EFFECT OF POTATO SPINDLE TUBER VIRUS ON POLLEN FUNCTION

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ABSTRACT

EFFECT OF POTATO SPINDLE TUBER VIRUS ON POLLEN FUNCTION

By

Pornsawan Nimnoi

Potato spindle tuber virus (PSTV) is a pollen and seed transmitted virus. However, little is known concerning the function of pollen from PSTV infected plants and also cytology of pollen-mother-cells.

Pollen grains from *Lycopersicum esculentum* Mill., cv. *Rutgers* and from 2 symptomless hosts of PSTV, namely, *Physalis floridana* L. and *Solanum dulcamara* L., were compared with healthy plants for percentage of pollen germination and length of pollen tubes. Flowers, at anthesis stage, were picked between 10:00-11:00 a.m. from healthy control plants and also from PSTV infected plants. Pollen grains from each source were germinated separately in a medium and incubated under light and at approximately 21-30°C. Pollen germination was observed at 0.5 hr intervals over a period of 2 hrs. Germ tube lengths were determined at the end of the 2 hrs incubation period. Pollen grains from healthy Rutgers tomato plants germinated with higher

percentage and formed longer pollen tubes than did those from PSTV infected plants. In *P. floridana* L. and *S. dulcamara* L. pollen germination was impaired. PSTV infected pollen tubes from *S. dulcamara* L. were shorter than those of healthy pollen.

Stainability with I_2-KI solution indicated higher percentage of viable pollen from healthy Rutgers tomato plants than from those infected with PSTV. Germinability tests and stainability tests were not in agreement.

Cytological study of PSTV infected pollen-mother-cells was made with fresh smear and squash preparation 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.

Infectivity of pollen grains from PSTV infected Rutgers tomato and *S. dulcamara* L. plants was shown by grinding the pollen grains and inoculating cotyledons of tomato seedlings. Approximately 40% of seedling plants developed symptoms following inoculation with pollen from PSTV infected Rutgers tomato and 20% became infected from PSTV infected *S. dulcamara* L. pollen. When the pollen grains from PSTV infected *S. dulcamara* L. were used directly

without grinding, only 1% infection of the plants was obtained.

Virus concentration within tomato ovaries, 14 days after pollinating healthy female with PSTV infected pollen, was apparently very low when the ovaries were ground and used as inoculum. However, frequency of infection was high as 7 of 9 ovaries tested contained PSTV. Many ovaries aborted when stigmas were pollinated with PSTV infected pollen.

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Pornsawan Nimnoi

A DISSERTATION

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To

My Parents

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INTRODUCTION

Potato spindle tuber virus (PSTV) is one of the smallest known infectious entities consisting simply of RNA without a protein coat. Its molecular weight is 5×10^4 daltons. Diener (1971) called the infectious entity of PSTV a "viroid" because of its minuscule size, but its action resembles a virus. The most important disease caused by PSTV is spindle tuber of potato (*Solanum tuberosum* L.). The bunchy top disease of tomato (*Lycopersicum esculentum* Mill. cv. Rutgers) (Benson *et al.*, 1965) is caused by the same virus. The disease is transmitted through seed tubers of potatoes and symptoms are extremely difficult to detect. In potato, the disease is important because of difficulty in maintaining seed stocks free from the virus and resultant deleterious effects on yields.

PSTV is carried through the true seed and is also transmitted through pollen. A relatively small number of viruses in other plants are known to be seed and pollen transmitted. In comparison to the large literature on other aspects of plant virus infection, relatively little is known concerning influences of virus infection on

pollen germination, germ tube length, and possibly cytological effects. Transmission of virus from infected pollen by mechanical means has been demonstrated for a number of fruit viruses. A few studies of pollen viability have been made with a limited number of other viruses. The influence of virus infection on germ tube length has not previously been reported. Cytological investigations of pollen-mother-cells of pollen transmitted viruses have not yet been published. Furthermore, no investigations have been made on pollen germination or cytology of PSTV infected pollen.

For these reasons, pollen of PSTV infected plants was investigated. This reports a study of pollen viability from PSTV infected plants of 3 species and cytology of PSTV infected pollen-mother-cells in tomato.

LITERATURE REVIEW

The potato spindle tuber disease was first described by Schultz and Folsom in 1923 as follows. Potato plants that come from tubers showing the disease are different from healthy plants in having more erect and more spindling shoots. The leaves are smaller, more erect and early in the season are somewhat darker green with more rugosity, that is, with the leaf surface raised between the veins. Later in the season, leaves are even more dwarfed but the rugosity is not so marked. The most striking effect of the disease is on the tubers, which are made spindling, long, and cylindrical, with a more irregular or bumpy outline, more spindle-shaped or tapering ends and more conspicuous eyes. Usually the skin of a spindle tuber is smoother and more tender, and in the spring the flesh cuts more easily. Diener and Raymer (1971) also observed that small leaflets often overlap. Foliage from late spring to mid-summer frequently turns slate gray with dull leaf surface. Tubers are elongate with prominent bud scales ('eyebrows') and may have severe growth cracks.

Potato unmottle curly dwarf (Folsom, 1946) is probably caused by a strain of PSTV. The disease, described by

Folsom (1946), lacked the mottling of a mosaic and in comparison with spindle tuber produced more dwarfing, more leaf distortion, more leaf burning, more stem streaking, and more gnarling and cracking of the tubers, and it was somewhat more easily transmitted with the leaf-mutilation method.

The tomato bunchy top disease, described by McClean in 1931, was shown (Benson *et al.*, 1965) to be caused by PSTV. O'Brien and Raymer (1964) pointed out similarity in host range and symptoms of PSTV and the virus causing bunchy top disease of tomato. Raymer and O'Brien (1962) first experimentally demonstrated PSTV infection of a plant other than potato. This was accomplished by mechanical transfer to tomato and thus provided an efficient assay method for working with the disease. PSTV was later named "potato gothic virus" (Leont'eva, 1964).

Symptoms of PSTV in Saco potato (Hunter and Rich, 1964) included slow development of sprouts and slow emergence of plants. Infected plants were stunted and spindly with a sharp angle of branching, and the leaves developed tip burn. Infected plants died prematurely and the number and size of tubers was smaller than expected. Infected hills yielded only 35% as much as healthy hills. Yield reduction was due to fewer tubers per hill but mostly to their smaller size. This factor, plus the spindle shape, would place many of them in an inferior

grade. Percentage of yield reduction (Singh *et al.*, 1971) depended on severity of the strain of PSTV.

Diener (1971) called the infectious entity of PSTV a "viroid." It was a particle only one-eightieth the size of the smallest known virus, the so-called Q-beta, which infects certain bacteria. Despite its miniscule size, however, the particle resembled the virus in its action. The potato viroid (Diener, 1971) consists simply of a fragment of RNA with no protein coat. Its molecular weight was only 50,000 daltons, as compared with 4 million of the Q-beta virus. Nevertheless, the viroid invades living cells and disrupts their metabolic processes as efficiently as a typical virus.

PSTV causes spindle tuber of potato (Schultz and Folsom, 1923) and bunchy top of tomato (McClean, 1931). At present, no other disease of crop plants is recognized as being caused by this virus. Similarity of host symptoms induced by citrus exocortis and PSTV in potato, tomato, and six species of *Scopolia* has been reported (Singh *et al.*, 1972). Diener (1974) believes that sufficient differences exist so that the two viruses, PSTV and citrus exocortis, should be considered separate entities.

Host-range

The first host identified for PSTV other than potato was Rutgers tomato (Raymer and O'Brien, 1962). Symptoms

in tomato showed first in 10-14 days or up to 5 weeks after plants were graft inoculated. Symptoms consisted of epinasty and rugosity of new leaves with stunting, followed by yellowing and necrosis of the midrib and lateral veins of rugose leaflets, stunting and rugosity of apical leaves. Diener and Raymer (1971) used the term 'bunchiness' of apical leaves to describe the appearance of infected tomato plants. Later 10 *Solanum* spp. (Easton and Merriam, 1963) were also found to be susceptible to PSTV. Subsequently O'Brien and Raymer (1964) found that thirteen plant varieties and species commonly used as virus indicators were susceptible to sap inoculation with the PSTV. Infection with PSTV was determined by inoculating Rutgers tomato. Susceptible but symptomless genera in the Solanaceae included *Capsicum, Datura, Nicotiana, Petunia,* and *Physalis.* One host in the Amaranthaceae, *Gomphrena globosa* L., was highly resistant but not immune. All inoculated hosts were symptomless except *Nicotiana glutinosa* L., in which a break in flower color occurred. Infection in 2 species of *Datura* was more readily detected by grafting to tomato than by sap inoculation to tomato. Additional symptomless hosts (O'Brien, 1972) for PSTV were found in the families *Solanaceae, Nolanaceae,* and *Scrophulariaceae.* Inoculated plants of *S. melongena* (eggplant "Black Beauty") were dwarfed and epinastic. Recently, Singh (1973) tested 232 plant selections

(species and/or varieties) for susceptibility to PSTV. Susceptible plants were found in the families *Boraginaceae, Campanulaceae, Caryophyllaceae, Compositae, Convolvulaceae, Dipsaceae, Sapindaceae, Scrophulariaceae, Solanaceae,* and *Valerianaceae.* Most susceptible selections were symptomless carriers of PSTV. He also found that the PSTV developed local lesions in *Scopolia sinensis.*

Transmission

PSTV has been transmitted by two species of aphids (Schultz and Folsom, 1925), grasshopper (Goss, 1928), flea beetles, tarnished plant bug, leaf beetle, and Colorado potato beetle (Goss, 1931). Aphid transmission has not been confirmed. The agent is easily spread by foliage contact (Folsom, 1946), by machinery (Bonde and Merriam, 1951; Merriam and Bonde, 1954; Manzer and Merriam, 1961), by cutting knives and seed piece contact (Goss, 1926; Bonde, 1927). Diener (1973) has proposed that when PSTV is transmitted mechanically, transmission was not through free RNA but rather viroid RNA was firmly associated with chromatin (pieces of nuclei). This explanation results from purification procedures in which as long as extraction was made with low ionic strength buffer, the infectious agent was not released from the chromatin. When combined with chromatin, the viroid RNA was actually quite insensitive to ribonuclease denaturation.

by seed: -

The virus has long been known to be transmitted by vegetative means through potato "seed" tubers. Transmission from infected seed tubers should not be confused with transmission through true seed. Virus survival of tomato bunchy top in or on true seed was first demonstrated by McClean (1948). He ground seed of infected plants in a mortar and inoculated tomato plants. He demonstrated virus in seeds of 5 solanaceous species and that infectivity persisted in seeds of *Solanum incanum* L. for at least 6 years and in *S. aculiatissimum* Jacq. for over 4 years. He showed, over a period of 3 years, 63% transmission occurred with new and 39% with old seed of *S. incanum* L. With *Physalis peruvianum* L. 11% transmission was obtained with one year old seed. Benson and Singh (1964) reported 11% transmission of PSTV in tomato seed. Singh (1966) reported 11% transmission through potato seed, while Hunter *et al.* (1969) obtained 87 to 100% transmission through potato seed when both parents were infected.

by pollen: -

Fernow *et al.* (1970) found that potato progeny can be infected with PSTV either through pollen or the ovule, and that 0-100% of the seed might be infected. This is the only known pollen and seed-transmitted virus infecting potato. Most seed transmitted viruses also appear to be transmitted through the pollen from infected plants though not all have been adequately tested (Matthews, 1970).

Reddick and Stewart (1918) first suggested that bean common mosaic might be carried in the pollen of diseased plants and that plants so infected may not show typical symptoms of disease but only show it in the progeny. However, it is true that cross-pollination is of common occurrence and it is possible that pollen so carried might germinate and enter the style and infection might be effected in this way.

Reddick (1931) reported pollen transmission of a virus causing disease of beans (*Phaseolus vulgaris*) by artificial cross pollination experiments and by observations of progenies of natural hybrids in mosaic-immune varieties.

A short time later, Nelson and Down (1933) studied seed-transmission of bean common mosaic virus in crosses between Refugee and early Prolific varieties of navy pea bean. If one parent was infected, about 25% of the F_1 progeny carried the virus, regardless of which parent supplied the virus. This indicated that transmission of the virus through pollen and ovule was about equal in effectiveness in the varieties tested.

Medina and Grogen (1961) obtained relatively high percentage of bean seed infection with Bean virus 1 and/or the New York strain 15 using either infected ovules or pollen in the cross. Pollen usually transmitted virus to a larger number of progeny than did ovules.

Gold *et al.* (1954) observed rod shaped particles of false stripe disease of barley in pollen and infected pistils. Rods were present in seed produced from healthy pistils pollinated by pollen from diseased plants.

Pollen transmission of elm mosaic virus through elm seeds (Callahan, 1957) was 30% effective when pollen from diseased plants was pollinated with a pollen gun to healthy flowers. Since seed set was drastically reduced in flowers on diseased plants, pollen transmission was apparently of greater importance in infecting seed than was infection through the ovules.

Lychnis ringspot virus was transmitted readily through pollen and seed of the dioecious species, *Lychnis divericata* and *Silene noctiflora*. Infected plants of *Tetragonia expansa* and *Stellaria media* produced no seed, and seed production was drastically reduced in *Beta vulgaris*, *Callistephus chinensis*, and *S. noctiflora* (Bennett, 1959).

Ryder (1964), using male sterile plants as female parents, obtained less than 0.5% transmission of lettuce mosaic virus from infected pollen and over 5.0% through the ovules. No pollen viability counts were made.

Way and Gilmer (1958) found that 5 of 18 cherry seedlings from a cross of healthy English Morello (female parent) by virus infected Montmorency (male parent) were infected with necrotic ringspot virus when indexed on cucumber seedlings.

Gilmer and Way (1960) identified relatively low infectivity in virus infected sour cherry seedlings. These were grown from seeds produced on virus free trees which had been pollinated with sour cherry pollen from necrotic ringspot and/or prune dwarf virus infected trees.

They reported (1963) evidence of tree-to-tree transmission by pollen of cherry yellows virus in sour cherry. Foliage symptoms were delayed 2 and 3 years following pollination.

Das, Milbrath and Swenson (1961) studied seed-transmission of Prunus ringspot virus by pollen. They obtained no seed infection in 3152 seeds when healthy squash plants were pollinated with pollen from virus infected plants. When ovaries were infected, virus was transmitted in the seed. They concluded that virus in the ovary appeared to be the controlling factor in seed transmission. However, 8 of 49 squash plants became infected after pollination with diseased pollen. Mechanical inoculation during pollination was not precluded. They do not report observations on pollen germination from virus infected plants.

Das and Milbrath (1961), using precautions to prevent mechanical transmission during pollination, observed 9 infected squash plants out of 97 so pollinated. There was also impairment of fruit development following pollination with virus infected pollen.

George and Davidson (1963) reported transmission of necrotic ringspot and sour cherry yellows viruses from tree-to-tree through pollination. They also observed that the diseases did not spread to neighboring trees from which blossom buds were removed.

Davidson and George (1964) presented additional evidence of virus spread in sour cherry orchards by pollen in the Niagara Peninsula. They found that both viruses can spread over a considerable distance, necrotic ringspot virus at least 800 yd and sour cherry yellow virus about 100 yd, but most infections occur within 50 ft of a known source. They suggested that some factor, possibly low pollen viability, might affect the pollen carrying ability of the sour cherry yellows virus. This might explain the slow spread of sour cherry yellows virus as compared with necrotic ringspot virus.

Cameron *et al.* (1973) demonstrated marked reduction in natural transmission of Prunus ringspot in Montmorency cherry by removal of blossoms.

Tests for pollen viability

Converse (1973) tested pollen viability from black raspberry plants infected with raspberry bushy dwarf virus. Pollen was germinated on a 10% sucrose, yeast extract, salts agar and observed by direct microscopic examination of pollen grains after 7 hr incubation at 25°C. He

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obtained a statistically nonsignificant reduction in germination of infected over control pollen.

Freeman, Daubeny, and Stace-Smith (1969), using acetocarmine staining, observed statistically significant difference between pollen collected from healthy and from diseased plants. Differences in viability were observed in response to pollen infection in individual clones of 4 red raspberry cultivars infected with black raspberry necrosis, raspberry mosaic, tomato ringspot and raspberry vein necrosis.

Transmission of certain stone fruit viruses by mechanical methods from ground Prunus pollen was demonstrated by Ehlers and Moore (1957). Gilmer and Way (1960) demonstrated that triturated pollen from necrotic ringspot virus was infectious to buttercup squash.

Virus from pear pollen stored for more than 5.5 years was recovered (Williams and Smith, 1967) by grinding in mercaptoacetic acid and by mechanical inoculation to cucumber cotyledons.

Converse (1967), using triturated black raspberry pollen and mechanical transmission to *Chenopodium quinoa* leaves, demonstrated the presence of virus in pollen in several symptomless red and black raspberry varieties. Converse and Lister (1969) used triturated black raspberry pollen in mechanical transmission tests. They obtained black raspberry latent virus in the pollen of six out of

six infected black and two out of two red raspberry stocks.

Fernow *et al.* (1970), in very limited trials which deserve confirmation, reported pollen transmission by grinding potato pollen from PSTV infected plants and rubbing this to tomato.

Frequently seed set (Kostoff, 1933; Caldwell, 1952; Swaminathan, 1959) is markedly reduced in seed and pollen transmitted viruses. Occasionally fruit shape is modified (Das and Milbrath, 1961). Possibly reduced seed set may be associated with failure of pollen function. The probability is high that pollen function may be impaired when pollen is infected by virus. Information concerning virus effects on pollen function is needed. Studies on percentage of germination of virus infected pollen are limited. Observations have not been reported on germ tube length from infected pollen nor on cytology of virus infected pollen-mother-cells.

Germination of pollen

The process of pollen germination *in vitro* could be considered as a model for the first step in progamic phase of fertilization in higher plants (Linsken, 1969b). It also could be used to investigate the activation of the dehydrated and metabolically inactive pollen grains. The activation process, called germination in all types of diaspores (Marre, 1967), varied in time from species to

species and depended on other controlling external factors. Pollen germination was accompanied by the initiation of protein synthesis (Linsken, 1967; Mascarenhas and Bell, 1969), and the inactive pollen grain is equipped with a ribosomal system which becomes active immediately after hydration. Germination and subsequent growth (Sansten, 1909) of the pollen tubes were very similar to germination of ordinary spores and the growth of the hyphal thread of a fungus.

Pollen grains of different species (Sansten, 1909) germinate in a solution of cane sugar. The concentration of sucrose optimum for pollen germination differed with plant species. With tomato pollen germination is best in a slightly acidulated 10% solution of cane sugar. Irish potato pollen (King and Johnson, 1958) germinated best on agar media containing 14-16% sucrose. Sugar in the medium (Sansten, 1909) may control the osmotic pressure and/or provide substrate for the metabolism of developing pollen tubes. Pollen brought into a sucrose solution caused hydrolysis of sucrose (Dickinson, 1967; Tupy, 1960; Vasil, 1960).

Among inorganic substance in the germination medium, boron, supplied as boric acid or borate, had the most dramatic effect on pollen germination and tube growth. Schmucker (1933) was the first to discover the importance of boron for proper germination of pollen grains and the

growth of tubes. In boron deficient media, germination percentage was low and a high proportion of pollen tubes burst. The optimum concentration of boron in medium varied between 10-200 ppm. A solution of 20% sucrose plus 50 ppm boric acid was suitable medium for germinating *Solanum* pollen *in vitro* (Mortenson *et al.*, 1964).

Calcium also had a remarkable effect on pollen germination and tube growth. Pollen cultured in large populations (Brink, 1924) germinated at higher percentage and formed longer tubes than pollen from small populations on the same medium. This, called the "crowding effect", suggested that a substance which stimulated germination and tube growth diffused into the medium away from the grains or growing tubes. Brewbaker and Kwack (1963) found that calcium was involved. The optimum level varied from 0.03-0.5% $Ca(NO_3)_2$ for different plant species. Pollen grains contained only small amounts of calcium in comparison with other flower parts. It was assumed that calcium diffused rapidly out of pollen in aqueous media leaving an amount inside the grain which was insufficient to support optimal growth.

The most favorable temperature (Smith and Cochran, 1935) for best germination of tomato pollen *in vivo* was 70-85°F. The maximum rate of pollen tube growth occurred at 70°F, with 85°, 50° and 100° ranging in decreasing order.

Cytological abnormalities and virus infection

Costoff (1933) called attention to the fact that in some TMV diseased tobacco varieties and related *Nicotiana* species non-functional pollen was formed. He observed degeneration of chromosomes of pollen-mother-cells during diakinesis.

Caldwell (1952), working in cytology of pollen-mother-cells of tomato severely attacked by aspermy virus, found that at pachytene stage the chromosomes aggregated themselves into an irregular mass which was then followed by a disintegration of the cell.

Swaminathan (1959) studied meiosis of pollen-mother-cells of *Capsicum annuum* L. infected with mosaic and leaf-roll virus. He reported several abnormalities of chromosomes in the pollen-mother-cells such as reduced chiasma frequency, formation of chromosome mosaic cells, binucleate cells and restitution nuclei, irregular anaphase separation, and the presence of monads, dyads, micronuclei and linear titrads.

The 3 viruses which are known to cause impairment in meiosis, namely, TMV, tomato aspermy, and *Capsicum* mosaic and leaf roll, have not been reported pollen transmitted.

Observation of mitotic abnormality in virus infected somatic cells is limited. Wilkinson (1953) studied dividing cells of root tips 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. Metaphase collapse was also observed. He concluded that there was competition between virus particles and chromonematal material for nucleoprotein contained in the nucleolus. In 1960 he studied mitotic abnormalities in a range of Solanaceous species infected respectively with TMV and aspermy virus. Abnormalities observed ranged from persistence of nucleolar material as far as early anaphase to complete amitosis encountered in stemenations of one species examined, viz., *Petunia violacea*. He suggested virus particles, multiplying within dividing cells, competed with the nuclear DNA for the supply of RNA located in the nucleolus.

MATERIALS AND METHODS

Representative isolates of PSTV were obtained from a number of sources as indicated below:

Isolate designation	Original source of inoculum
Canada	Dr. N. S. Wright, Canada Agriculture Station, Vancouver, Canada
Wisconsin	Dr. H. M. Darling, University of Wisconsin
Schultz	Miss M. J. O'Brien (isolate #48 of E. S. Schultz collection), Potato Investigation Laboratory, USDA, Beltsville, Maryland
Diener	Dr. T. O. Diener, Crops Research Division, ARS, USDA, Beltsville, Maryland

These 4 isolates were very similar, producing strong symptoms of bunchy top, and frequently veinal necrosis in tomato.

Mechanical inoculation of a plant

Seed of tomato (*Lycopersicum esculentum* cv. Rutgers), *Physalis floridana* L., and *Solanum dulcamara* L. were germinated in small pots between 27-33°C and grown in continuous light for approximately 10-14 days after which they were transplanted.

Leaves from inoculated PSTV infected tomato were ground in a mortar with a small amount of 0.1 M phosphate buffer pH 7.4. After dusting with carborundum, cotyledons were rubbed gently with a sterile spatula dipped in the crude sap and were then rinsed with distilled water. Plants were then grown under normal greenhouse conditions of alternating air temperature (21-27°C) and natural daylight. With each group of inoculated plants, non-inoculated control plants were included. Controls were treated the same as inoculated plants except that phosphate buffer was used as inoculum instead of the crude sap with buffer.

Collection of pollen grains

PSTV inoculated plants were grown in the greenhouse until flowering. The flowers, at the anthesis stage, were picked between 10:00-11:00 a.m. from PSTV infected and also from control plants. Flowers were then brought into the laboratory and used the same day to determine percentage of pollen germination and length of pollen tubes.

Methods for germination of pollen grains

The medium described by Brewbaker and Kwack (1963) was used. Stock mineral solution composed of H_3BO_3, 0.1 gm; $Ca(NO_3)_2 \cdot 4H_2O$, 0.3 gm; $MgSO_4 \cdot 7H_2O$, 0.2 gm; KNO_3, 0.1 gm; and distilled water, 100 ml. This stock was diluted 1-10 and 1 gram of sucrose was added per 10 ml of the above solution.

A drop of medium was put on a coverslip. Anthers were then shaken just above the coverslip, to dehisce mature, dry pollen over the medium. In order to retard evaporation and maintain a high level of humidity of the medium, the coverslip was inverted and placed on a Van Tieghum cell on a slide. The slide was again placed in a closed petri dish with a moist filter paper in the bottom. The petri dish was placed under light and at a temperature of approximately 21-30°C for pollen germination (Smith and Cochran, 1935).

Percentage of pollen germination

Germinating pollen grains were observed at 30 minute intervals over a 2 hr period with 10X objective. The percentage of pollen germination was determined by counting 100 pollen grains in a field. Pollen grains with germ tube lengths less than the average diameter of the grain were considered as non-germinated. Cultures from both PSTV infected and healthy pollen grains were prepared and examined in the same trial. Statistical treatment of data was made using the student t-test analysis as described by W. Mendenhall (1968).

To obtain percentage of viability, pollen grains were stained in an aqueous I_2-KI solution (I_2, 2 gm; KI, 2 gm, distilled water, 50 ml). A flower was shaken to dehisce pollen grains into a drop of the I_2-KI solution. The drop was then covered with a cover glass and warmed gently over

an alcohol flame. Observation was made under the 10X objective of a compound microscope. Pollen grains which stained brown or dark purple were considered viable and those which stained yellow, non-viable.

Measurement of pollen tube lengths

Measurement of pollen tube lengths was done using an ocular micrometer 2 hr after initiation of pollen germination. Fifty pollen tubes of each culture were measured in microns (μm).

Cytological preparation pollen-mother-cells

Rutgers tomato plants about 10 inches high were inoculated mechanically with PSTV. They were then grown until flowering at 21-27°C on the greenhouse bench illuminated with approximately 1000 ft-c light at 16-17 hrs day length. Plants showed mild symptoms of PSTV infection 3-4 wks after inoculation. Flowers from these plants were collected between 10:00 a.m. and noon and 3:00-4:00 p.m. They were then fixed with farmer's fixative solution (Sass, 1968) overnight. Flowers from healthy plants, growing in the same environment, were collected and treated in the same manner.

All cytological observations were made on pollen-mother-cells stained with iron-acetocarmine. Freshly-made preparations were used in non-permanent form for observing chromosomes. The method consisted of smearing an anther in a drop of the acetocarmine stain. Pieces of anther

wall were removed with fine forceps which left only masses of sporocytes. These were then covered with a cover glass, pressed to separate clumps of pollen-mother-cells and the slide was flamed over an alcohol lamp several times. The edges of the cover glass were sealed with paraffin to prevent evaporation of the dye.

Infectivity of pollen in mechanical transmission

Flowers from PSTV infected plants were picked at the anthesis stage and shaken to dehisce mature pollen grains into a mortar. These were ground with a small amount of 0.1 M phosphate buffer pH 7.4. Cotyledons of 10-14 day old tomato seedlings were inoculated with a glass spatula as described earlier. Control plants were inoculated similarly with mature pollen from healthy plants. In other trials, intact pollen was rubbed on cotyledons as before.

All tomato plants were then grown under normal greenhouse conditions for 2-3 weeks. Tops were then removed and infection determined when the new growth developed in the next 3 weeks.

Ovary infection by PSTV infected pollen grains

Healthy Rutgers tomato plants and PSTV infected plants were grown in the greenhouse and pollinated when flowers on healthy plants were at anthesis stage. Pollen from flowers on PSTV infected plants was shaken onto a

clean slide with a small vibrator commonly used for collecting pollen. Calyx, corolla, and stamens of healthy tomato plants were removed one day before pollination. After 2 wks from pollination flowers were picked. Only the ovaries were saved as other parts of the flowers were removed using sterile razor blades and forceps. Each ovary was then ground separately in a mortar with a small amount of 0.1 M potassium phosphate buffer pH 7.4. This crude sap was used to inoculate cotyledons of tomato testers.

RESULTS

PSTV symptoms on various plant species

Rutgers tomato: - Infected plants were noticeably stunted. Inoculated leaves remained symptomless. Apical leaves which formed after inoculation showed epinasty and rugosity within 4 weeks. Gradually leaves developed mild necrosis of midribs and lateral veins but leaves did not die. These symptoms are in agreement with those described by Raymer and O'Brien (1962). All 4 isolates in these trials incited similar severe symptoms.

Physalis floridana L.: - This is a symptomless host of PSTV (O'Brien and Raymer, 1964). However, in our trials, the infected plants were stunted.

Solanum dulcamara L.: - This has been shown recently to be another symptomless host (Singh, 1973) as well as overwintering host (Yang, 1974) of PSTV. It was symptomless in our trials.

Pollen germination

Pollen grains from healthy and from infected Rutgers tomato were compared using the 4 different isolates of

PSTV. For counting percentages of germination and measurements of pollen germ tube lengths, the low power field (10X) of a compound microscope was used. Fields for study were chosen at random from both healthy and PSTV infected pollen cultures. One hundred pollen grains from such fields of each culture were counted for germination at 30 min intervals over a period of 2 hrs. Germ tube lengths were determined at the end of the 2 hrs incubation period. After that pollen tubes became so long that germ tube lengths could not be accurately determined.

Pollen grains from cultures of healthy and from PSTV infected plants respectively are shown after 2 hrs of incubation (Figures 1 and 2). Some of the pollen grains from PSTV infected plants were shrunken and did not appear as large and as well filled out as those from healthy plants. Shrunken pollen grains did not germinate. Shrunken grains were most noticeable when first shaken from the flowers. Later, during incubation, they became hydrated and more nearly normal in appearance.

At the first 30 min observation the percentage of pollen germination in a total of 100 grains was low. Apparently pollen did not regularly germinate in this short time. In general, more pollen grains from healthy flowers germinated than did those from PSTV infected flowers (Tables 1, 2 and 4). The only exception was with pollen from tomato infected with the Canada isolate of

Figure 1. Pollen germination from healthy Rutgers tomato plants after 2 hrs of incubation. Bar indicates 100 µm.

Figure 2. Pollen germination from PSTV infected Rutgers tomato plants after 2 hrs of incubation. Shrunken pollen grains (arrow) were most apparent soon after removal from the anthers. These usually did not germinate. Note the short germ tubes as compared to those of the healthy pollen (Figure 1). Bar indicates 100 µm.

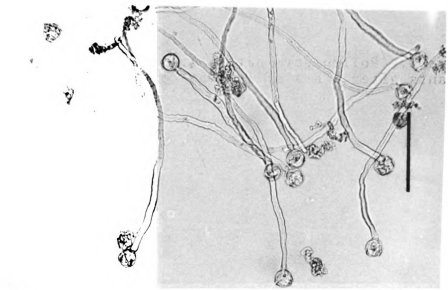


Figure 1

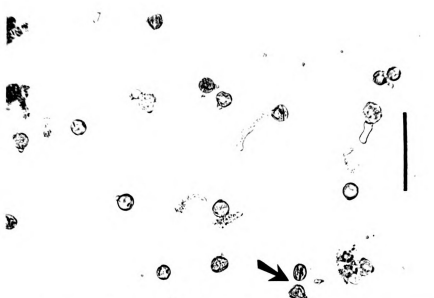


Figure 2

Table 1. Pollen germination of Rutgers tomato from healthy plants and from those infected with PSTV (Diener isolate)

Experiment No.	Type of pollen	Percentage germination after incubation (hrs) 0.5	1.0	1.5	2.0
1	Healthy	82	85	89	89
	PSTV infected	47	49	49	49
	% of control[a]	(57.32)	(57.65)	(55.06)	(55.06)
2	Healthy	79	85	85	85
	PSTV infected	34	48	48	48
	% of control	(43.04)	(56.47)	(56.47)	(56.47)
3	Healthy	46	78	78	78
	PSTV infected	22	25	28	29
	% of control	(47.83)	(32.05)	(35.90)	(37.18)
4	Healthy	68	83	84	87
	PSTV infected	67	67	67	69
	% of control	(98.53)	(80.72)	(79.76)	(79.31)
5	Healthy	0	49	58	65
	PSTV infected	0	12	17	18
	% of control	(0)	(24.49)	(29.31)	(27.69)
6	Healthy	0	61	68	76
	PSTV infected	0	16	20	26
	% of control	(0)	(26.23)	(29.41)	(34.21)

Table 1 (continued)

Experiment No.	Type of pollen	Percentage germination after incubation (hrs) 0.5	1.0	1.5	2.0
7	Healthy	0	62	70	71
	PSTV infected	0	3	7	10
	% of control	(0)	(4.84)	(10.00)	(14.09)
8	Healthy	0	71	76	76
	PSTV infected	0	2	11	13
	% of control	(0)	(2.82)	(14.47)	(17.11)
Average	Healthy	34.40	71.80	76.00	78.40**
	PSTV infected	21.30	27.80	30.90	32.80**
	% of control	(61.92)	(38.72)	(40.66)	(41.84)

[a]Numbers in parentheses indicate percentage of germination from PSTV infected plant as compared to germination from healthy plants.

T test analysis made from observations 2 hrs after induction of germination.

**$t = 9.21$; $P \leq 0.01$.

Table 2. Pollen germination of Rutgers tomato from healthy plants and from those infected with PSTV (Wisconsin isolate)

Experiment No.	Type of pollen	Percentage germination after incubation (hrs) 0.5	1.0	1.5	2.0
1	Healthy	6	53	60	60
	PSTV infected	0	13	24	24
	% of control[a]	(0)	(24.53)	(40.00)	(40.00)
2	Healthy	0	65	91	91
	PSTV infected	0	0	0	0
	% of control	(0)	(0)	(0)	(0)
3	Healthy	0	65	91	91
	PSTV infected	0	44	48	48
	% of control	(0)	(67.69)	(52.75)	(52.75)
4	Healthy	42	64	64	64
	PSTV infected	26	32	42	42
	% of control	(61.91)	(50.00)	(65.63)	(65.63)
5	Healthy	0	83	91	91
	PSTV infected	0	19	28	29
	% of control	(0)	(22.89)	(30.77)	(31.87)

Table 2 (continued)

Experiment No.	Type of pollen	Percentage germination after incubation (hrs) 0.5	1.0	1.5	2.0
6	Healthy	0	83	91	91
	PSTV infected	0	13	20	20
	% of control	(0)	(15.66)	(21.98)	(21.98)
Average	Healthy	8.00	68.80	81.30	81.30**
	PSTV infected	4.30	20.10	23.70	27.20**
	% of control	(53.75)	(29.22)	(29.15)	(33.46)

[a]Numbers in parentheses indicate percentage of germination from PSTV infected plants as compared to germination from healthy plants.

T test analysis made from observations 2 hrs after induction of germination.

**$t = 10.31$; $P \leq 0.01$.

Table 3. Pollen germination of Rutgers tomato from healthy plants and from those infected with PSTV (Canada isolate)

Experiment No.	Type of pollen	Percentage germination after incubation (hrs) 0.5	1.0	1.5	2.0
1	Healthy	5	42	52	62
	PSTV infected	1	9	9	11
	% of control[a]	(20.00)	(21.43)	(17.31)	(17.74)
2	Healthy	4	64	65	65
	PSTV infected	11	42	49	53
	% of control	(275.00)	(65.63)	(75.38)	(81.54)
3	Healthy	19	50	66	77
	PSTV infected	5	39	45	46
	% of control	(26.32)	(78.00)	(68.18)	(59.74)
4	Healthy	6	53	60	60
	PSTV infected	0	0	1	2
	% of control	(0)	(0)	(1.67)	(3.33)
5	Healthy	0	65	91	91
	PSTV infected	0	7	28	50
	% of control	(0)	(10.77)	(30.77)	(54.95)

Table 3 (continued)

Experiment No.	Type of pollen	Percentage germination after incubation (hrs) 0.5	1.0	1.5	2.0
6	Healthy	0	83	91	91
	PSTV infected	24	60	60	60
	% of control	(2400.00)	(72.29)	(65.93)	(65.93)
Average	Healthy	5.70	59.50	70.80	74.30**
	PSTV infected	6.80	29.50	35.30	43.30**
	% of control	(119.30)	(49.58)	(49.86)	(58.28)

[a]Numbers in parentheses indicate percentage of germination from PSTV infected plants as compared to germination from healthy plants.

T test analysis made from observations 2 hrs after induction of germination.

**$t = 5.57$; $P \leqslant 0.01$.

Table 4. Pollen germination of Rutgers tomato from healthy plants and from those infected with PSTV (Schultz isolate)

Experiment No.	Type of pollen	Percentage germination after incubation (hrs) 0.5	1.0	1.5	2.0
1	Healthy	31	79	87	87
	PSTV infected	0	2	4	4
	% of control[a]	(0)	(2.53)	(4.6)	(4.6)
2	Healthy	45	82	89	89
	PSTV infected	24	60	60	60
	% of control	(53.33)	(73.17)	(64.42)	(64.42)
3	Healthy	6	53	60	60
	PSTV infected	0	1	1	2
	% of control	(0)	(1.89)	(1.67)	(3.33)
4	Healthy	0	65	91	91
	PSTV infected	0	1	2	2
	% of control	(0)	(1.54)	(2.20)	(2.20)
5	Healthy	42	64	64	64
	PSTV infected	9	11	22	22
	% of control	(21.43)	(17.19)	(34.38)	(34.38)

Table 4 (continued)

Experiment No.	Type of pollen	Percentage germination after incubation (hrs) 0.5	1.0	1.5	2.0
6	Healthy	42	64	64	64
	PSTV infected	20	32	32	32
	% of control	(40.62)	(50.00)	(50.00)	(50.00)
Average	Healthy	27.70	67.80	75.80	75.80**
	PSTV infected	8.80	17.80	20.20	20.20**
	% of control	(31.77)	(26.25)	(26.65)	(26.65)

[a]Numbers in parentheses indicate percentage of germination from PSTV infected plants as compared to germination from healthy plants.

T test analysis made from observations 2 hrs after induction of germination.

**$t = 5.28$; $P \leq 0.01$.

PSTV (Table 3). In this case pollen grains from infected flowers germinated earlier in 2 out of 6 trials than did those from healthy flowers.

During the next 30 min period (1 hr), many pollen grains in the cultures from healthy plants germinated and also additional pollen grains germinated from PSTV infected plants. At this time pollen germination from PSTV infected plants was consistently lower than that of healthy plants.

Between 1.5 and 2.0 hrs, only a few additional pollen grains in both cultures germinated. Frequently the percentages of pollen germination were essentially similar between the 1.5 and 2.0 hrs observations.

After 30 min of incubation, germination of both infected and healthy pollen was low, but the percent reduction in germination in diseased as compared to healthy pollen was not as great as that obtained in the following observations. Germination of pollen infected with the Diener isolate (Table 1) was reduced to approximately 40% of the healthy control during 1 hr to 2 hrs of incubation. Differences were highly significant after 2 hrs of incubation. Results were essentially similar with the Wisconsin isolate (Table 2) except that the reduction in germination of diseased pollen was greater than with the Diener isolate. Differences between healthy and diseased pollen were highly significant at 2 hrs after germination. The Canada

isolate (Table 3) was somewhat less inhibitory to pollen germination after 1 to 2 hrs of incubation and germination approximated 50% of the control. Differences were again highly significant. The Schultz isolate (Table 4) had the lowest germination as expressed in the percent of control. Differences between virus infected and healthy pollen were highly significant.

In all these trials, approximately 74% of germination of healthy pollen was obtained after 2 hrs of incubation. This is in contrast to the 31% germination obtained with PSTV infected pollen.

Pollen viability may be determined by staining for starch with I_2-KI. This has the advantage of being somewhat less time consuming than direct observation of pollen germination. In a preliminary trial, stainability of pollen from several PSTV infected plants was compared to that from healthy plants (Table 5). Pollen stainability from virus free plants was higher (98%) than that from infected plants (81%). Levels of stainability of healthy and diseased pollen were considerably higher than those obtained by direct germination (Tables 1 through 4). Also differences in pollen stainability from virus infected and from healthy plants were not as great as those obtained earlier by direct germination.

A second comparison between pollen stainability and pollen germination was made simultaneously using tomato

Table 5. Pollen stainability of Rutgers tomato with I_2-KI solution

Type of pollen	Experiment No.	Pollen grains Observed(No.)	Pollen grains Stained(No.)	Percent
Healthy	1	216	215	
	2	284	277	
	3	300	298	
	4	322	316	
	5	285	280	
	6	258	254	
	Total	1165	1640	98.5
PSTV infected	1	268	206	
	2	222	178	
	3	100	92	
	4	328	234	
	5	292	263	
	6	204	171	
	Total	1414	1144	80.9

infected with the Schultz isolate of PSTV. In this trial also, germinability (Table 6) was considerably higher as estimated by the starch staining method than it was by direct observation of pollen germination. Also PSTV infected pollen was more severely affected as compared to the healthy control when measured by direct germination than it was when measured by the I_2-KI viability test. The reason for this discrepancy is not immediately clear.

Pollen germination of *Physalis floridana* L.

Two PSTV isolates were used in these trials, i.e., the Schultz and Canada isolates. Germination of pollen from healthy plants was good (Figure 3) and poor from infected plants (Figure 4). *Physalis floridana* L. is a symptomless host and no flower abnormalities were evident in infected plants. Anthers from infected plants dehisced abundant pollen. However, pollen grains from diseased plants appeared shrunken, not well filled out, and were somewhat smaller than pollen from healthy plants. Shrunken pollen grains did not germinate. Pollen did not germinate in these trials within 30 min of incubation. Germination of healthy *P. floridana* L. pollen was significantly (1% level of probability) higher than germination of pollen infected with either the Canada isolate (Table 7) or with the Schultz isolate (Table 8). Germination reduction (% of control) with the Canada isolate in *P. floridana* L. (Table 7) was considerably greater (22%)

Table 6. Comparison of stainability and direct germination in assessing viability of tomato pollen

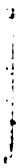
Method of assay	Test No.	Pollen viability	
		Healthy	PSTV infected[a]
Direct germination	Test 1	77/100	19/100
	Test 2	68/100	9/100
	Average	65%	14%
I_2-KI staining	Test 1	430/435	269/319
	Test 2	390/395	151/203
	Average	99%	80%

[a]Schultz isolate of PSTV infected Rutgers tomato.

x/y x = no. of viable pollen grains
y = no. of pollen grains counted

Figure 3. Pollen germination from healthy *Physalis floridana* L. plants after 2 hrs of incubation. Bar indicates 100 µm.

Figure 4. Pollen germination from PSTV infected *Physalis floridana* L. plants after 2 hrs. Note reduced frequency of germination as well as the shorter germ tubes as compared to healthy pollen. Bar indicates 100 µm.



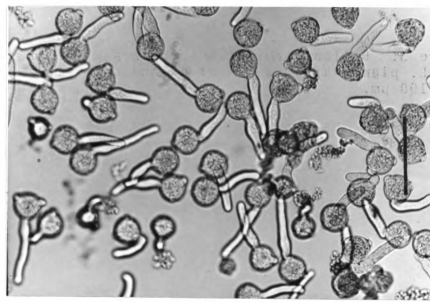


Figure 3

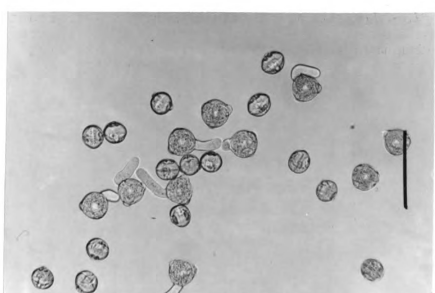


Figure 4

Table 7. Pollen germination of *Physalis floridana* L. from healthy plants and from those infected with PSTV (Canada isolate)

Experiment No.	Type of pollen	Percentage germination after incubation (hrs) 0.5	1.0	1.5	2.0
1	Healthy	0	47	74	88
	PSTV infected	0	10	21	24
	% of control[a]	(0)	(21.27)	(28.37)	(28.27)
2	Healthy	0	76	94	94
	PSTV infected	0	6	16	16
	% of control	(0)	(7.89)	(17.02)	(17.02)
3	Healthy	0	30	78	87
	PSTV infected	0	7	18	23
	% of control	(0)	(23.33)	(23.07)	(26.43)
4	Healthy	0	30	46	63
	PSTV infected	0	0	0	4
	% of control	(0)	(0)	(0)	(0)
5	Healthy	0	30	78	87
	PSTV infected	0	1	40	40
	% of control	(0)	(3.33)	(51.28)	(45.97)

Table 7 (continued)

Experiment No.	Type of pollen	Percentage germination after incubation (hrs) 0.5	1.0	1.5	2.0
6	Healthy	0	10	46	63
	PSTV infected	0	0	0	1
	% of control	(0)	(0)	(0)	(1.58)
7	Healthy	0	22	61	86
	PSTV infected	0	2	7	25
	% of control	(0)	(9.09)	(10.44)	(29.06)
8	Healthy	0	21	39	56
	PSTV infected	0	0	3	6
	% of control	(0)	(0)	(7.69)	(10.71)
Average	Healthy	0	33.25	64.50	78.00**
	PSTV infected	0	3.25	13.13	17.38**
	% of control	(0)	(9.77)	(20.36)	(22.29)

[a]Numbers in parentheses indicate percentage of germination from PSTV infected plants as compared to germination from healthy plants.

T test analysis made from observations 2 hrs after induction of germination.

**t = 19.75; P<0.01.

Table 8. Pollen germination of *Physalis floridana* L. from healthy plants and from those infected with PSTV (Schultz isolate)

Experiment No.	Type of pollen	Percentage germination after incubation (hrs) 0.5	1.0	1.5	2.0
1	Healthy	0	47	74	88
	PSTV infected	0	5	28	34
	% of control[a]	(0)	(10.63)	(37.83)	(38.63)
2	Healthy	0	47	74	88
	PSTV infected	0	19	22	29
	% of control	(0)	(40.42)	(29.72)	(32.95)
3	Healthy	0	76	94	94
	PSTV infected	0	6	23	23
	% of control	(0)	(7.89)	(24.46)	(24.46)
4	Healthy	0	68	78	78
	PSTV infected	0	18	57	57
	% of control	(0)	(26.47)	(73.07)	(73.07)
5	Healthy	0	47	92	92
	PSTV infected	0	14	48	48
	% of control	(0)	(29.78)	(52.17)	(52.17)

Table 8 (continued)

Experiment No.	Type of pollen	Percentage germination after incubation (hrs) 0.5	1.0	1.5	2.0
6	Healthy	0	22	61	86
	PSTV infected	0	2	9	22
	% of control	(0)	(9.09)	(14.75)	(25.58)
7	Healthy	0	21	39	56
	PSTV infected	0	1	4	19
	% of control	(0)	(4.76)	(10.25)	(33.92)
8	Healthy	0	22	61	86
	PSTV infected	0	0	5	23
	% of control	(0)	(0)	(8.19)	(26.74)
Average	Healthy	0	43.75	71.63	83.50**
	PSTV infected	0	8.13	24.50	31.88**
	% of control	(0)	(18.36)	(34.34)	(38.18)

[a]Numbers in parentheses indicate percentage of germination from PSTV infected plants as compared to germination from healthy plants.

T test analysis made from observations 2 hrs after induction of germination.

**$t = 8.8$; $P<0.01$.

than it was in tomato (59%) (Table 3). Germination reduction of pollen of *P. floridana* L. with the Schultz isolate (Table 8) was not as great as that with tomato pollen (Table 4). *Physalis floridana* L. pollen from control plants and from plants infected with the Schultz isolate germinated generally poorer than did tomato pollen.

Pollen germination of *Solanum dulcamara* L.

During the first half hour of incubation pollen from neither healthy nor PSTV infected (Schultz isolate) *S. dulcamara* L. germinated (Table 9). At this time pollen tubes were just beginning to emerge but they were not sufficiently long to count. One hour after incubation pollen tubes extended sufficiently to permit accurate counting and percentage pollen germination of healthy *S. dulcamara* L. plants was already high (82%). That of PSTV infected pollen averaged 50%. During the next hour more PSTV infected pollen germinated. After 2 hrs germination of both healthy and PSTV infected *S. dulcamara* L. pollen was high (Figures 5 and 6). The difference in percent germination 2 hrs after incubation was significant at the 5% level. However, the percent germination in PSTV infected pollen was considerably higher than that with tomato (Table 4) or with *P. floridana* L. (Table 8). Since *S. dulcamara* L. is a symptomless host of PSTV, it may be that pollen is not as severely affected as is pollen of hosts reacting more strongly to the virus.

Table 9. Pollen germination of *Solanum dulcamara* L. from healthy plants and from those infected with PSTV (Schultz isolate)

Experiment No.	Type of pollen	Percentage germination after incubation (hrs) 0.5	1.0	1.5	2.0
1	Healthy	0	76	84	91
	PSTV infected	0	6	16	73
	% of control[a]	(0)	(7.89)	(19.04)	(80.21)
2	Healthy	0	43	73	73
	PSTV infected	0	68	77	77
	% of control	(0)	(158.13)	(105.47)	(105.47)
3	Healthy	0	95	98	98
	PSTV infected	0	72	91	91
	% of control	(0)	(75.78)	(92.85)	(92.85)
4	Healthy	0	92	95	95
	PSTV infected	0	22	33	64
	% of control	(0)	(23.91)	(34.73)	(67.36)
5	Healthy	0	92	99	99
	PSTV infected	0	62	92	92
	% of control	(0)	(62.62)	(92.92)	(92.92)

Table 9 (continued)

Experiment No.	Type of pollen	Percentage germination after incubation (hrs) 0.5	1.0	1.5	2.0
7	Healthy	0	76	80	80
	PSTV infected	0	60	65	70
	% of control	(0)	(78.94)	(81.25)	(87.50)
Average	Healthy	0	81.90	89.40	90.40**
	PSTV infected	0	50.00	64.00	77.30**
	% of control	(0)	(61.05)	(71.58)	(85.50)

[a]Numbers in parentheses indicate percentage of germination from PSTV infected plants as compared to germination from healthy plants.

T test analysis made from observations 2 hrs after induction of germination.

** $t = 2.97$; $P<0.05$.

Figure 5. Pollen germination from healthy *S. dulcamara* L. plants after 2 hrs of incubation. Bar indicates 100 µm.

Figure 6. Pollen germination from PSTV infected *S. dulcamara* L. after 2 hrs of incubation. The frequency of germination contrasts with that obtained with tomato or *P. floridana* L. Bar indicates 100 µm.

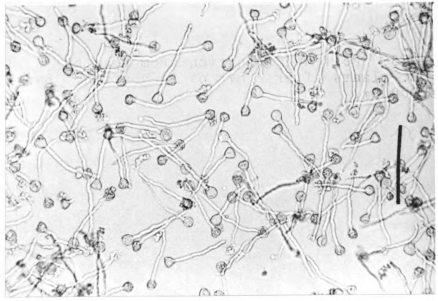


Figure 5

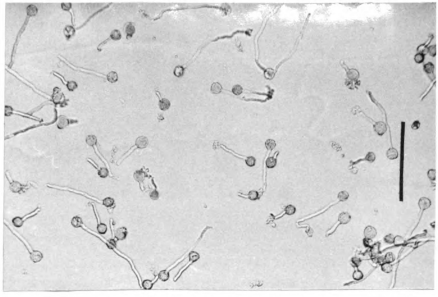


Figure 6

Pollen tube length of Rutgers tomato

Lengths of pollen tubes were measured after 2 hrs incubation. This incubation period was selected because by this time most tubes capable of germinating had done so and yet the tubes had not grown too long for accurate measurement. Fifty pollen tubes of each culture were measured. Experiments were repeated 6-8 times for each isolate of PSTV used (Tables 10, 11, 12 and 13).

Growth of pollen grains infected with the Diener isolate of PSTV was poor as measured by pollen tube length (Table 10, Figure 7). Many pollen grains from PSTV infected plants failed to germinate as previously shown (Table 1) and of those which did germinate pollen germ tubes were usually shorter than those from healthy pollen. Infected and healthy pollen had approximately equal numbers of pollen germ tubes in the range of 114-170 μm. Below that germ tube length category (Table 10) considerably more PSTV infected pollen grains were present than were healthy pollen grains. Above that germ tube length category (114-170 μm) frequency of healthy pollen was much higher than that of diseased pollen. Over 40% of the healthy pollen grains reached the maximum germ tube length category. In contrast, only 1% of the germ tubes from diseased plants reached germ tube length of this order.

Similarly the pollen from the Wisconsin, Canada, and Schultz isolates (Tables 11, 12 and 13) produced short germ

Table 10. Pollen tube length of Rutgers tomato from healthy plants and from those infected with PSTV (Diener isolate)

Experiment No.	Type of pollen	Frequency distribution of pollen tube lengths (μm)					
		0-56	57-113	114-170	171-226	227-282	over 282
		No.	No.	No.	No.	No.	No.
1	Healthy	0	0	6	9	24	11
	PSTV infected	12	30	7	1	0	0
2	Healthy	0	3	4	5	5	33
	PSTV infected	22	20	5	3	0	0
3	Healthy	0	6	2	2	6	34
	PSTV infected	24	5	12	7	0	2
4	Healthy	0	0	6	4	6	34
	PSTV infected	17	3	4	11	13	2
5	Healthy	0	0	7	9	9	25
	PSTV infected	37	11	2	0	0	0
6	Healthy	0	10	0	7	13	20
	PSTV infected	25	12	7	4	1	0

Table 10 (continued)

Experiment No.	Type of pollen	Frequency distribution of pollen tube lengths (μm)					
		0-56	57-113	114-170	171-226	227-282	over 282
		No.	No.	No.	No.	No.	No.
7	Healthy	0	0	27	10	10	3
	PSTV infected	43	2	4	0	1	0
8	Healthy	0	10	7	20	10	3
	PSTV infected	39	10	1	0	0	0
Total	Healthy	0 (0%)	29 (7%)	59 (15%)	66 (16%)	83 (21%)	163 (41%)
	PSTV infected	219 (55%)	93 (23%)	42 (10%)	26 (7%)	15 (4%)	5 (1%)

Table 11. Pollen tube length of Rutgers tomato from healthy plants and from those infected with PSTV (Wisconsin isolate)

Experiment No.	Type of pollen	Frequency distribution of pollen tube lengths (μm)					
		0-56	57-113	114-170	171-226	227-282	over 282
		No.	No.	No.	No.	No.	No.
1	Healthy	0	38	10	2	0	0
	PSTV infected	39	10	1	0	0	0
2	Healthy	2	0	0	1	7	40
	PSTV infected	50	0	0	0	0	0
3	Healthy	2	0	0	1	7	40
	PSTV infected	30	18	2	0	0	0
4	Healthy	0	4	22	11	7	6
	PSTV infected	17	21	9	2	1	0
5	Healthy	0	1	5	4	3	37
	PSTV infected	15	30	4	1	0	0
6	Healthy	0	1	5	4	3	37
	PSTV infected	4	26	12	7	0	1
Total	Healthy	4 (1%)	44 (15%)	42 (14%)	23 (8%)	27 (9%)	160 (53%)
	PSTV infected	155 (52%)	105 (35%)	28 (9%)	10 (3%)	1 (1%)	1 (0%)

Table 12. Pollen tube length of Rutgers tomato from healthy plants and from those infected with PSTV (Canada isolate)

Experiment No.	Type of pollen	Frequency distribution of pollen tube lengths (μm)					
		0-56	57-113	114-170	171-226	227-282	over 282
		No.	No.	No.	No.	No.	No.
1	Healthy	0	0	5	7	8	30
	PSTV infected	16	30	4	0	0	0
2	Healthy	0	5	4	3	15	23
	PSTV infected	39	5	6	0	0	0
3	Healthy	0	38	10	2	0	0
	PSTV infected	50	0	0	0	0	0
4	Healthy	0	0	0	1	7	40
	PSTV infected	15	16	10	5	4	0
5	Healthy	0	1	5	4	3	37
	PSTV infected	14	27	3	1	5	0
Total	Healthy	2 (1%)	44 (17%)	24 (10%)	17 (7%)	33 (13%)	130 (52%)
	PSTV infected	134 (54%)	78 (31%)	23 (9%)	6 (2%)	9 (4%)	0 (0%)

Table 13. Pollen tube length of Rutgers tomato from healthy plants and from those infected with PSTV (Schultz isolate)

Experiment No.	Type of pollen	Frequency distribution of pollen tube lengths (μm)					
		0-56	57-113	114-170	171-226	227-282	over 282
		No.	No.	No.	No.	No.	No.
1	Healthy	0	5	9	13	21	2
	PSTV infected	46	4	0	0	0	0
2	Healthy	0	6	9	7	7	21
	PSTV infected	0	30	12	3	1	4
3	Healthy	0	38	10	2	0	0
	PSTV infected	49	0	1	0	0	0
4	Healthy	2	0	0	1	7	40
	PSTV infected	50	0	0	0	0	0
5	Healthy	0	4	22	11	7	6
	PSTV infected	38	11	1	0	0	0
6	Healthy	0	4	22	11	7	6
	PSTV infected	13	30	5	1	1	0
Total	Healthy	2 (1%)	57 (19%)	72 (24%)	45 (15%)	49 (16%)	75 (25%)
	PSTV infected	196 (65%)	75 (25%)	19 (6%)	4 (2%)	2 (1%)	4 (1%)

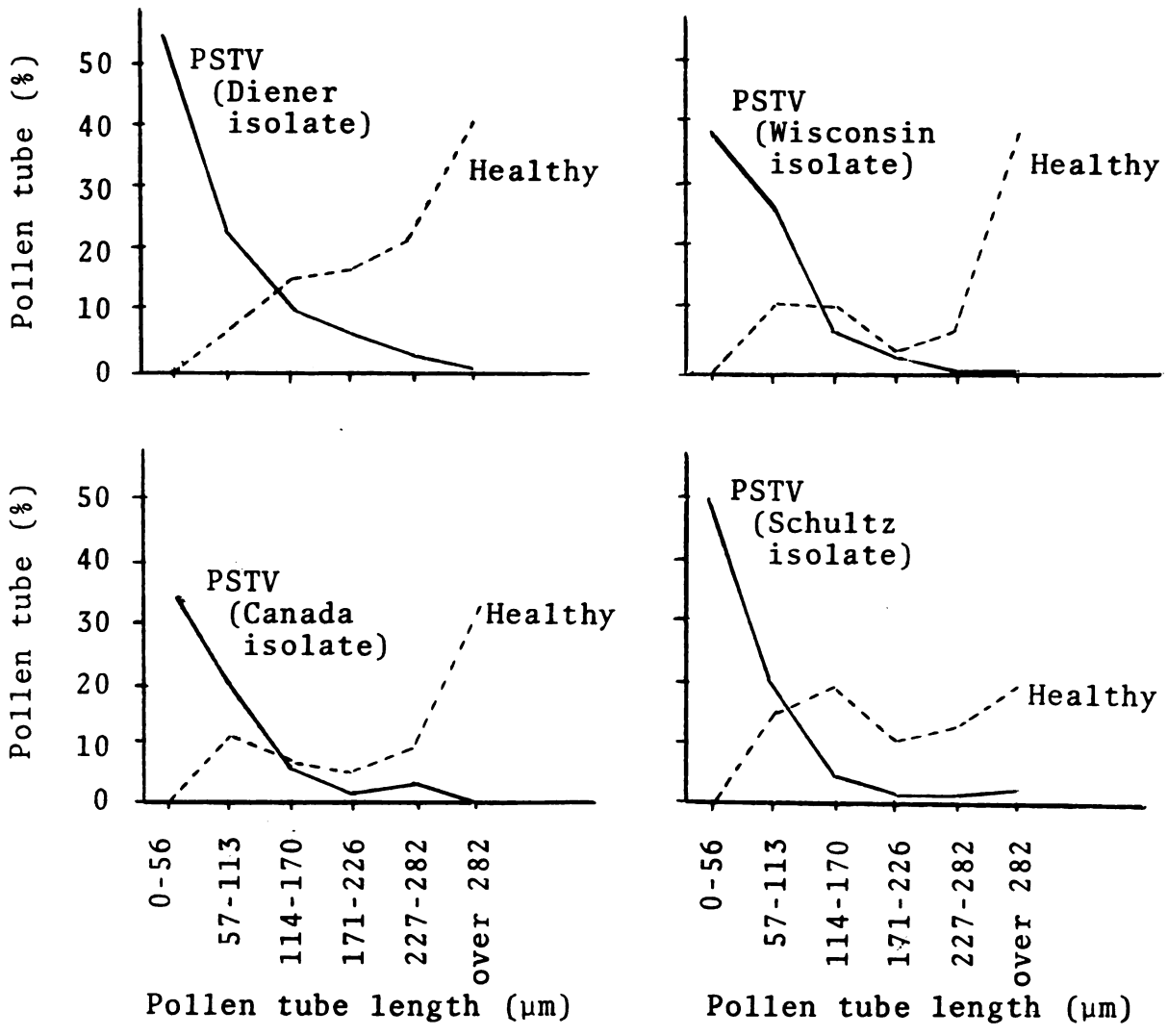


Figure 7. Frequency distribution of pollen tube lengths of Rutgers tomato 2 hrs after incubation.

tubes. Pollen from plants infected with these isolates of PSTV had respectively 87%, 85%, and 90% of the germ tubes less than 114 µm in length. In contrast, healthy controls with these same isolates had respectively 16%, 18%, and 20% of the germ tubes less than 114 µm in length.

Pollen tube lengths of *Solanum dulcamara* L.

Measurements of pollen tube lengths of *S. dulcamara* L. were made after 2 hrs of incubation and the experiment was repeated 6 times (Table 14). No measurements were made of pollen which had not germinated. One-half of the diseased pollen tubes were less than 57 µm in length whereas 80% of the healthy pollen tubes exceeded 57 µm. Germ tubes of pollen grains from PSTV infected plants were shorter than those from healthy plants. In general, pollen tube lengths of *S. dulcamara* L. were considerably shorter than were those of Rutgers tomato plants. None of the pollen in this experiment had tube lengths over 282 µm (Table 14). It was previously shown that pollen grains from PSTV infected *S. dulcamara* L. plants germinated slower than did those from healthy plants (Table 8). Even though PSTV was essentially symptomless in most respects, pollen germ tube length was severely reduced by infection. This is interesting in that the percent pollen germination was not severely reduced in infected plants.

Table 14. Pollen tube length of *Solanum dulcamara* L. from healthy plants and from those infected with PSTV (Schultz isolate)

Experiment No.	Type of pollen	Frequency distribution of pollen tube length (μm) 0-56	57-113	114-170	171-226
1	Healthy	0	50	0	0
	PSTV infected	50	0	0	0
2	Healthy	0	3	47	0
	PSTV infected	18	29	3	0
3	Healthy	21	19	10	0
	PSTV infected	24	25	1	0
4	Healthy	0	0	50	0
	PSTV infected	14	36	0	0
5	Healthy	30	20	0	0
	PSTV infected	15	31	4	0
Total	Healthy	51 (20%)	92 (37%)	107 (43%)	0 (0%)
	PSTV infected	126 (51%)	116 (46%)	8 (3%)	0 (0%)

Cytology of PSTV infected pollen grains

Normal meiosis in Rutgers tomato plants is comparable with that of other diploids. The normal chromosome number of 24 forms 12 bivalents (Figures 8 and 9). These bivalents align themselves on a single equatorial plate (Figure 13) followed by 12-12 bipolar anaphase I disjunction of chromosomes. Meiosis is synchronized so that sister nuclei proceed simultaneously to metaphase II and anaphase II. Cytoplasmic cleavage then occurs and thus typical four-celled quartets are formed.

The early prophase stage of PSTV infected Rutgers tomato pollen appeared to be fully regular. All cells entered the diakinesis stage with 12 normal appearing bivalents (Figures 8 and 9). However, these bivalents tended to form into groups. A total of 308 cells was observed at this stage; grouping was found in 151 cells or 49% (Table 14). Chromosome grouping was abnormal in that each group functioned more or less independently within the cell. The most common type of this abnormality evidenced by grouping, which accounted for 60 of the 151 abnormal cells at diakinesis, was separation into two groups of 1 and 11 (Figure 10), 5 and 7, 4 and 8 (Figure 11), 6 and 6, or 3 and 9.

At metaphase I, instead of forming the normal one metaphase plate (Figure 13), microplates of varying chromosome numbers were formed (Figures 14 and 15). At

Figure 8. Normal prophase I (diplotene) showing 12 bivalents.

Figure 9. Normal prophase I (diakinesis).

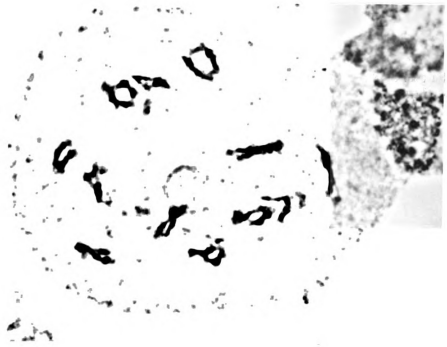


Figure 8

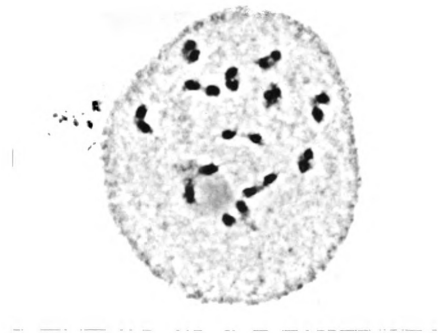


Figure 9

Figure 10. Multipolar prophase I (diakinesis) showing 1-11 separation of chromosomes and cell furrowing (arrow).

Figure 11. Multipolar meiosis I (diakinesis) showing (8-4) separation of chromosomes.

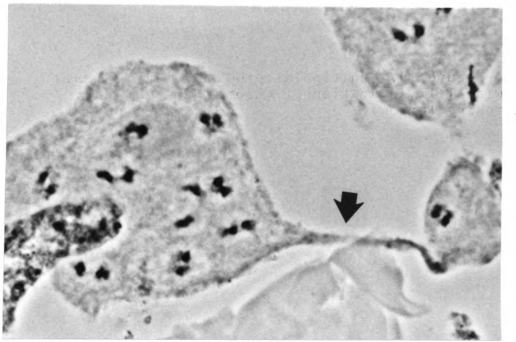


Figure 10

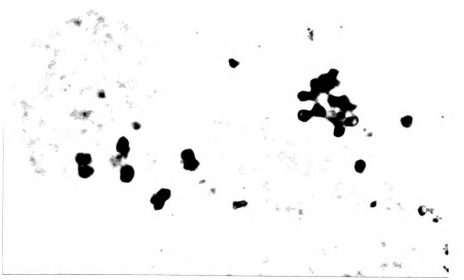


Figure 11

Figure 12. Anaphase I. An intact cell with 6 univalents.

Figure 13. Normal metaphase I with one metaphase plate.

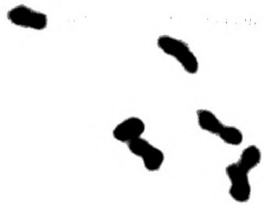


Figure 12

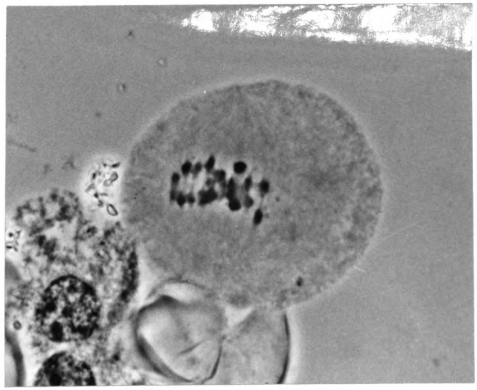


Figure 13

Figure 14. Multipolar metaphase I with micro-plates of (4-8).

Figure 15. Multipolar metaphase I with (2-7-3) separation of chromosomes into 3 groups.

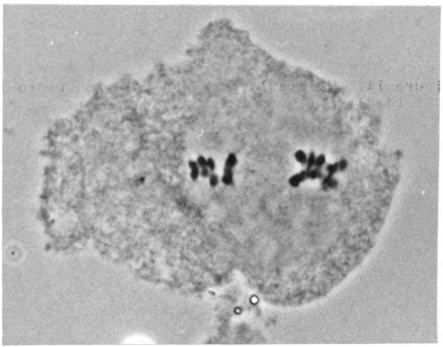


Figure 14

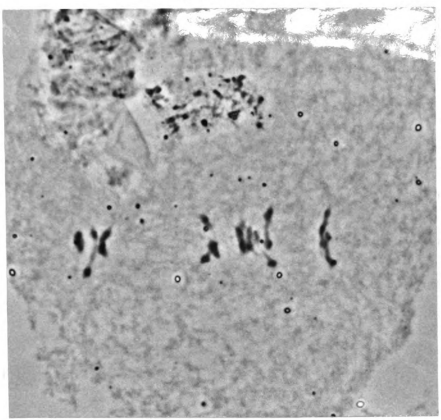


Figure 15

metaphase I chromosomes in 17 of the 45 cells observed (38%) formed into groups (table 15). Two group separation was still the most common (Figure 14). Occasionally 3 group separation (Figure 15) was observed, but it was not as frequent as 2 group separation. Separation into more than 3 groups was not observed.

At anaphase I, grouping of chromosomes, if present, was apparently rare, as none was found. Chromosome abnormality at this stage was unequal segregation of chromosomes, stickiness of chromosomes, and 'disorganized anaphase.' Unequal segregation of chromosomes occurred in 28 of the 70 cells examined or 40% (Table 15). Chromosomes segregated into 11-13 or 10-14 at this stage. Lack of synchronization of sister cells in diseased plants was also observed. Cytokinesis could occur also at this time. This gave rise to sister cells with from 1 to 11 univalents. Figure 12 shows an intact cell with 6 normal univalents.

In healthy pollen grains of Rutgers tomato there was grouping of chromosomes at diakinesis and metaphase I. However, the percentage of cells with abnormal chromosome behavior was very low (Table 15) as compared to that in PSTV infected pollen. In addition, unequal segregation of chromosomes at anaphase I and non-synchronized division were apparently rare, as none was found in healthy pollen grains.

Table 15. Abnormal chromosome behavior at different meiotic stages in PSTV infected pollen grains

Meiotic stages and cell characteristics		Healthy Rutgers tomato pollen grains	PSTV infected Rutgers tomato pollen grains
	Cells observed (no.)	259	308
Diakinesis	Cells with grouping of chromosomes (no.)	9	151
	Cells with grouping of chromosomes (%)	3.5	49
	Cells observed (no.)	75	45
Metaphase I	Cells with grouping of chromosomes (no.)	2	17
	Cells with grouping of chromosomes (%)	2.7	37.8
	Cells observed (no.)	18	70
Anaphase I	Unequal segregation (no.)	0	28
	Unequal segregation (%)	0	40

Infectivity of ground pollen from PSTV infected plants

Rutgers tomato: - PSTV infected flowers dehisced relatively small amounts of pollen as compared to those of healthy plants. Pollen grains from PSTV infected flowers ground in a small amount of 0.1 M phosphate buffer was used as inoculum. For this many flowers were required for each trial. Cotyledons of tomato indicator plants were inoculated and only those showing typical symptoms of PSTV infection were considered positive (Table 16). Percentage of plants infected by mechanical inoculation of ground pollen was 26% and is considered relatively high infectivity for such small amounts of inoculum.

Table 16. Infectivity by mechanical inoculation of ground pollen from PSTV infected Rutgers tomato

Experiment No.[a]	Number of plants showing symptoms	Number of plants inoculated
1	1	9
2	0	8
3	4	8
4	1	4
5	7	19
6	6	25
Total	19	73

[a]On each day the pollen obtained from a number of flowers was ground and a single group of plants was inoculated.

Solanum dulcamara L.: - The experiment was performed in the same manner as was done by using tomato pollen. Due to the fact that *S. dulcamara* L. is a symptomless host for PSTV and flowered continuously in the greenhouse, there was a considerable amount of pollen to work with. The number of tester plants was therefore larger than the trial using tomato pollen grains. PSTV was transmitted through pollen grains of *S. dulcamara* L. (Table 17) in 7 out of the 9 inoculation trials. Out of 86 inoculated plants, 17 showed PSTV symptoms (18%). Thus the percentage of transmission of PSTV through *S. dulcamara* L. pollen grains is quite high but was not as high as with tomato pollen.

Table 17. Infectivity by mechanical inoculation of ground pollen from PSTV infected *Solanum dulcamara* L.

Experiment No.[a]	Number of plants showing symptoms	Number of plants inoculated
1	1	18
2	0	9
3	1	12
4	0	6
5	1	21
6	5	12
7	9	18
Total	17	96

[a]In each experiment pollen obtained from a number of flowers was ground and a single group of plants was inoculated.

Infectivity of intact *Solanum dulcamara* L. pollen

Intact pollen grains collected from blossoms on infected plants were dusted directly on tomato cotyledons which had been rubbed with 600 mesh carborundum in distilled water before inoculation. After pollen grains had been applied leaf surfaces were again rubbed with a glass spatula. Symptoms developed after 4-6 weeks and results were collected (Table 18). Out of 77 plants tested, only 1 plant showed positive infection (1.4%).

Table 18. Infectivity from intact pollen grains of *Solanum dulcamara* L.

Experiment No.	Number of plants showing symptoms	Number of plants inoculated
1	0	32
2	0	21
3	1	24
Total	1	77

Ovary infection of Rutgers tomato by PSTV infected pollen

Flowers from healthy Rutgers tomato were pollinated from PSTV infected flowers. Two weeks after pollination, fruits were picked if pollination had been successful. Female parts of healthy flowers pollinated with infected pollen frequently turned yellow in approximately 2 days

and soon the flowers fell off the plant. Thus not all pollinations attempted were successful. In fact, frequency of successful pollination was very low, and apparently diseased pollen was relatively ineffective and the ovary abcised. Failure of pollination may have been due in part to the shrunken pollen grains which did not germinate, or pollen tubes may not have elongated sufficiently to reach the ovule.

Following successful pollination, usually 14 days, the ovary was removed with sterile razor blades and ground separately in a mortar with a small amount of 0.1 M phosphate buffer pH 7.4. This was used as inoculum on cotyledons of tomato seedlings. Following inoculation, typical PSTV symptoms developed (Table 19). The virus inoculated tester plants (10%) showed symptoms. This low percentage of tomato infection suggests that the virus concentration within a given ovary must have been very low 2 weeks after pollination.

The low frequency of fruit set made it difficult to obtain sufficient numbers of ovaries for extensive testing. However, in these 9 ovaries obtained 78% carried the virus.

Table 19. Percentage of ovary infection of Rutgers tomato flowers with PSTV

Ovule No.	Number of plants with symptoms	Number of plants tested
1	1	15
2	1	14
3	2	15
4	4	15
5	5	12
6	2	24
7	0	20
8	1	20
9	0	20
Total	16	155

DISCUSSION

Relatively few virus diseases are transmitted by infected pollen. Where pollen transmission has been identified, relatively little attention has been given to the several effects of virus infection on pollen function. These effects might well modify germinability, germ tube length, pollen-mother-cell cytology, etc.

Although in this study of PSTV the extent of reduction of pollen and seed set was not determined, it became evident that infected tomato plants produced considerably less pollen and seed than did healthy plants. Other pollen transmitted viruses which have been reported to cause low production of pollen and seed set in infected plants are elm mosaic virus (Callahan, 1957), lychnis ringspot virus of *Beta vulgaris*, *Callistephus chinensis*, and *Silene noctiflora* (Bennett, 1959), aspermy virus of tomato (Kostoff, 1933; Caldwell, 1952) and mosaic and leaf roll virus of *Capsicum annuum* L. (Swaminathan, 1959).

Stainability of pollen grains from Rutgers tomato plants was determined by the I_2-KI starch staining method. Percentage reduction in pollen stainability of PSTV

infected tomato plants was 81% as compared to healthy plants (99%). Freeman *et al.* (1969), using acetocarmine staining, also observed reduction in stainability of pollen grains from 4 raspberry cultivars infected with black raspberry necrosis, raspberry mosaic, tomato ringspot, and raspberry vein necrosis. Differences between percentage of abortion in the control and in diseased plants, although statistically significant, were relatively small and virus infection did not drastically increase abortion. Freeman did not attempt to germinate pollen grains. His results are in essential agreement with those I obtained in that differences between stainability of healthy and diseased pollen were not widely separated.

The effect of virus on pollen grain germinability was recently examined by Converse (1973), who worked with raspberry bushy dwarf virus infected raspberry plants. He obtained, by streaking pollen grains on sucrose-yeast extract agar and checking for germination after 7 hrs of incubation at 25°C, a statistically nonsignificant difference in germination rate between pollen collected from virus-free and from virus-infected plants. In this study with PSTV, the effect on pollen germination was demonstrated using liquid media. Statistically significant differences were obtained from comparisons made between healthy and PSTV infected pollen of 3 different

plant species. Even though plants developed severe symptoms (Rutgers tomato), mild symptoms (*P. floridana* L.), or no symptoms at all (*S. dulcamara* L.), responses as measured by pollen germination were relatively similar.

Tomato pollen viability as determined by direct observation of germinating spores was considerably lower than that determined by the I_2-KI starch staining method. Pollen from healthy plants germinated approximately 76% while, in contrast, stainability tests indicated 99% viability. Diseased pollen responded 33% germination and 81% stainability. The reason for this discrepancy is not readily apparent. This discrepancy might be understood by postulating that starch content of pollen cells may have been sufficient to support germination and give a positive stain test, but that chromosome aberration in meiosis may have precluded germination.

The effect of virus on pollen germ tube length has not been investigated previously to the best of my knowledge. With Rutgers tomato and *S. dulcamara* L., differences in germ tube lengths between healthy and PSTV infected plants after 2 hrs incubation were statistically significant. Possibly PSTV infected pollen failed to grow rapidly enough to reach the ovule before abcission.

Mechanical transmission of viruses to suitable indicator plants by using triturated pollen has been

demonstrated for a number of viruses, cherry pollen (Gilmer and Way, 1960), pear pollen (Williams and Smith, 1967), and black raspberry pollen (Converse, 1967).

Pollen collected from flowers of PSTV infected plants contained sufficient virus to infect tomato when small amounts of pollen were ground in the conventional manner and rubbed on tomato seedlings. In 1970 Fernow *et al.* in limited trials ground pollen from PSTV infected potato and rubbed to tomato seedlings. He inoculated 13 pairs of plants and obtained infection in 2 pairs (approximately 31% recovery). In subsequent tests from the symptomless inoculated tomato he identified additional plants infected with a mild strain of PSTV. I used the same method as that of Fernow *et al.* with tomato pollen. I inoculated over 70 tomato plants and obtained 26% recovery. It is difficult to understand why virus was more readily recovered from potato and tomato pollen than it was from *S. dulcamara* L. pollen (18% recovery). Possibly PSTV did not reach as high a titre in pollen or did not as frequently infected pollen of *S. dulcamara* L. plants as it did pollen of potato and tomato. It is interesting that germination of pollen tubes of *S. dulcamara* L. was not as severely reduced by PSTV infection as was that of tomato.

Pollen grains from PSTV infected *S. dulcamara* L. plants, when used directly without grinding for inoculating

cotyledons of tomato seedlings, were relatively non-infectious. This suggests release of infectious material by grinding of the pollen grains. Possibly most of the virus RNA was inside the pollen grains.

The virus could be recovered from ovaries soon after pollination. This indicates that pollen grains from PSTV infected plants, even though impaired in function, contained sufficient virus to infect ovaries. Apparently virus was present in very low concentration as very few tester plants ultimately developed symptoms. However, 7 out of the 9 ovaries carried at least some infective virus suggesting that infection occurred with relatively high frequency.

The only cytological studies of virus infection which I have found are those of Kostoff (1933), Caldwell (1952) and Swaminathan (1959). Kostoff investigated cytological details of pollen-mother-cells of tobacco plants severely attacked by mosaic virus. He observed degeneration of chromosomes during the diakinesis. Caldwell worked with tomato pollen-mother-cells infected with aspermy virus. He reported chromosome aggregation at pachytene stage of meiosis. Swaminathan (1959) worked with mosaic and leaf roll virus of *Capsicum annuum* L. He found several abnormalities of chromosomes in the pollen-mother-cells such as reduced chiasma frequency, formation of chromosome mosaic cells, binucleate cells

and restitution nuclei, irregular anaphase separation, and the presence of monads, dyads, micronuclei, and linear titrads.

Pollen disfunction was severe in tomato infected by PSTV. Cytology of the pollen-mother-cells of PSTV infected tomato plants demonstrated multipolar-meiosis. The phenomenon of multipolar-meiosis (Tai, 1970) has been described by various terms including "double-plate metaphase" (Vaarama, 1949), "reductional groupings" (Wilson, 1950), "complement fractionation" (Thompson, 1962) and "multipolar-spindles" (Kabarity, 1966). Multipolar-meiosis (Tai, 1970) is a phenomenon by which the meiotic or mitotic chromosome complement is sub-divided into two or more groups that function more or less independently within the cell. This phenomenon is characterized by the formation of two or more meta-phase plates, appropriately called "microplates", within a single cell. The consequence of multiple plates and spindles is the production of daughter cells lacking the full chromosome complement. Pollen grains with reduced number of chromosomes should not be expected to function normally and could very well cause reduction in percentage pollen germination and shortening of pollen germ tubes.

Multipolar divisions have been reported in both animal and more frequently in plant species. They can occur spontaneously (Clayberg, 1959; Thompson, 1962;

Vasek, 1962) or they can be induced artificially by temperature shock (Huskins and Cheng, 1950), low concentration of colchicine (Ostergren, 1950), antibiotics (Wilson, 1950), irradiation (Puza and Srb, 1964), and other chemical agents (Kabarity, 1966). The low frequency of aberrations (Table 15) in healthy pollen-mother-cells indicates a low level of spontaneous multipolar division in tomato. Clayberg (1959) reported that the proportion of stainable pollen from plants with multipolar meiosis varied from zero to ten percent and that some stainable pollen was probably functional.

Clayberg (1959) and Thompson (1962) suggested that the mechanism of multipolar divisions consisted of one or any combination of phenomena occurring in pre-meiotic cells or in meiotic cells at the first or second division. These phenomena were (1) subdivision of the chromosome complement into 2 or more groups that function independently within the cell; (2) non-disjunction of chromosomes; and (3) unequal distribution of chromosomes to the 2 polar regions at anaphase.

The multipolar meiosis observed in the pollen-mother-cells of PSTV infected tomato plants offers an explanation for the reduction in both pollen production and germination as well as the shortening of pollen germ tubes. PSTV infection caused multipolar meiosis and resulted in pollen cells with variable numbers of chromosomes. Some

of them may have functioned normally up to an undetermined stage, or they may have aborted before maturity. My observations differ from those of Kostoff and Caldwell with pollen-mother-cells from tomato plants infected with aspermy virus in that degeneration of chromosomes was not observed at any stage of meiosis. To the best of my knowledge, multipolar meiosis has not yet been reported to occur in pollen-mother-cells of virus infected plants. Conceivably reduced seed set in PSTV infected tomato plants results from any or a combination of possible disfunctions. These might include: (1) the low percentage of pollen germination may have resulted in less than complete fertilization of the many ovules in a tomato fruit; (2) the germ tubes capable of elongation may have failed in fertilization of ovules because germ tubes grew so slowly that they did not reach the ovules; (3) abnormal chromosome complements within the pollen germ tube may have failed to stimulate ovule development; (4) should meiosis in the egg mother cell of PSTV infected plants be as severely impaired as is meiosis in the pollen-mother-cell, failure of ovule development may be independent of aberrations in pollen function.

SUMMARY

Pollen grains from healthy *Lycopersicum esculentum* Mill., cv. *Rutgers* germinated with significantly higher percentage than did those from plants infected with each of the 4 strains of PSTV tested.

Pollen tube lengths from healthy tomato plants were consistently longer than those from PSTV infected plants.

Pollen grains from two symptomless hosts of PSTV, namely, *Physalis floridana* L. and *Solanum dulcamara* L., were tested against healthy plants for percentage of pollen germination and length of pollen tubes. Pollen grains from these two symptomless hosts responded similarly to those from PSTV infected Rutgers tomato. Germination was reduced from 84% in healthy to 32% in PSTV (Schultz isolate) infected *P. floridana* L.; 90% in healthy to 77% in PSTV (Schultz isolate) infected *S. dulcamara* L. Of the healthy pollen tubes of *S. dulcamara* L., 43% were 114-170 μm in length whereas 51% of those from PSTV infected plants were less than 56 μm long.

Stainability with I_2-KI solution of healthy pollen grains from Rutgers tomato plants indicated that 99% were viable as compared to 65% germination in media. Similarly

the stain test of PSTV infected pollen indicated 80% viability whereas only 14% of the pollen tube germinated in media.

PSTV infected Rutgers tomato pollen-mother-cells exhibited abnormal behavior of chromosomes in meiosis. At the diakinesis stage, chromosomes tended to form groups. Grouping of chromosomes was also observed at the metaphase I. At the anaphase I, chromosomes segregated unequally. This led to the formation of pollen grains with chromosome number less than the normal number of 12.

Pollen from PSTV infected Rutgers tomato and from *S. dulcamara* L. infected 26% and 18% of the plants respectively when ground and used as inoculum. When the pollen grains were used directly without grinding for inoculation cotyledons of tomato seedlings, less than 1% of the plants became infected.

PSTV infected pollen was ineffective in stimulating fruit set in healthy plants and most ovaries abscised. Of many pollinations attempted only 9 ovaries were set. After 14 days these were ground and inoculated to tomato seedlings. Of the 9 ovaries obtained, 7 ovaries contained sufficient virus to infect tester plants. Virus concentration in the ovaries at this time was apparently very low, as only 10% of the tester plants became infected.

LITERATURE CITED

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