths. Symptoms developed slower and much less distinctly on plants

incubated at 6-hr day lengths. When a young PSTV-inoculated tomato plant was kept under either natural light or 12-hr day artificial light for 10 days to 2 weeks and then incubated in the continuous light of approximate 1,000 ft-c, new growth of infected plants became albino white in approximately one month. When the diseased albino plants grown in continuous light were returned to the 12-hr day length, the young growth as it formed progressively became more green until it exhibited typical PSTV-symptoms. Four PSTV isolates used in this research induced similar degrees of albino white shoots on inoculated Rutgers tomato plants in continuous light. Other tomato varieties tested also developed albino white symptoms when inoculated with PSTV and kept in continuous light.

Virus infectivities of extracts from white portions were higher than those from green portions of the same plant.

Severe internal necrosis developed in parenchyma tissue of systemically invaded petioles and the stems of PSTV-inoculated S. acaule. Localized internal necrotic areas were examined in PSTV-inoculated potato and tomato plants. No virus inclusion-like materials were consistently present in infected tomato, potato, or S. acaule plants.

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