ALBINISM OF POTATO SPINDLE TUBER VIROID-INFECTED RUTGERS TOMATO IN CONTINUOUS LIGHT[1]

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Abstract

Potato spindle tuber viroid (PSTV) caused albinism in the new growth of Rutgers tomato under continuous light. The albinism response was obtained with 4 isolates causing severe symptoms in tomato and with 1 mild isolate which is normally symptomless.

Rutgers tomato inoculated with the 4 severe strains of PSTV developed typical 'bunchy top' symptoms in 12 to 16-hr day lengths. Symptoms developed slower and much less distinctly on plants incubated in 6-hr days. New growth in PSTV-infected plants was green in short days (12-hr illumination) and chlorotic to white in continuous light.

Infectivities of extracts from white portions of plants were from 3 to 10 times greater than those from green portions of infected plants.

Albino symptoms also developed in other tomato varieties when inoculated with PSTV and kept under continuous light. Albinism was most pronounced under continuous light at 30°C, somewhat less intense at 24° , and at 16°, new growth of infected leaves and stems remained green. Albinism did not develop in other *Solanum* species which are symptomless hosts of PSTV nor in seedling potato plants grown in continuous light.

Resumen

El viroide causante del tubérculo ahusado en la papa (PSTV) produjo albinismo en los nuevos brotes de la variedad de tomate Rutgers cuando se tuvo bajo luz contínua. La reacción de albinismo se obtuvo con cuatro aislamientos que producen síntomas severos en tomate y con un aislamiento benigno que no causa síntomas normalmente.

La variedad de tomate Rutgers al ser inoculada con las cuatro razas severas de PSTV desarrolló síntomas típicos de cogollo en ramillete (bunchy top) bajo 12-16 horas de luz por día. Los síntomas se produjeron más lentamente y con mucho menos intensidad en plantas que tuvieron la incubación con días de 6 horas de luz. Los nuevos brotes en las plantas infectadas con PSTV fueron verdes en condiciones de días cortos (12 horas

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de luz) y fueron cloróticos o blancos si la luz era contínua. La infectividad de los extractos de las porciones blancas de las plantas fue de 3 a 10 veces mayor que la de las porciones verdes en las plantas infectadas.

Los síntomas de albinismo también se desarrollaron en otras variedades de tomate cuando se inocularon con PSTV conservándolas bajo luz contínua. El albinismo se pronunció al máximo bajo luz contínua a 30°C, fue algo menos intenso a 24°C y a 16°C las hojas y tallos de los nuevos brotes permanecieron verdes. El albinismo no se desarrolló en otras especies de *Solanum* que son huéspedes sin síntomas del PSTV ni tampoco en plántulas de papa cultivadas bajo luz contínua.

Introduction

Rutgers tomato was first demonstrated by Raymer and O'Brien (8) as susceptible to the potato spindle tuber viroid (PSTV) and the plant has since been widely used as an indicator host. Under natural light conditions symptoms consist of epinasty, rugosity, vein necrosis, and severe stunting. Raymer and O'Brien (8) indicated that symptoms developed at Beltsville, MD in early fall within 10 days and that longer incubation periods up to 42 days were required in November through January. Raymer, O'Brien and Merriam (9) reported that greenhouse conditions of bright sunlight (12 to 15-hr days) at temperatures of 75 to 85°F (24 to 30°C) were optimum for symptom expression in tomato. Whitney and Peterson (14) pruned inoculated tomato plants after 14 days to induce new growth in which symptoms were frequently severe within a second 14-day period. Lee and Singh (5) enhanced diagnostic symptoms by adjusting the Mn/Fe ratios and the Mn content of the nutrient medium in which tomatoes were grown. Isolates producing very mild symptoms or no symptoms at all in tomato (2, 6, 12) reduce effectiveness of tomato in PSTV diagnosis. Because mild isolates remain symptomless in tomato, other methods of testing become necessary, such as double inoculations of tomato (2) or polyacrylamide gel diagnosis for PSTV-RNA (6).

Materials and Methods

Isolates of PSTV, causing severe symptoms in tomato referred to as 1) Canadian, 2) Wisconsin, 3) Shultz, and 4) Diener, respectively were obtained from: 1) Dr. N. S. Wright, Agriculture Canada Research Station, Vancouver, British Columbia; 2) Dr. H. M. Darling, University of Wisconsin, Madison; 3) Miss M. J. O'Brien (isolate #48 of E. S. Shultz collection), Potato Investigation Laboratory, ARS, USDA, Beltsville, MD; and 4) Dr.

T. O. Diener, Crops Research Div., ARS, USDA, Beltsville, MD. One additional isolate causing mild symptoms in tomato was described by Morris and Wright (6). Virus isolates were maintained on Rutgers tomato plants in the greenhouse.

Rutgers tomato (*Lycopersicon esculentum* Mill.) was grown in greenhouse potting soil with supplemental fertilizer applied in aqueous solution. Plants were mechanically inoculated with glass spatulas using 400-mesh silicon carbide as abrasive. Day length was regulated in growth chambers with automatic light and temperature controls. Light intensity of approximately 1000 ft-c was supplied with 40-watt cool white fluorescent tubes.

Relative viroid infectivity was determined on Rutgers tomato in 10-fold serial dilutions and is expressed as the number of infected plants per 30 plants inoculated.

Chlorosis was expressed either as the percent chlorotic leaflets per plant or by spectrophotometric measurements of chlorophyll in leaf extracts (1).

Experimental Results

Symptoms in 12- to 16-Hour Day — Bunchy top symptoms with the 4 severe isolates appeared approximately 2-3 weeks after inoculation (Fig. 1, E). Plants were in distinct contrast to healthy appearance of non-inoculated controls (Fig. 1 A). They were essentially similar to those described earlier (8) consisting of epinasty and slight twisting of petioles, rugosity of leaf surfaces, downward rolling of leaves, veinal necrosis, and reduction or cessation of apical meristematic growth producing a rosette or 'bunchy top'. Such symptoms were slower in developing by at least a week to 10 days than those seen under continuous light. Growth of subterranean parts of plants was poor. Plants inoculated with the mild isolate (Fig. 1, C) remained symptomless in a 12-hr day and plants grew as well as did the non-infected controls.

Symptoms in Continuous Light — The 4 severe isolates of PSTV were compared relative to white symptom development in inoculated tomato plants growing in continuous light (Fig. 1 F). After cotyledon inoculation, plants were kept in natural day length for 10 days then incubated in continuous light for 30 days. Percentages of white leaflets on 15 plants inoculated separately with each of the severe isolates of PSTV, Wisconsin, Schultz, Canadian, or Diener, were 81, 78, 74, and 79 respectively. Response of Rutgers tomato to the 4 PSTV isolates was consistent and no appreciable differences were evident. During subsequent work, the 4 isolates were consistently similar when compared in several trials under slightly different growing conditions. Non-infected control plants grown under the same conditions were normal in color and lacked white leaflets (Fig. 1 B).

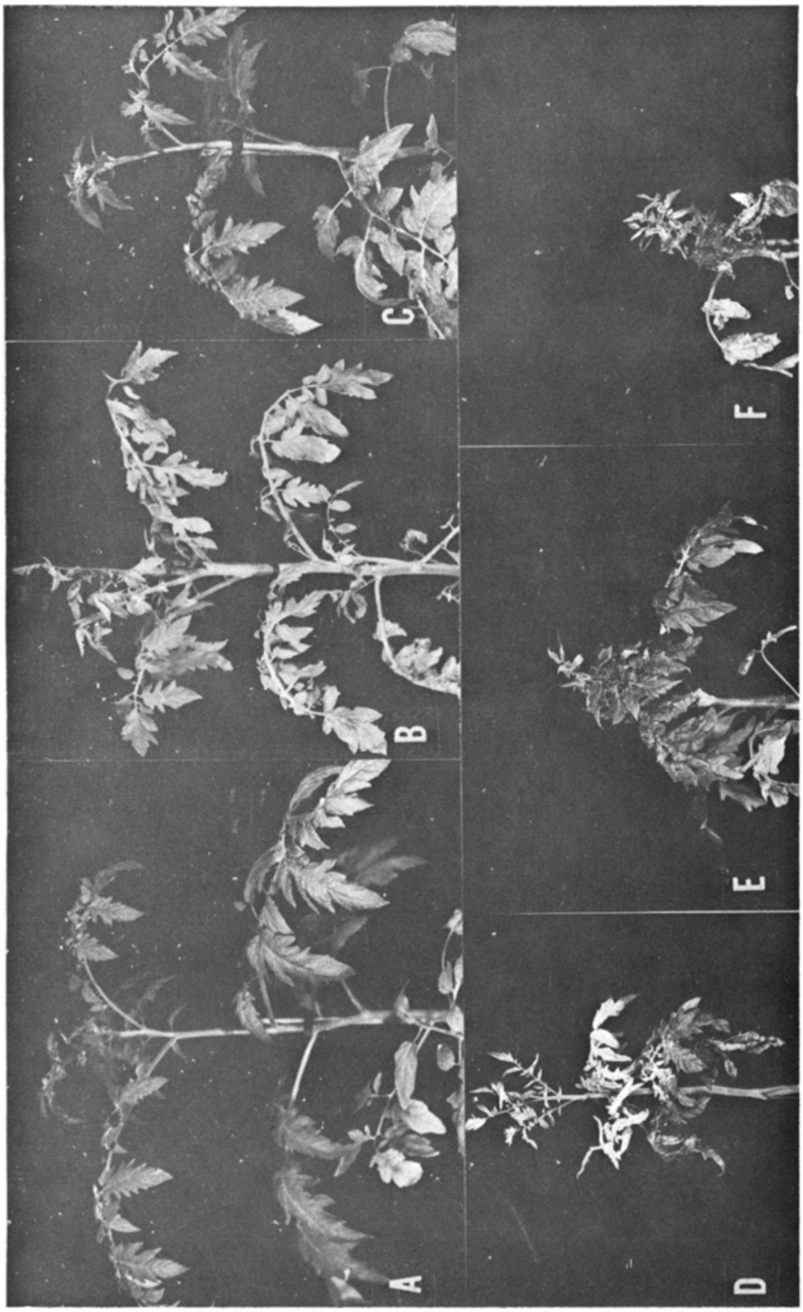

FIG. 1. Rutgers tomato inoculated 25 days previously with 2 strains of PSTV and grown under different day lengths: Non-inoculated controls in A) 12 hr day, and B) in continuous light. Mild isolate of PSTV in C) 12 hr day, and D) in continuous light. Severe isolate of PSTV in E) in 12 hr day and F) in continuous light.

Small plants inoculated with severe strains of the viroid and grown on the greenhouse bench for 10-14 days were moved into growth chambers with continuous light. Three to 4 days later, young green leaves became darker in color, increased in rugosity, reduction in apical growth became evident, and plants became bunchy topped. After approximately 10 days of continuous light, newly developed portions of leaflets were either very low or lacking in chlorophyll. As subsequent leaves developed new tissue was white and extremely chlorotic. Lower leaf surfaces became somewhat purple, particularly on the veins. Newly formed white leaflets were small, rolled upward, and frequently twisted. However, such leaflets were noticeably less rugose and bunched than green terminal portions of plants growing in short days.

Leaves completely expanded at initiation of continuous light consistently maintained the normal green color and those incompletely expanded before long day exposure were partially green and partially white or yellow. Veinal necrosis was equally severe in green leaves of plants infected with the severe PSTV isolates in short days as well as in continuous light. Veinal necrosis was not evident in white leaves of diseased plants in continuous light. Occasionally albino secondary branches developed in continuous light from axillary buds on the lower green parts of plants. Albino plant parts survived for approximately 3 months when maintained in continuous light. Plants survived longer when moved away from the continuous light and kept under 12 to 16-hr days so that new growth could develop green tissue.

The mild isolate (6) in continuous light although less extensively tested, induced mild to severe symptoms of 'bunchy top', occasional veinal necrosis, and distinct albinism and strong chlorosis in Rutgers tomato similar to symptoms of the severe isolates (Fig. 1 D). To determine that the mild PSTV had not changed in pathogenicity and had actually remained 'mild' in the long day treatment, parallel transfers were made from plants infected with the mild strain growing in the 12-hr day (Fig. 1 C) and in continuous light (Fig. 1 D). Inoculated and control Rutgers plants were incubated in both 12-hr day and in continuous light. Regardless of inoculum source, symptoms were identical within an environment. Inoculated plants in the 12-hr days remained symptomless while those in continuous light developed 'bunchy top', some necrosis in lower leaves, and white terminal new growth.

Plants inoculated with severe PSTV and exposed to continuous light usually developed rugosity in green leaves followed by white new growth. However, diseased plants occasionally developed white symptoms first particularly when older plants were inoculated or when a highly diluted viroid preparation was used.

Albinism developed in inoculated tomato plants in continuous light under different conditions in several growth chambers as well as under banks of light suspended over greenhouse benches. However, continuous light of low intensity such as light over nearby benches did not induce white symptoms.

White leaves developed more rapidly when small plants were inoculated than was the case following inoculation of larger plants. Since plants infected with the mild isolate continued to grow more vigorously they developed the white symptom earlier than plants infected with the severe isolates in which new growth was reduced due to stunting associated with the 'bunchy top' symptom.

Under certain conditions, not well understood, small non-inoculated tomato seedlings grown under continuous, fluorescent light of 1,000 ft-c sometimes developed chlorosis beginning at the center of the leaflets while the margins remained green. This was avoided by growing plants for 10 to 14 days on the greenhouse bench before inoculation and exposure to continuous light.

Symptoms with Different Day Lengths — This trial was made with the severe PSTV isolate (Diener) in a walk-in growth chamber at 27°C with continuous light of 2,000 ft-c. Inoculated and control plants, 3 plants per 10-cm diameter pot, 3 pots per treatment, were put in wooden frames with the sides covered with aluminum foil. After inoculation the top of each frame was covered every day with an aluminum foil cap providing day lengths of 6, 12, 18 and 24 hr duration.

Plants exposed to a 6-hr light period were slightly etiolated and the usual symptoms of rugosity did not develop within 3 weeks. At 12 and 18 hrs light per day 'bunchy top' PSTV symptoms developed in approximately 2 weeks and there were no appreciable differences in symptom severity. In continuous light, some leaves of both healthy control plants and infected plants had a slight grayish cast and rolled upward somewhat. Leaflets of inoculated plants developing after continuous illumination became white, characteristic of the albinism described previously. No white symptoms developed in diseased plants held in the shorter day-length regimes.

Symptoms from Changing Day Lengths — When green, PSTV-infected tomato plants were transferred from 12-hr days to continuous light, very young leaves of the plants became cholortic and subsequent new growth was white. This change first became evident in as short a time as 5 days, but usually longer periods of continuous light were required. Plants with severe rugosity but still green in color produced new growth which was chlorotic and white more rapidly than did plants in which the symptoms were still mild. Healthy plants incubated under similar conditions remained green and growth was normal.

Conversely, when diseased plants with white apical growth were transferred from 24-hr illumination to a 12-hr day, new young growth, as it progressively developed, was green and leaves were crinkled and rugose.

The chlorophyll content of newly developed young leaves rapidly increased (Table 1). Frequently the lower white leaves wilted and dried out after plants were changed from continuous light to short day length. Results were similar whether plants were transferred to either the greenhouse bench under natural light, or to a 12-hr day in growth chamber. Expanded white leaves of PSTV inoculated plants which had developed under continuous light did not later become green in shorter days. PSTV-infected plants initially in 12 to 16-hr day length (Fig. 2), then incubated in continuous light, and later returned to the intermittent light exhibited green leaves with typical PSTV symptoms on the lower and upper parts of the plants and white symptoms in the middle portions. Reversals in color of new growth as influenced by day length were obtained in plants inoculated with all 4 severe isolates.

TABLE 1.—*Chlorophyll content of new leaf tissue forming on PSTV-infected plants after changing from continuous light to 12-hr day*[1].

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

[1] Diener isolate.

Viroid Infectivity from Green and from White Leaf Tissue—Green and white leaflets were individually removed from tomato plants infected with the severe PSTV isolates. All these plants were inoculated and grown under comparable conditions. One gram of leaf tissue was thoroughly triturated in 9 ml dist water and serially diluted 1-10 so as to provide 3 final dilutions of 1-10, 1-100, and 1-1000. For each dilution, 30 plants were mechanically inoculated. After inoculation, plants were kept on the greenhouse bench under natural light. Plants exhibiting bunchy top and leaf rugosity were counted as diseased (Table 2). Some of the plants not showing symptoms may have been infected but inoculum levels were too low to initiate symptoms promptly. As incubation periods increased frequency of symptoms increased.

Comparisons of infectivity were as follows: 1) White branches of Rutgers tomato plants inoculated by top grafts (Canadian isolate) and incubated under continuous light were compared to green axillary branches of another graft-inoculated plant within the same growth chamber. Selection of green and white tissue was possible because axillary buds did not develop chlorotic

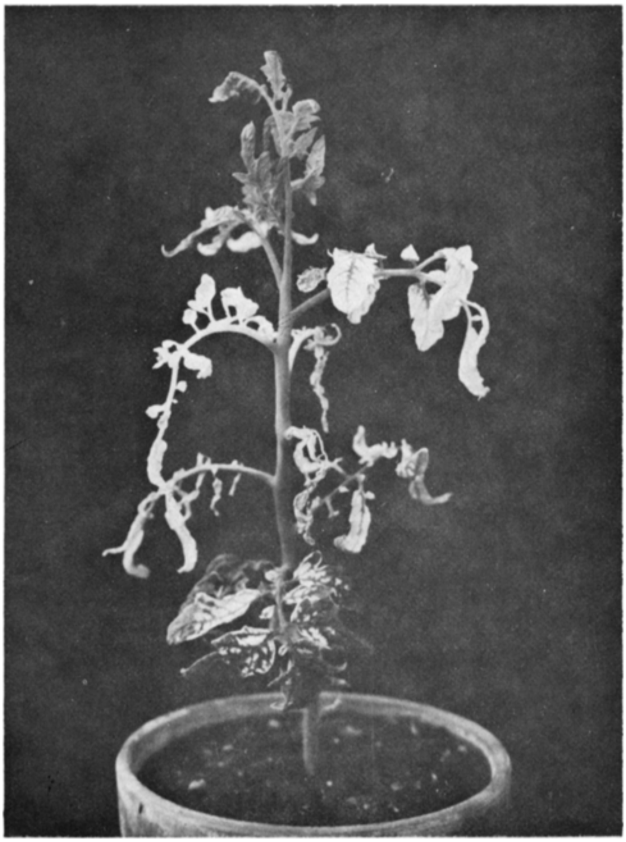

FIG. 2. Rutgers tomato inoculated with PSTV and grown for 30 days on the greenhouse bench, grown for 25 days in continuous light, and returned to the greenhouse bench for 13 days. The albino leaves in the center of the plant developed in continuous light.

tissue uniformly following inoculation. 2) A group of Rutgers plants infected with the Diener PSTV isolate grown in a growth chamber with continuous light was divided and one-half was covered for 12 hr each night and the remaining half was left in continuous light. After approximately 2 weeks, young green leaves from plants in 12-hr light were compared to white leaves of comparable age from plants in continuous light. 3) Green and white leaflets infected with the Diener strain of PSTV from plants in continuous light were assayed after being paired for comparable age, size, and vigor.

In all tests, infection frequency was higher in triturates from white tissue than in comparable triturates from green tissue. In the first comparison, dilution of green tissue triturates seldom exceeded 1-100 while triturates of white tissue reached 1-1000 suggesting approximately a 10-fold increase

TABLE 2.—*Infectivity of green and white leaf tissue from PSTV-infected Rutgers tomato.*

Inoculum source	Incubation period[1]	Infectivity[2] of green tissue at inoculum dilution			Infectivity[2] of white tissue at inoculum dilution		
		1/10	1/100	1/1000	1/10	1/100	1/1000
Axillary shoots on graft inoculated plants	20	5	1	0	10	6	5
	30	10	6	0	19	13	12
	38	19	13	3	28	20	21
Green leaves from 12-hr day and white leaves from continuous light	15	3	2	0	16	10	7
	25	18	8	3	25	22	16
	35	18	8	5	29	27	22
Leaflets matched for comparable size, age and vigor	30	—	—	0	—	—	4
	40	—	—	1	—	—	8
"	30	—	—	1	—	—	22
	40	—	—	4	—	—	25
"	30	—	—	4	—	—	5
	40	—	—	8	—	—	10

[1]Days of incubation of test plants on greenhouse bench under natural day lengths.

[2]Each figure represents number of Rutgers plants with symptoms of bunchy top and leaf rugosity from a population of 30 plants inoculated at each indicated dilution of inoculum.

in viroid concentration. In the second test, infectivity of green tissue was slightly higher than in the first trial, but was still approximately 25% the infectivity of viroid extracts of white tissue. The third comparison also indicated that infectivity was considerably lower in extracts from green tissue as compared to those of white tissue.

Influence of Temperature in Continuous Light — Non-inoculated and PSTV-inoculated (Schultz isolate) Rutgers tomato plants were grown in growth chambers at 16°, 24°, and 30°C with 24-hr illumination at approximately 1,000 ft-c. After 3 weeks, most of the non-inoculated plants were essentially similar to those grown under natural light conditions on the greenhouse bench except that at 30°C the plants possessed smaller, somewhat more chlorotic and thicker leaves than those grown at 24°C. At 16°C, leaves were dark and purplish, and growth was retarded. No non-inoculated control plants developed white or chlorotic tissue in continuous light.

New leaves and shoots of PSTV-inoculated plants in continuous light at both 24° and 30°C were white. However, the tissues at 30°C were more intensely white than those at 24°C. No chlorotic or white tissue formed in inoculated plants under continuous light at 16°C. As in previous trials, veinal necrosis was not present in white leaves of diseased plants incubated at any temperature. Veinal necrosis of inoculated plants was more severe in green leaves formed before exposure to continuous light at 24°C than at 30°C.

Response of Other Tomato Varieties — Several commercially available tomato varieties, Bonny Best, Michigan-Ohio, Fantastic, Beefsteak, Marglobe, Jubilee, Machine Harvest #1, and Rutgers were inoculated with the Diener PSTV isolate to determine varietal responses, particularly development of white symptoms. Plants were grown in continuous light of approximately 1000 ft-c and at 27-30°C. After 4 weeks incubation, the 8 tomato varieties exhibited similar white symptoms under continuous light. All plants of each variety developed the white symptoms except those of Marglobe in which only 3 of the 9 inoculated plants developed white symptoms.

Plants Other than Tomato — Attempts were made to determine if continuous light would induce white symptoms in certain other species of host plants. Inoculated and non-inoculated plants of *Solanum acaule* Bitt. were maintained under continuous light. No white symptoms were obtained in 3 months. When *S. acaule* plants were top grafted to diseased Rutgers tomato plants and kept under continuous light, the *S. acaule* scion often became chlorotic but no white symptoms developed. No white and only slight chlorotic symptoms were obtained when seedling potato plants (*Solanum tuberosum* L.) grown from selfed seed of the Onaway variety were similarly treated. Otherwise symptomless host plants of PSTV, *Datura tatula* L., *D. metel* L., *Physalis floridana* Rydb. syn. (*P. pubescens* L.) and *Solanum dulcamara* L., were inoculated with the Diener PSTV isolate and incubated under continuous light. No symptoms developed in any of the inoculated plants, although PSTV was consistently recovered from such plants using tomato as the index plant.

Discussion

Symptom expression of PSTV in tomato has been reported (8,9) to be most pronounced during the longer day lengths of the greenhouse growing season and also when light is of greater intensity. In potato, symptoms are most evident in higher temperatures (28°C) (3). Other *Lycopersicon* spp. (13) respond by stronger symptoms at high temperatures. We are unaware of previous reports of albinism or of extreme chlorosis in PSTV infected tomato under continuous light.

Other symptomless hosts of PSTV, notably *S. dulcamara* (10,15), *P. floridana*, *D. metel*, and *D. tatula*, remained symptomless under continuous light in our trials, and PSTV was consistently recovered using tomato as index host. O'Brien and Raymer (7) report high frequency of recovery of PSTV from *P. floridana*, and very low recovery frequency from *D. metel* and *D. tatula*. Singh (10) reported *D. stramonium* var. *tatula* to be resistant to both mild and severe PSTV strains. It is possible that viroid concentration increased sufficiently under continuous light to cause increased frequency of transmission when indexed on tomato.

A selection of *Solanum acaule* (PI 230-544) from the U.S.D.A., Regional Plant Introduction Station, Sturgeon Bay, Wisconsin was essentially symptomless under usual greenhouse conditions, but became bunchy topped in continuous light (Hooker and Yang, unpublished data) suggestive of symptoms described elsewhere for tomato (8) and those illustrated for *Solanum rostratum* (11).

Lee and Singh (5) studied effects of Mn nutrition and Fe/Mn ratios on development of PSTV induced leaf necrosis in tomato. Increasing levels of Mn dramatically increased the characteristic veinal necrosis induced by both mild as well as severe strains of PSTV. Their photographs suggest chlorosis at the higher Mn levels. Since chlorosis is not mentioned in their descriptions and since modified Mn nutrition increased veinal necrosis, the response they report is apparently distinct from the influence of continuous light reported herein. Under continuous light, veinal necrosis of tomato was consistently absent in albino leaves in our trials.

Under continuous light, white symptoms developed in all tomato plants of the varieties inoculated except for Marglobe and all varieties responded essentially similarly. Among these varieties, Bonny Best, Michigan-Ohio, and Fantastic have been reported as PSTV resistant (13). The former 2 varieties, however, were later found susceptible (5).

Basal portions of young leaves of very young seedling tomatoes developed interveinal chlorosis under continuous fluorescent light prior to PSTV inoculations. Older plants grown on the greenhouse bench under normal light and later placed under continuous light were not so affected. The reason for this chlorosis was not studied further, but may have been similar to that described by Housley (4).

Symptom expression of PSTV in Rutgers tomato is enhanced by incubation of infected plants in continuous light of 1000 ft-c at 24-30°C. Although mild strains of the viroid have not been widely tested it seems probable that additional symptomless strains may express symptoms under such environments. In all cases, tomatoes grown under continuous light developed much more pronounced symptoms than under 12-16 hr days. Dr. R. P. Singh (unpublished data) has confirmed in independent trials in his laboratory that mild PSTV strains from eastern Canada cause distinct symptoms of rugosity and albinism in tomato when grown under continuous light. The use of continuous light in tomato culture for indexing PSTV is proposed as a means of enhancing sensitivity of the host and improving reliability of the tomato inoculation test for diagnosing PSTV infections in potato improvement programs.

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Literature Cited

1. Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1-15.
2. Fernow, K. H. 1967. Tomato as a test plant for detecting mild strains of potato spindle tuber virus. Phytopathology 57: 1347-1352.
3. Goss, R. W. , and G. L. Peltier. 1925. Further studies on the effect of environment on potato degeneration diseases. Nebr Agr Exp Stn Res Bull 29. 32 pp.
4. Housley, S. 1973. Controlled environment for biological research and teaching: Development of a technology for the future. Lab Pract 22: 59-63.
5. Lee, C. R. , and R. P. Singh. 1972. Enhancement of diagnostic symptoms of potato spindle tuber virus by manganese. Phytopathology 62: 516-520.
6. 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.
7. O'Brien, M. J., and W. B. Raymer. 1964. Symptomless hosts of the potato spindle tuber virus. Phytopathology 54: 1045-1047.
8. Raymer, W.B., and M.J. O'Brien. 1962. Transmission of potato spindle tuber virus to tomato. Amer Potato J 39:401-408.
9. Raymer, W. B., M. J. O'Brien, and D. Merriam. 1964. Tomato as a source of and indicator plant for the potato spindle tuber virus. Amer Potato J 41: 311-314.
10. Singh, R. P. 1973. Experimental host range of the potato spindle tuber 'virus'. Amer Potato J 50: 111-123.
11. 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.
12. 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.
13. Singh, R. P., and M. J. O'Brien. 1970. Additional indicator plants for potato spindle tuber virus. Amer Potato J 47: 367-371.
14. Whitney, E. D., and L. C. Peterson. 1963. An improved technique for inducing diagnostic symptoms in tomato infected by potato spindle tuber virus. Phytopathology (abstract) 53: 893.
15. Yang, T. C., and W. J. Hooker. 1973. Overwintering of potato spindle tuber virus in *Solanum dulcamara* L. Amer Potato J (abstract) 50: 387.