MITOSIS IN POTATO SPINDLE TUBER VIROID INFECTED TOMATO

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ABSTRACT

MITOSIS IN POTATO SPINDLE TUBER VIROID

INFECTED TOMATO

By

Ming Yu

Cytological effects of potato spindle tuber viroid infection were studied on shoot and root tips in Rutgers tomato. Mitotic figures and mitotic indices were examined in temporary slides using the acetocarmine smear technique.

Rutgers tomatoes infected by PSTV were severely stunted and had very poor root systems. Mitotic indices and numbers of cells at prophase from both root and shoot tips were similar in controls and infected plants. Numbers of cells in anaphase were significantly higher in healthy than in diseased root tips 14 and 47 days after inoculation. Numbers of cells in metaphase were significantly higher in healthy than in diseased shoot tips.

Frequency of cells from infected plants with abnormal mitosis was very low. In a relatively small number of infected cells, 26 chromosomes were observed instead of the normal 24. Cells with numerous short chromosomes or chromosome fragments were observed in root tips from infected plants.

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To

My Parents

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INTRODUCTION

The potato spindle tuber viroid disease (PSTV) causes heavy losses of the potato crop. Yield reduction is due in part to fewer tubers per hill and in part to reduction in size of tubers. The reduction in tuber size and the spindly shape lowers the market grade of many tubers. On tomatoes, diseased plants produced small fruits with few seeds or no seeds at all.

PSTV as well as chrysanthemum stunt and citrus exocortis are caused by low-molecular-weight RNAs. These infectious RNAs without protein coats were designated by Diener as viroids and they may be either related or identical. The best known diseases caused by the potato spindle tuber viroid are spindle tuber of potato and bunchy top of tomato.

Mechanical transmission and transmission through seed tubers are mainly responsible for the spread of PSTV in nature. PSTV is transmitted either through the pollen or the ovules of infected potato or tomato plants. Nimnoi (1974) reported effects of the infectious agent on pollen germination, germ tube length, and cytology of pollen-mother-cells.

Cytological investigations have not been made on meristems of PSTV infected plants. Nimnoi observed aberrant meiosis in pollen-mother-cells of PSTV infected tomato. Relatively few cytological studies of virus infection have been made with other viruses.

Since PSTV in tomato causes stunting of apical growth, leaf distortion and reduced root growth and since the viroid is known to be mainly associated with the nuclei (Diener 1971a), investigations were made in the possibility that mitotic aberrations might be involved in abnormal plant growth.

LITERATURE REVIEW

The potato spindle tuber disease was first described by Schultz and Folsom and Fernow in 1923. The disease is largely symptomless or produces very mild symptoms in potato until the infectious agent had been propagated for a number of tuber generations. The vine symptoms are characterized by spindliness and uprightness, and often by a darker green color and slight rugosity. Tubers are abnormally spindle-shaped, cylindrical, and supplied with conspicuous eyes, these symptoms varying somewhat with the variety. It was at first considered to be a disease closely related to mosaic, leafroll, and other similar 'degeneration' diseases of potato. The disease was then believed to be of virus etiology since the symptoms and natural means of transmission were basically similar to those of many virus diseases of plants. During the next 40 years, transmission of PSTV by insects and by mechanical means was investigated further (Schultz and Folsom 1925, Goss 1926, Bonde 1927, Bonde and Merriam 1951, and Manzer and Merriam 1961). No host other than potato was reported in these studies.

McClean first described the bunchy top disease of tomato in South Africa in 1931. Because of marked similarities in host range, symptoms, thermal inactivation point *in vitro* (75-80° C), and transmission, tomato bunchy top virus and PSTV were considered to be identical or strains of the same virus (Raymer and O'Brien 1962, O'Brien and Raymer 1964, Benson *et al.* 1965).

In 1962, Raymer and O'Brien reported tomato to be also susceptible to PSTV. Rutgers tomato produced marked systemic symptoms 2—5 weeks after mechanical or graft inoculation with the virus from potato. The symptoms included epinasty, rugosity, vein necrosis of the leaves and severe stunting.

Subsequently, the host range of PSTV has been extensively studied. The known hosts are mostly solanaceous species although susceptible plants in other genera have been identified (Easton and Merriam 1963; Singh and O'Brien 1970; Diener, Smith, and O'Brien 1972; O'Brien 1972; Singh 1973). A number of symptomless hosts were also reported by O'Brien and Raymer (1963, 1964), and Singh and Bagnall (1968). The citrus exocortis disease is caused by a related or similar viroid (Singh, Clark, and Weathers 1972; Semancik, Magnuson, and Weathers 1973).

Two groups or strains of PSTV are recognized with symptoms of tomato being used as criteria for classifying as mild or severe (Fernow 1967, Singh et al. 1970, Morris and Wright 1975). The severe strain causes a rosette appearance, severe epinasty and downward curling, and severely wrinkled leaves. This type was rarely found in field-grown potatoes. Symptoms with mild strains developed slowly and were likely to be missed. Infected plants usually showed slight epinasty and twisting of terminal leaflets and a general reduction in growth. Plants in the field were mostly infected with the mild strain (Fernow 1967). Yang (1974) enhanced PSTV symptom expression on tomato by growing plants under continuous light. Symptoms of albinism were obtained in addition to symptoms described above for shorter day length.

Diener and coworkers (Diener and Raymer 1967; Diener 1972, Diener and Smith 1973; and Sogo, Koller, and Diener 1973) reported the

infectious agent of the disease to be a free single-stranded RNA with molecular weight within the range of 7.5×10^4 to 8.5×10^4 daltons, an average length of about 500A, and some kind of hairpin-like structure which Diener named a 'viroid' (1971b). He proposed the term 'viroid disease' for the pathological condition incited by PSTV-RNA to distinguish diseases incited by typical viruses.

In tissue extracts infectivity was mainly associated with nuclei and purified chromatin. Fractions that contained soluble constituents, ribosomes, mitochondria, or chloroplasts were virtually noninfectious. These observations suggested that the infectious agent might be located within the nucleus (Diener 1971a). Takahashi and Diener (1975) identified an RNA-synthesizing system in nuclei purified from healthy and PSTV-infected tomato leaves and showed that PSTV could be replicated in the nuclei of infected cells. Diener (1974) suggested that a specific interference with host metabolic functions might be caused by PSTV. Viroids might, for example, act as abnormal transfer RNAs, with the result that faulty proteins are synthesized; or they might interfere with host genome transcription, either by repressing normally expressed cistrons, or by derepressing normally repressed cistrons. Alternatively, interference might be via a polypetide translated from the viroid RNA.

PSTV is transmitted either through the pollen or the ovules of infected potato or tomato plants. Benson and Singh (1964) reported that both fruits and seed from diseased female parents of tomato were smaller than normal. Germination of seed originating from diseased females was reduced 24 to 28%. Evidence of both ovule and pollen transmission was observed. Hunter et al. (1969) also reported that PSTV

could be transmitted via true seed of potato. Fernow et al. (1970) reported PSTV to be present in seed and pollen of diseased potato plants. They observed that transmission through the seed from open-pollinated female parents to the seedlings occurred frequently (average 31%) but varied in individual collections from zero to 100%. Singh (1970) confirmed that PSTV could be transmitted through seed in both tomato and potato.

The relationship between cytological abnormalities and infective agents was demonstrated by Nimoni (1974). She studied the effect of PSTV on pollen function, and found that pollen grains from healthy Rutgers tomato plants germinated with higher percentage and formed longer pollen tubes than did those from PSTV infected plants. Cytological studies of PSTV infected pollen-mother-cells were made with fresh smears and squash preparations stained with acetocarmine. Multipolar meiosis of chromosomes of PSTV infected pollen-mother-cells was observed. This is the first report of multipolar meiosis of pollen-mother-cells associated with virus infection. The chromosomes separated into groups. This led to formation of pollen grains with chromosome numbers less or more than the normal chromosome number of 12. Infection of tomato was obtained by inoculating plants with triturates of infected pollen grains.

Other plant viruses have been reported to cause meiotic abnormalities. Kostoff (1933) reported that mosaic virus caused meiotic aberrations in some Nicotiana species. The chromosome number changed from normal 24 to 23, 25, 26, and 27 during the second division. Lagging chromosomes were observed. Caldwell (1952) reported that

tomato aspermy virus caused a variable number of chromosomes in microspore-mother-cells, and none of these cells formed normal pollen grains. He also reported aggregation of the chromosomes at pachytene accompanied by precocious nucleolar disintegration in megaspore-mother-cell nuclei. Wilkinson (1953a) reported abnormal meiosis in Nicotiana glutinosa infected with tomato aspermy virus. The pachytene threads of spore mother-cells were collapsed, accompanied by abnormal multiplication of nucleolar bodies. Pairing failed in one and sometimes two or three bivalents, resulting in a proportion of chromosome-deficient gametes, with consequent production of misshapen pollen grains, together with microcytes. Swaminathan et al. (1959) reported that three varieties of Capsicum annuum L. infected with mosaic and leafroll viruses showed reduced chiasma frequency, and irregular anaphase separation.

Observation of mitosis in PSTV infected somatic cells has not yet been made, and studies of mitotic abnormality in other virus infected cells are limited. In Vicia faba infected with bean mosaic virus, Continho (1940) noted chromosome fragments and 'strange lateral bridges' in somatic metaphase and anaphase figures in root tips. Wilkinson (1953) reported widespread nucleolar, arrested metaphase, collapse at metaphase, spindle malformation, and breakdown, and a tendency to form giant nuclei in somatic divisions of tomato and tobacco plants infected with aspermy virus. In root tips of tomato heavily infected with the aspermy virus, the nucleolar material instead of dispersing during prophase, persisted through anaphase in the form of one or more prominent and somewhat elongated vesicles. He also

observed distorted spindles and metaphase collapse. Similar results were also obtained in tobacco species infected with the same virus. He suggested that competition might exist for RNA between the virus and the chromonematal material of the chromosomes. Wilkinson (1960) observed mitotic abnormalities in a range of solanaceous species infected respectively with tobacco mosaic virus and aspermy virus. The first indications of abnormality appeared at prometaphase. After this stage in healthy cells the fully contracted chromatids converge towards the equatorial plane of the spindle accompanied simultaneously by dispersal of the nucleolar material. Of 200 mitoses observed in diseased cells, 5 - 15% exhibited a persistence at least as far as the commencement of anaphase. The nucleolus did not retain its spherical form characteristic of early prophase, but presented a lobed and distorted appearance. This often extended as a finger-like vesicle across the equatorial plane of the spindle. At anaphase, 'thin' chromosomes and chromatin bridges occurred as abnormalities. Sometimes spindle breakdown resulted in either a mere scattering of the chromosomes, or else failure of the disjoining chromosome groups to separate, with the consequent production of giant nuclei. Petunia violacea infected with aspermy virus gave the most remarkable abnormality apparently undergoing amitosis with many elongated and constricted nuclei.

Severe symptom expression in tomato infected with PSTV suggested possibly also that mitotic aberrations could be anticipated. For this reason, meristematic tissues of tomato following PSTV-infection were examined for mitotic figures.

MATERIALS AND METHODS

A severe isolate of potato spindle tuber viroid (PSTV) was used throughout the study. This isolate was obtained from Miss M. J. O'Brien (Potato Investigation Laboratory, USDA, Beltsville, Maryland) as #48 of E. S. Schultz collection. Seeds of tomato (Lycopersicum esculentum Mill cv. Rutgers) were germinated in greenhouse potting soil composed of equal parts of perlite and peat. After about 10-14 days, seedlings were transplanted into 4-inch pots. Small plants were inoculated in the 2-4 leaf stage. Inoculum was obtained from infected plants with distinct symptoms by grinding leaves in a sterilized mortar and pestle. A small amount of water was added to the triturate. The upper surfaces of leaves of plants to be inoculated were dusted with carborumdum, and gently rubbed with a glass spatula dipped into inoculum. A few layers of paper towel were used to support the leaves during inoculation. After a few seconds, residual inoculum was rinsed away using water. Plants were kept in the greenhouse under natural day-night length or continuous light. For cytological studies, root tips and shoot tips were collected at different times from both control and diseased plants during the following three months. Shoot tips were excised and immediately put into Farmer's fixing solution, glacial acetic acid (1 part) : absolute ethanol (3 parts) (Sass 1961). Root tips approximately 1 cm in length were excised using forceps, rinsed in the water, and fixed in Farmer's solution. They were stored in corked small

glass bottles. Stem and root tips were held in Farmer's fixing solution at room temperature overnight, and then hydrolyzed by 1N HCl at 60°C for 5-7 minutes. Some specimens were treated with 0.01% colchicine (O'Mara 1939) before fixing in Farmer's solution in order to synchronize cell division and shorten the chromosomes. Only the apical 1 mm portion of root tip was put on a slide with a small drop of acetocarmine stain (Darlington and LaCour 1969). A coverslip was placed over the root tip, the slide was heated on an alcohol lamp for a few seconds, and then firmly pressed to squash the root and the edges sealed with wax. A similar treatment was applied to shoot tips after dissecting out the apex of the shoot tip under a microscope. Slides were examined under phase contrast illumination. The mitotic index was defined as percentage of cells with mitotic figures $\left(\frac{\text{cells in mitosis}}{\text{total number of cells}} \times 100\right)$. Cell counts were made from three different random fields per slide and the data were combined and expressed as one number. Photographs of mitotic figures were made with Kodak Panatomic-X (FX 135-36) film and printed on Rapidoprint papers (a Fotorite Product. Agfa-Gevaert).

RESULTS

The Schultz isolate of PSTV caused severe systemic symptoms on Rutgers tomato similar to those described by Raymer and O'Brien (1962). Plants inoculated in the 2-4 leaf stage developed rugosity, epinasty, slight downward rolling of leaves, and severe stunting within 2-4 weeks after inoculation. As infected plants grew older, vein necrosis of leaves developed, and leaves appeared lighter greenish in color as compared with healthy ones. The entire plant developed a bunchy-top appearance.

Roots of PSTV infected tomato were reduced in total volume and showed considerable stunting. The primary root was very stunted, and there were many small white secondary roots, which were coarse, stubby, and with a very light brown color. Poor root systems were previously reported by Yang (1974).

Root tips from healthy seedlings grown under natural daylight conditions on the greenhouse bench were used to determine proper sampling time of mitotic division. Every two hours for 24 hours, root tips were excised, fixed, hydrolyzed, stained, and quickly screened under the microscope. Cell divisions were found most frequently between 10:00 p.m. and 2:00 a.m. Collections were made between 10:00 p.m. and 1:00 a.m. throughout the trials reported herein.

Normal mitosis in root tips of tomato plants is similar to that in other higher plants with the normal (2N) somatic chromosome number

being 24. During prophase, chromosomes become thickened and shortened, and the nuclear membrane disappears (Figure 1). At metaphase (Figures 2 and 3) the chromosomes become oriented at the equatorial plate. Anaphase (Figure 4) begins when the centromeres divide and start to move toward opposite poles. When movement ceases, cytokinesis occurs giving rise to two daughter cells.

The root meristems from PSTV infected plants in the prophase (Figure 5), metaphase (Figure 6), anaphase and prophase (Figure 7) stages were similar to those of the healthy controls. Only in a few cases, 26 chromosomes (Figure 8) were counted instead of the normal 24. Cells with chromosome fragments (Figure 9) which were not observed in the noninoculated controls were present in root tip cells of infected plants 26 days after inoculation. This phenomenon was found towards the end of this study. Proportionally, frequency of cells with abnormal mitotic figures was very low.

Figure 1. Prophase in a healthy plant showing 24 chromosomes. X 1,250.

Figure 2. Metaphase in a healthy plant (polar view). X 2,000.

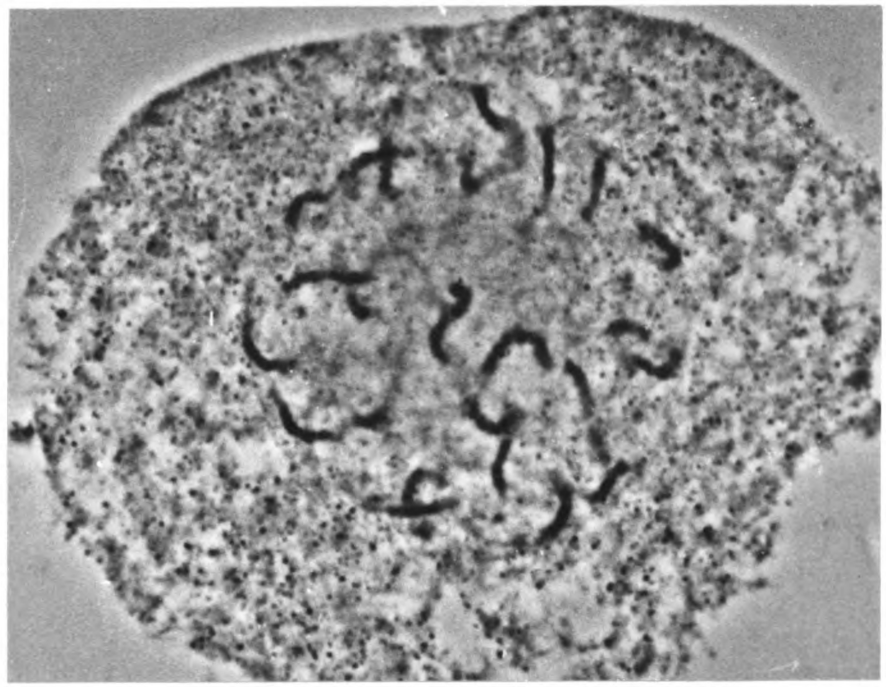

Figure 1

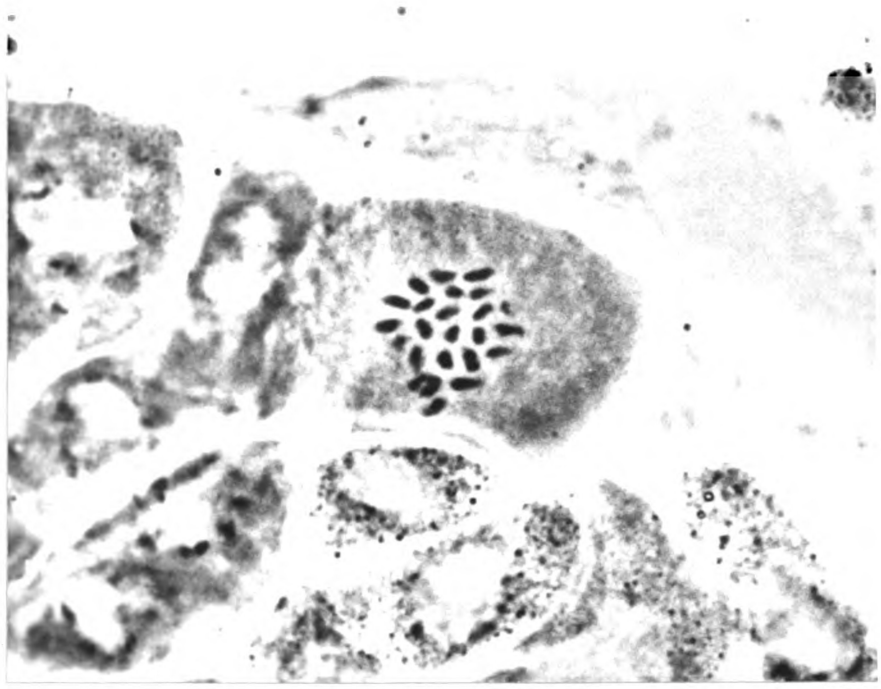

Figure 2

Figure 3. Metaphase in a healthy plant (lateral view). X 2,000.

Figure 4. Anaphase in a healthy plant. X 2,000.

Figure 3

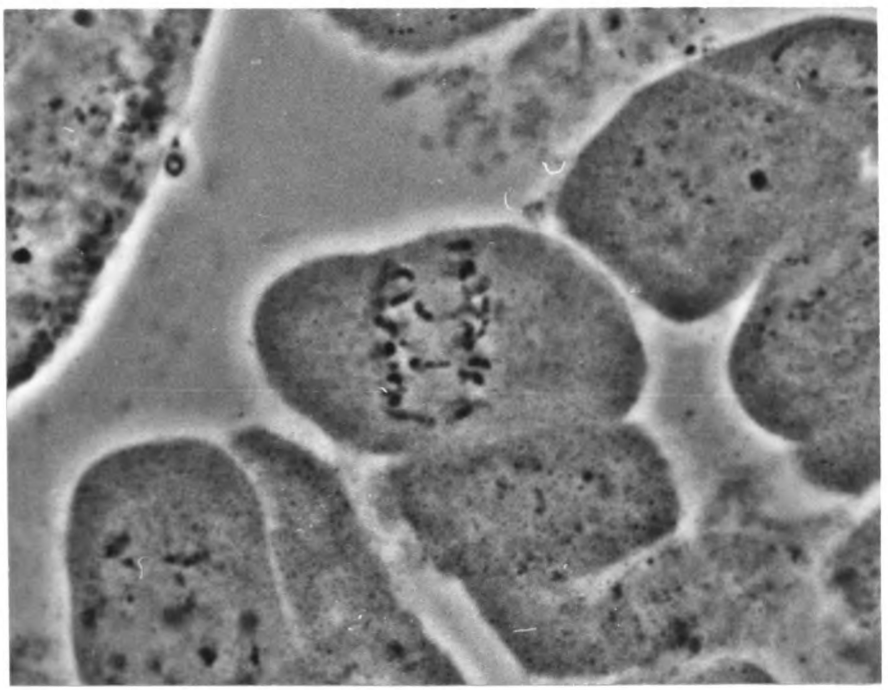

Figure 4

Figure 5. Prophase from a PSTV-infected plant 26 days after inoculation showing usual 24 chromosomes similar in number to the control. X 3,520.

Figure 6. Metaphase from PSTV-infected tomato 26 days after inoculation. X 2,000.

Figure 5

Figure 6

Figure 7. Anaphase (right) and prophase (center) from PSTV-infected tomato 26 days after inoculation. X 2,000.

Figure 8. Prophase from PSTV-infected tomato 26 days after inoculation showing 26 chromosomes. X 3,200.

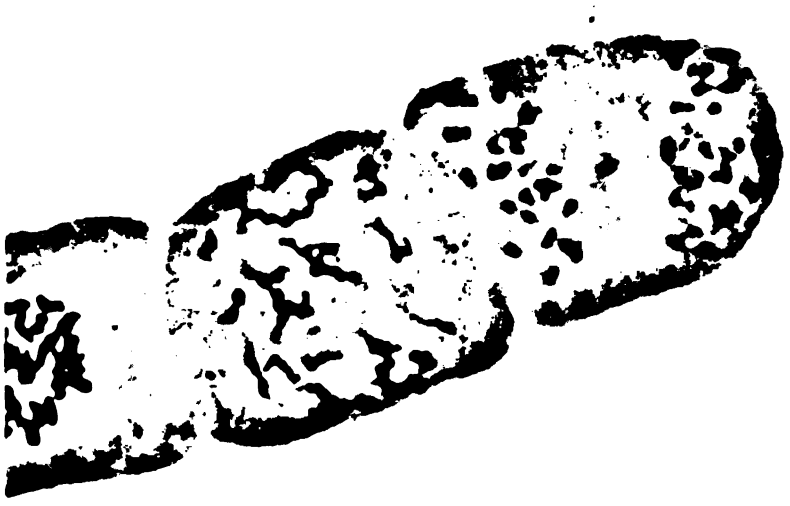

Figure 7

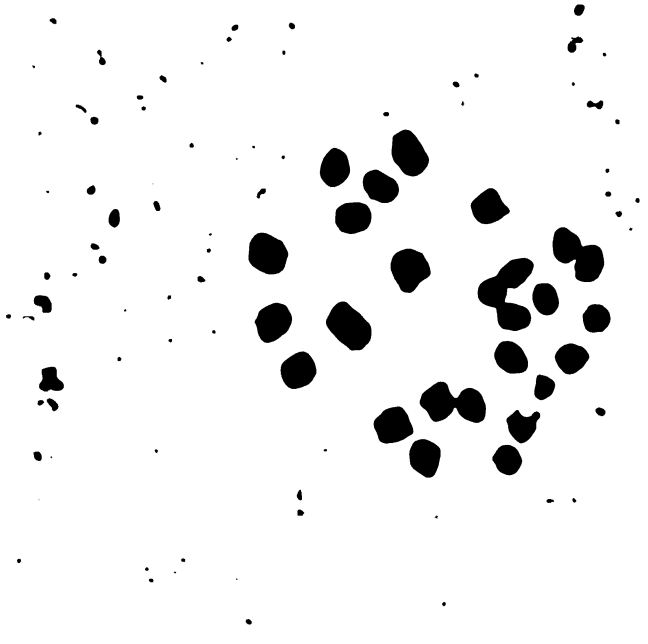

Figure 8

Figure 9. Cells from a PSTV-infected plant 26 days after inoculation showing numerous chromosomes and some chromosomes not included in the main group (arrow). X 2,000.

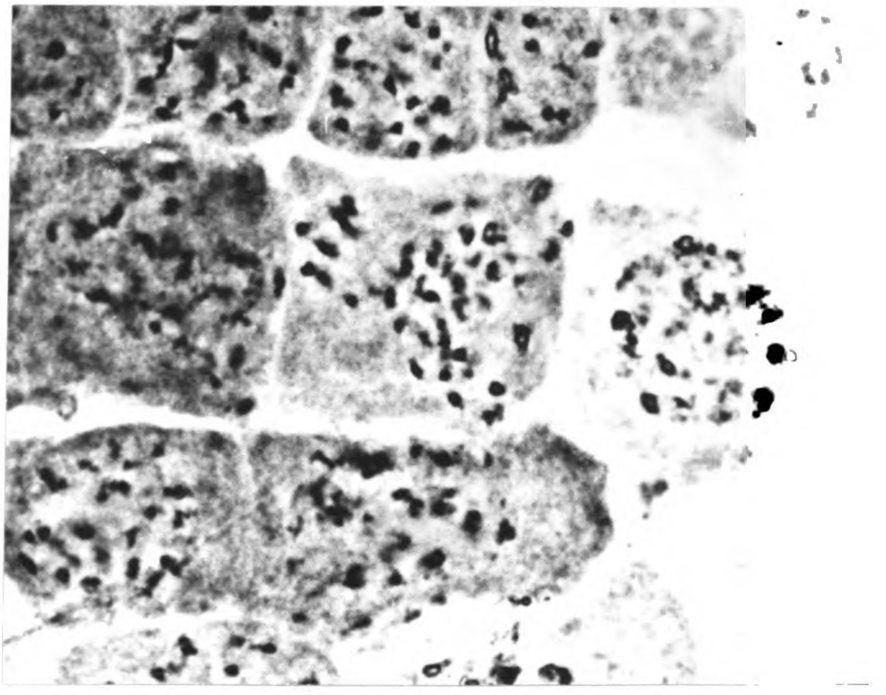

Figure 9

PSTV infected tomato plants exhibited severe stunting and possessed very reduced root systems suggesting reduced frequency of cell division. It was more difficult to get as many root tips from PSTV infected plants as from the controls. The mitotic index was calculated to provide information in comparing the effect of PSTV on meristematic cells. Root and shoot tips collected from control and from PSTV infected plants were prepared as temporary slides. A Wild-Heerbrugg phase contrast microscope was used to examine cells in estimating mitotic index. Because the small size of chromosomes, colchicine treatment (0.01%, 1 hr., room temp.) was applied to obtain more metaphase figures and make chromosomes thicker and thereby easily observed. Three different random fields were observed on each slide and the total number of cells observed as well as number of mitotic figures at each stage of division were recorded. The numbers from the three fields then were added to obtain total numbers of cells in prophase, metaphase, and anaphase, respectively.

Specimens were collected 14 days (Table 1) after inoculation when the above ground parts of inoculated plants started to show slight symptoms. Symptoms of PSTV infection in inoculated plants become more severe with age of infection. Plants 96 days after inoculation were severely diseased with the lower leaves becoming dry and dead. Only the apical part remained green. Root systems were very restricted. Growing root tips were infrequent. However, it was still possible to get measurable numbers of dividing cells.

Table 1. Mitotic figures in root tip cells of Rutgers tomato 14 days aftr PSTV inoculation.

Source	Total number of cells observed	Non-dividing cells		Dividing cells				
		Total	%	Prophase	Metaphase	Anaphase	Total	Mitotic index %
Control	416	316	76	83	12	5	100	24
	537	450	84	79	4	4	87	16
	329	300	91	24	2	3	29	9
	358	333	93	20	3	2	25	7
	357	323	90	23	5	6	34	10
	159	130	82	25	3	1	29	18
	470	420	89	45	1	4	50	11
	502	443	88	54	0	5	59	12
	382	362	95	13	6	1	20	5
Total	3510	3077	88	366	36	31	433	12
PSTV infected	444	433	98	8	2	1	11	2
	282	240	85	41	1	0	42	15
	244	171	70	73	0	0	73	30
	102	101	99	0	1	0	1	1
	241	233	97	4	1	3	8	3
Total	1313	1178	90	126	5	4	135	10

For significance at 95% level of probability t value should equal or exceed 2.179 (t = 1.03 for prophase, 1.875 for metaphase, 2.8 for anaphase, 1.288 for total).

A statistical analysis (t test) was used to compare two means between infected and healthy control plants. The null hypothesis states $H_o: \mu_1 = \mu_2$, the test statistic $t = \frac{\bar{x}_1 - \bar{x}_2}{S\sqrt{1/n_1 + 1/n_2}}$ is used to show whether the population means differ statistically. Comparisons were made between numbers of cells in prophase, metaphase, anaphase, and in total number of dividing cells between PSTV infected and healthy plants. The t value for the prophase (Table 1) was calculated to be 1.03. For significance in population mean t should equal or exceed 2.197 ($\alpha = .05$). Since the value of 1.03 with a 95% probability does not exceed the value of 2.179, $\mu_1 = \mu_2$. As above, comparisons of metaphase and anaphase as well as total number of cells with mitotic figures were calculated. The only significant difference was found in anaphase. In later trials, root tips were collected 26, 47, and 96 days after inoculation (Tables 2, 3, 4, and 5). The t test was applied as in Table 1 and the results were shown in each subsequent Table. Only in Table 4 was there a significant difference between infected and healthy plants at anaphase. In other comparisons there were no distinct differences in mitotic indices between controls and plants with distinct PSTV symptoms.

Table 2. Mitotic figures in root tip cells of Rutgers tomato 26 days after PSTV inoculation. (Test 1)

Source	Total number of cells observed	Non-dividing cells		Dividing cells				
		Total	%	Prophase	Metaphase	Anaphase	Total	Mitotic index %
Control	394	302	77	87	4	1	92	23
	391	365	93	25	1	0	26	7
	79	73	92	6	0	0	6	8
Total	864	740	86	118	5	1	124	14
PSTV infected	79	78	99	0	1	0	1	1
	213	191	90	17	1	4	22	10
	320	318	99	2	0	0	2	1
Total	612	587	96	19	2	4	25	4

For significance at 95% level probability t value should equal or exceed 2.776 (t = 1.34 for prophase, .8 for metaphase, .74 for anaphase, 1.226 for total).

Table 3. Mitotic figures in root tip cells of Rutgers tomato 26 days after PSTV inoculation. (Test 2)

Source	Total number of cells observed	Non-dividing cells		Dividing cells				
		Total	%	Prophase	Metaphase	Anaphase	Total	Mitotic index %
Control	219	190	87	28	1	0	29	13
	246	235	96	11	0	0	11	4
	279	238	85	36	2	3	41	15
Total	744	663	89	75	3	3	81	11
PSTV infected	261	206	79	48	1	6	55	21
	292	273	93	19	0	0	19	7
	271	260	96	11	0	0	11	4
Total	824	739	90	78	1	6	85	10

For significance at 95% level of probability t value should equal or exceed 2.776 (t = .08 for prophase, 1.06 for metaphase, .46 for anaphase, .062 for total).

Table 4. Mitotic figures in root tip cells of Rutgers tomato 47 days after PSTV inoculation.

Source	Total number of cells observed	Non-dividing cells		Dividing cells				
		Total	%	Prophase	Metaphase	Anaphase	Total	Mitotic index %
Control	559	551	99	3	3	2	8	1
	408	400	98	4	3	1	8	2
	325	325	100	0	0	0	0	0
	263	257	98	1	4	1	6	2
	366	257	70	107	1	1	109	30
Total	1921	1790	93	115	11	5	131	7
PSTV infected	290	290	100	0	0	0	0	0
	367	364	99	2	1	0	3	1
	463	461	100	1	1	0	2	0
	375	375	100	0	0	0	0	0
	342	331	97	10	0	1	11	3
	284	273	96	7	4	0	11	4
Total	2121	2094	99	20	6	1	27	1

For significance at 95% level of probability t value should equal or exceed 2.262 (t = 1.053 for prophase, 1.25 for metaphase, 2.34 for anaphase, 1.11 for total).

Table 5. Mitotic figures in root tip cells of Rutgers tomato 96 days after PSTV inoculation.

Source	Total number of cells observed	Non-dividing cells		Dividing cells				
		Total	%	Prophase	Metaphase	Anaphase	Total	Mitotic index %
Control	301	213	71	78	7	3	88	29
	233	178	76	53	0	2	55	24
	317	302	95	13	1	1	15	5
Total	851	693	81	144	8	6	158	19
PSTV infected	293	273	93	10	7	3	20	7
	199	184	92	9	2	4	15	8
	256	253	99	1	0	2	3	1
Total	748	710	95	20	9	9	38	5

For significance at 95% level of probability t value should equal or exceed 2.776 (t = 2.157 for prophase, .101 for metaphase, 1.25 for anaphase, 1.84 for total).

Shoot tips were collected on different days after inoculation. There were no differences in mitotic indices between shoot tips of controls and PSTV-infected plants (Table 6) except that in metaphase. Because the smaller size of the apical meristematic cells and the difficulty to separating from neighboring cells, only limited work with apical meristems was possible.

Table 6. Mitotic figures in shoot tip cells of Rutgers tomato after PSTV inoculation.

Source	Days after inoculation	Total number of cells observed	Non-dividing cells		Dividing cells				
			Total	%	Prophase	Metaphase	Anaphase	Total	Mitotic index %
Control	14	191	177	93	14	0	0	14	7
	14	643	616	96	22	4	1	27	4
	14	462	446	97	14	1	1	16	3
	39	471	453	96	15	2	1	18	4
	47	444	444	100	0	0	0	0	0
	49	693	673	97	11	3	6	20	3
Total		2904	2309	96.7	76	10	9	95	3.3
PSTV infected	14	529	505	95	19	4	1	24	5
	14	429	419	98	4	4	2	10	2
	14	1215	1172	96	24	9	10	43	4
	39	973	955	98	7	8	3	13	2
	47	663	654	99	3	3	3	9	1
	49	616	598	97	13	3	2	18	3
Total		4425	4303	97.2	70	31	21	122	2.8

For significance at 95% level of probability t value should equal or exceed 2.228 (t = .211 for prophase, 2.65 for metaphase, 1.186 for anaphase, and .64 for total).

DISCUSSION

Cytological aberrations following virus infection of plants have received relatively little attention. Continho (1940) observed chromosome fragments and 'strange lateral bridges' in somatic metaphases and anaphases in root tips of Vicia faba infected with bean mosaic virus. Wilkinson (1953b) examined somatic divisions in tomato and tobacco plants infected by aspermy virus and observed widespread nucleolar and other abnormalities. Some of these abnormalities suggest aberrations regarded as typical of cell divisions found in malignant animal tissue such as arrested metaphase, spindle malformation, and tendency to form giant nuclei, etc. He suggested that there is competition between the virus particles and chromosome material for nucleoprotein contained in the nucleolus. Wilkinson (1960) observed mitotic abnormalities in a number of solanaceous species infected respectively with tobacco mosaic virus and aspermy virus. These abnormalities ranged from persistence of the nucleolar material as far as early anaphase to complete amitosis in stem enations of one species of Petunia violacea. Wilkinson thought that virus particles, multiplying within dividing cells, competed with the nculear DNA for the supply of RNA located in thc nucleolus.

Changes in host cell chromosomes and mitosis have been studied more extensively in animals. Hampar and Ellison (1961), in hamster tissue culture cells infected by Herpes simplex virus, found

multiple breakages, chromatin deletions, and new chromosomes. Koprowski et al. (1962) and Todaro et al. (1963), using human tissue cultures; and Cooper and Black (1963), using hamster tissue culture, all infected by SV 40 virus, found chromosomal aberrations such as breaks, translocations, and deletions. Cooper and Black (1963) concluded that SV 40 produced a biochemical growth stimulus on which are superimposed processes producing aneuploidy. Stich et al. (1964), using hamster tissue culture infected by adenovirus, found chromatid and chromosome breaks in the metaphase plate which exhibited numerous exchanges and the formation of new chromosomes. They also reported fragmentation of the entire chromosome. Walt et al. (1964) reported that an extra chromosome, as well as a morphologically unusual chromosome, was found in the bone marrow cells of mice with virus leukemia. The infection by virus in Rhynchosciara angelae (a kind of fly) cause hypertrophy of the entire cell, including the nucleus and the chromosomes, and also caused specific constrictions which were not found in non-infected chromosomes (Pavan 1967).

Nimnoi (1974) reported cytological aberrations in PSTV infected pollen-mother-cells of Rutgers tomatoes. Multipolar meiosis of chromosomes of PSTV infected pollen-mother-cells was observed. The chromosomes separated into groups. This led to formation of pollen grains with chromosome numbers less or more than the normal chromosome number of 12. Meiotic abnormality observed by Nimnoi as well as PSTV symptoms on Rutgers tomato plants expressing very severe stunting on aboveground parts and very reduced root system logically led to search for mitotic aberrations or a study of mitotic figures in somatic tissues of tomato.

In my work, there were no significant differences in mitotic indexes from both root tips and shoot tips between healthy controls and PSTV infected tomatoes except in two comparisons at anaphase (Tables 1 and 4) from root tips and one comparison at metaphase (Table 6) from shoot tips. PSTV may have some effect on the process of mitosis. However, this effect is apparently slight. In three comparisons metaphase and anaphase which occurred in very low frequencies might have been delayed or blocked by the effect of pathogen. Distinct growth retardation of PSTV infected tomato plants may be caused by factors other than cytology, and may involve a disease effect on physiology or metabolism.

The most prominent result in this work was the chromosome number change from the normal 24 to the PSTV infected 26. Although it was in low frequency, this phenomenon was observed at least 4 - 5 times. This may be the first time that the virus infection of plants caused a change in chromosome number in plant somatic tissue. Changes in chromosome numbers such as new chromosome or extra chromosomes have been reported in virus infected animal tissues (Hampar and Ellison 1961, Stich et al. 1964, and Walt et al. 1964).

Another abnormality was presence of numerous chromosomes or chromosome fragments (Figure 9) exceeding the normal 24 chromosome number. These were only found in PSTV infected root tips 26 days after inoculation. Since this phenomenon was observed only towards the end of this study otherwise it could have been investigated further. Continho (1940) reported chromosome fragments in root tips of Vicia faba infected with bean mosaic virus. Fragmentation of the entire chromosome was also previously reported in animal cells (Stich et al. 1964).

Diener (1971) reported that in tissue extracts, infectivity was mainly associated with nuclei and purified chromatin. Hadidi et al. (1976) published an interesting and intriguing paper concerning hybridization of potato spindle tuber viroid to cellular DNA of normal plants. They detected a partial complementary action between PSTV and RNA from uninfected or infected tomatoes. They suggested that possibly the introduction of PSTV into cells could act as a regulatory signal, derepressing PSTV-specifying sequences and leading to viroid replication and disease development. They also suggested that the more distant, phylogenetically, plant species were from solanaceous plants, the fewer related sequences their DNAs contained and the more distant they were from PSTV. They proposed the hypothesis that PSTV originated from genes in normal solanaceous plants. The viroids of PSTV and citrus exocortis are identical (Singh et al. 1972). The hypothesis of Hadidi et al. (1976) requires further explanation if viroids of potato spindle tuber and citrus exocortis have common origin. If each viroid originated independently in separate hosts, the coincidence is remarkable that the end products are so similar.

SUMMARY

PSTV infected Rutgers tomato plants show typical symptoms with rugosity, epinasty, severe stunting, and very poor root systems. The longer after inoculation, the more severe are the symptoms of PSTV infection in inoculated plants. Although growing root tips were very restricted, it was still possible to get measurable numbers of dividing cells. No statistically significant differences were obtained in mitotic indices from both root and shoot tip cells between healthy controls and diseased plants. Comparisons between healthy and diseased root tips showed differences at anaphase 14 and 47 days after inoculation. There was a significant difference in metaphase in shoot tips between healthy and diseased plants.

Chromosome number change from the normal 24 to the PSTV infected 26 was observed 26 days after inoculation. This may be the first time that the virus infection on plants caused a change in chromosome number in plant somatic tissue. Numerous chromosomes or chromosome fragments were also observed in diseased root tips 26 days after PSTV inoculation. This phenomenon still needs more detailed studies.

LITERATURE CITED

LITERATURE CITED

Benson, A. P. and R. P. Singh. 1964. Seed transmission of potato spindle tuber virus in tomato. Amer. Potato J. 41: 294.

Benson, A. P., W. B. Raymer, W. Smith, E. Jones, and J. Munro. 1965. Potato diseases and their control. Potato Handbook 10: 32-33. The Potato Association of America c/o College of Agriculture, N. Brunswick, N. Jersey.

Bonde, R. 1927. The spread of spindle tuber by the knife. Amer. Potato J. 4: 151-152.

Bonde, R. and D. Merriam. 1951. Studies on the dissemination of the potato spindle tuber virus by mechanical inoculation. Amer. Potato J. 28: 558-560.

Caldwell, J. 1952. Some effects of a plant virus on nuclear division. Ann. Appl. Biol. 39: 98-102.

Coutinho, L. DEA. 1940. Os virus como agentes modificadores dos cromosomas. Revista Agronomica 28: 83.

Cooper, H. L. and P. H. Black. 1963. Cytogenetic studies of hamster kidney cell cultures transformed by the Simian Vacuolating Virus (SV 40). J. nat. Cancer Inst. 30: 1015-1043.

Darlington, C. D. and L. F. LaCour. 1969. The Handling of Chromosomes. 5th ed. George Allen & Unwin Ltd. London. Pp. 146.

Diener, T. O. 1971a. Potato spindle tuber virus: A plant virus with properties of a free nucleic acid. III. Subcellular location of PSTV-RNA and the question of whether virions exist in extracts or in situ. Virology 43: 75-89.

Diener, T. O. 1971b. Potato spindle tuber "virus": IV. A replicating, low molecular weight RNA. Virology 45: 411-428.

Diener, T. O. 1972. Potato spindle tuber viroid: VIII. Correlation of infectivity with a UV-absorbing component and thermal denaturation properties of the RNA. Virology 50: 606-609.

Diener, T. O. 1974. Viroids: The smallest known agents of infectious disease. Ann. Rev. Micro. 28: 23-39.

Diener, T. O. and W. B. Raymer. 1967. Potato spindle tuber virus: A plant virus with properties of a free nucleic acid. Science 158: 378-381.

Diener, T. O. and D. R. Smith. 1973. Potato spindle tuber viroid: IX. Molecular weight determination by gel electrophoresis of formylated RNA. Virology 53: 359-365.

Diener, T. O., D. R. Smith, and M. J. O'Brien. 1972. Potato spindle tuber viroid: VII. Susceptibility of several solanaceous plant species to infection with low molecular-weight RNA. Virology 48: 844-846.

Easton, G. D. and D. C. Merriam. 1963. Mechanical inoculation of the potato spindle tuber virus in the genus Solanum. Phytopathology 53: 349 (Abstr.).

Fernow, K. H. 1923. Spindling tuber or marginal leaf-roll. Phytopathology 13: 40 (Abstr.).

Fernow, K. H. 1967. Tomato as a test plant for detecting mild strains of potato spindle tuber virus. Phytopathology 57: 1347-1352.

Fernow, K. H., L. C. Peterson, and R. L. Plaisted. 1970. Spindle tuber virus in seeds and pollen of infected potato plants. Amer. Potato J. 47: 75-80.

Goss, R. W. 1926. Transmission of potato spindle tuber by cutting knives and seed piece contact. Phytopathology 16: 229-303.

Hadidi, A., D. M. Jones, D. H. Gillespie, F. Wong-Staal, and T. O. Diener. 1976. Hybridization of potato spindle tuber viroid to cellular DNA of normal plants. Proc. Natl. Acad. Sci. U.S.A. 73: 2453-2457.

Hampar, B. and S. A. Ellison. 1963. Cellular alterations in the MCH line of Chinese hamster cells following infection with Herpes Simplex Virus. Proc. Nat. Acad. Sci. 49: 474-430.

Hunter, D. E., H. M. Darling, and W. L. Beale. 1969. Seed transmission of potato spindle tuber virus. Amer. Potato J. 46: 247-250.

Koprowski, H., J. A. Ponten, F. Jensen, R. G. Ravdin, P. Moorhead, and E. Saksela. 1962. Transformation of cultures of human tissue infected with Simian Virus SV 40. J. Cell Comp. Physiol. 59: 281-292.

Kostoff, D. 1933. A contribution to the sterility and irregularities in the meiotic processes caused by virus diseases. Genetica 15: 103-114.

Manzer, F. E. and D. Merriam. 1961. Field transmission of the potato spindle tuber virus and virus X by cultivating and hilling equipment. Amer. Potato J. 38: 346-352.

McClean, A. P. D. 1931. Bunchy top disease of the tomato. Union Of South Africa, Dept. Agri. Sci. Bull. 100, 28 p.

Morris, T. J. and N. S. Wright. 1975. Detection on polyacrylamide gel of a diagnostic nucleic acid from tissue infected with potato spindle tuber viroid. Amer. Potato J. 52: 57-63.

Nimnoi, P. 1974. Effect of potato spindle tuber virus on pollen function. Ph.D. Thesis, Michigan State Univ.

O'Brien, M. J. 1972. Hosts of potato spindle tuber virus in suborder Solanineae. Amer. Potato J. 49: 70-72.

O'Brien, M. J. and W. B. Raymer. 1963. Symptomless hosts of the potato spindle tuber virus (PSTV). Phytopathology 53: 884 (Abstr.).

O'Brien, M. J. and W. B. Raymer. 1964. Symptomless hosts of the spindle tuber virus. Phytopathology 54: 1045-1047.

O'Mara, J. G. 1936. Observations on the immediate effects of colchicine. J. of Heredity 30: 35-37.

Pavan, C. 1967. Chromosomal changes induced by infective agents. Triangle 8: 42-48.

Raymer, W. B. and M. J. O'Brien. 1962. Transmission of potato spindle tuber virus to tomato. Amer. Potato J. 39: 401-408.

Raymer, W. B. and M. O'Brien. 1962. Transmission of potato spindle tuber virus to tomato. Phytopathology 52: 749 (Abstr.).

Sass, J. E. 1961. Botanical Microtechnique. 3rd ed. The Iowa State Univ. Press. Ames, Iowa. Pp. 19.

Schultz, E. S. and D. Folsom. 1923. Transmission , variation, and control of certain degeneration diseases of Irish potatoes. J. Agr. Res. 25: 43-118.

Schultz, E. S. and D. Folsom. 1923. Spindling-tuber and other degeneration diseases of Irish potatoes. Phytopathology 13: 40 (Abstr.).

Schultz, E. S. and D. Folsom. 1925. Infection and dissemination experiments with degeneration diseases of potatoes. Observations in 1923. J. Agr. Res. 30: 493-528.

Semancik, J. S., D. S. Magnuson, and L. G. Weathers. 1973. Potato spindle tuber disease produced by pathogenic RNA from citrus exocortis disease: Evidence for the identity of the causal agents. Virology 52: 292-294.

Singh, R. P. 1970. Seed transmission of potato spindle tuber virus in tomato and potato. Amer. Potato J. 47: 225-227.

Singh, R. P. 1973. Experimental host range of the potato spindle tuber 'virus'. Amer. Potato J. 50: 111-123.

Singh, R. P. and R. H. Bagnall. 1968. Solanum rostratum Dunal., a new test plant for the potato spindle tuber virus. Amer. Potato J. 45: 335-336.

Singh, R. P. and M. J. O'Brien. 1970. Additional indicator plants for potato spindle tuber virus. Amer. Potato J. 47: 367-371.

Singh, R. P., M. C. Clark, and L. G. Weathers. 1972. Similarity of host symptoms induced by citrus exocortis and potato spindle tuber causal agents. Phytopathology 62: 790 (Abstr.).

Singh, R. P., R. E. Finnie, and R. H. Bagnall. 1970. Relative prevalence of mild and severe strains of potato spindle tuber virus in eastern Canada. Amer. Potato J. 47: 289-293.

Sogo, J. M., T. Koller, and T. O. Diener. 1973. Potato spindle tuber viroid. X. Visualization and size determination by electron microscopy. Virology 55: 70-80.

Stich, H. F., G. L. Van Hoosier, and J. J. Trentin. 1964. Viruses and mammalian chromosomes: chromosome aberrations by human adenovirus type 12. Exp. Cell Res. 34: 400-403.

Swaminathan, M. S., T. Ninan, and M. L. Magoon. 1959. Effects of virus infection on microsporogenesis and seed fertility in Capsicum. Genetica 30: 63-69.

Takahashi, T. and T. O. Diener. 1975. Potato spindle tuber viroid: XIV. Replication in nuclei isolated from infected leaves. Virology 64: 106-114.

Todaro, G. J., S. R. Wolman, and H. Green. 1963. Rapid transformation of human fibroblasts with low growth potential into established cell lines by SV 40. J. Cell. Comp. Physiol. 62: 257-265.

Wald, N., A. C. Upton, V. K. Jenkins, and W. H. Borges. 1964. Radiation-induced mouse leukemia: consistent occurrence of an extra and a marker chromosome. Science 143: 810-813.

Wilkinson, J. 1953a. Some effects induced in Nicotiana glutinosa by the 'Aspermy' virus of tomato. Ann. Bot. N.S. 17: 219-223.

Wilkinson, J. 1953b. Virus-induced nucleolar abnormalities in tomato. Nature 171: 658-659.

Wilkinson, J. 1960. Virus-induced abnormalities of mitosis in certain Solanaceous species. Ann. Bot. N.S. 24: 516-521.

Yang, T. C. 1974. Potato spindle tuber virus in certain Solanum species. Ph.D. Thesis, Michigan State Univ.