Symptom Expression Enhanced and Low Concentrations of Potato Spindle Tuber Viroid Amplified in Tomato with High Light Intensity and Temperature

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ABSTRACT

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Indicator plants (*Lycopersicon esculentum* 'Rutgers') inoculated with a mild (PSTV-MA) or severe (PSTV-S) strain of potato spindle tuber viroid (PSTV) were grown in the summer and winter under prescribed temperature and light regimes to determine optimum conditions for symptom expression and viroid multiplication. Pruning the indicator plants increased detection based on symptoms 1,000-fold to 2.8×10^{-1} pg/ml initial inoculum of PSTV-S. Tomato plants inoculated with PSTV-MA and grown at high temperatures (24–39 C, diurnal fluctuation) and supplemented with sodium vapor lighting (650 μE m^{-2} s^{-1}) developed distinct symptoms. In summer and winter, symptoms developed earlier and with lower inoculum concentrations for both PSTV strains with sodium vapor supplemental lighting than without lighting or with fluorescent supplemental lighting. When winter-grown indicator plants were supplemented with high-intensity sodium vapor lights and indexed by polyacrylamide gel electrophoresis, detection of PSTV-S was enhanced 10,000-fold to 5.6×10^{-2} pg/ml compared with plants grown under fluorescent lighting (130 μE m^{-2} s^{-1}). It is suggested that sodium vapor lights should be used throughout the year to enhance detection of mild and severe PSTV strains.

Potato spindle tuber viroid (PSTV) constitutes a problem for certification programs and germ plasm collections of potato (*Solanum tuberosum* L.) because it is transmitted through tubers and botanical seed. Visual inspection to detect diseased potatoes is not reliable because symptoms may be subtle or absent (7). Tomato (*Lycopersicon esculentum* Mill. 'Rutgers') has been used as a diagnostic indicator of PSTV; however, the time required for symptoms to develop and severity of disease are dependent on the PSTV strain and environmental conditions (1,3,10,12).

Fernow (1) described a mild strain of PSTV that did not produce symptoms on Rutgers tomato and could be detected only by cross-protection. Yang and Hooker (12) detected a mild strain of PSTV by growing Rutgers tomato plants at 30 C under continuous light. These plants developed albinism, and extracts from albino leaves contained higher PSTV concentrations than extracts from green leaves. They also reported that symptoms developed slower when Rutgers tomato plants inoculated with a severe strain of PSTV were incubated at 6-hr photoperiods.

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Harris and Browning (2) found that viroid concentration increased faster in tomato plants grown at 31 than at 23 C and that low light intensity (photoperiods of 17–18 hr) did not "critically limit" viroid synthesis, but symptoms developed more slowly and were less severe at low than at high light intensities. Morris and Smith (3) determined that PSTV concentration was higher in inoculated Rutgers tomato plants grown at 30 than at 25 C, and the maximum viroid concentration was reached about 80 days after inoculation. Whitney and Peterson (11) reported that infected Rutgers tomato plants grown during the winter produced weak symptoms; however, distinct symptoms resulted when the plants were defoliated 2 wk after inoculation.

We have found consistently that inoculum containing low concentrations of a purified severe strain of PSTV (PSTV-S) produced weak or no symptoms on infected Rutgers tomato plants grown in a greenhouse with supplemental fluorescent lighting during the winter. A preliminary experiment demonstrated that distinct symptoms developed under these same conditions when the plants were supplemented with sodium vapor lighting. Therefore, experiments were designed to determine if a mild strain of PSTV (PSTV-MA) could produce diagnostic symptoms on Rutgers tomato when supplemented with sodium vapor lighting. In addition, we measured the lowest inoculum concentration of purified viroid (PSTV-S) that could be amplified in Rutgers tomato to levels detectable by polyacrylamide gel electrophoresis (PAGE) and determined the influence of temperature, light intensity, and defoliation 4 wk postinoculation on symptom expression. Tests were repeated in the summer and winter to determine possible differences in the dilution end points of the two strains and to determine whether sodium vapor lighting would enhance symptom expression in winter-grown tomatoes.

MATERIALS AND METHODS

Greenhouse conditions. Indicator Rutgers tomato plants inoculated with dilutions of PSTV-MA or PSTV-S (7) were grown in greenhouses in Madison, WI, during summer and winter of 1982. Inoculation dates were scheduled 4 wk before the summer and winter solstice. Summer-grown tomatoes inoculated with PSTV-S and supplemented with fluorescent lighting (16-hr photoperiod) were separated into pruned and unpruned groups. Pruning was done by defoliating plants with a sterile razor blade 4 wk after inoculation. Differential temperature regimes were established by growing tomatoes in non-air-conditioned (24–34 C, diurnal fluctuation) or air-conditioned (22–28 C, diurnal fluctuation) greenhouses during the summer. Photosynthetic photon flux density measurements were taken at canopy level with a Li-Cor Quantum photometer. Average summer ambient light intensity measured at noon was 530 μE m^{-2} s^{-1} on sunny days and 345 μE m^{-2} s^{-1} on overcast days. High or low supplemental light (16-hr photoperiod) provided by sodium vapor or fluorescent light, respectively, was used in summer and winter. Sodium vapor lights were arranged at 1.2-m centers above the plants and produced a light intensity of 650 μE m^{-2} s^{-1} at canopy height. Fluorescent lights were arranged end-to-end above the plants and produced a light intensity of 130 μE m^{-2} s^{-1} at canopy height.

Preparation of inoculum and inoculation technique. PSTV-S was purified according to the procedure of Niblett et al (4) and recovered from gel slices using a slight modification of the procedure of Pfannenstiel (6). PSTV-S was dialyzed against 0.1 M potassium phosphate buffer, pH 8.0, for 48 hr and the concentration was determined at 260 nm with a Varian model 635 spectrophotometer. Purified samples were stored at −20 C.

PSTV-MA was increased on Rutgers tomato. To minimize inoculum variability,

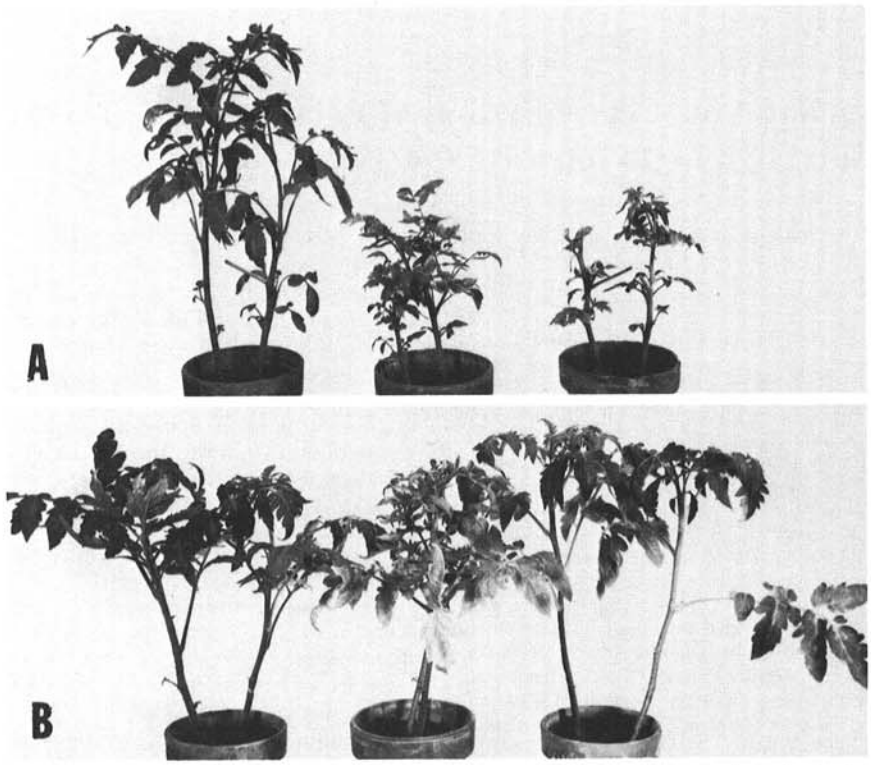

Fig. 1. Symptom severity on **(A)** pruned versus **(B)** unpruned Rutgers tomato plants 4 wk after inoculation with a severe strain of potato spindle tuber viroid (PSTV-S). Plants from left to right in A and B were inoculated with 0.1 M potassium phosphate buffer (pH 8.0), 2.8×10^{-2} μg/ml of PSTV-S, and 2.8×10^{-3} μg/ml of PSTV-S, respectively. All plants were greenhouse-grown in summer (24–39 C) under supplemental fluorescent light (16-hr photoperiods).

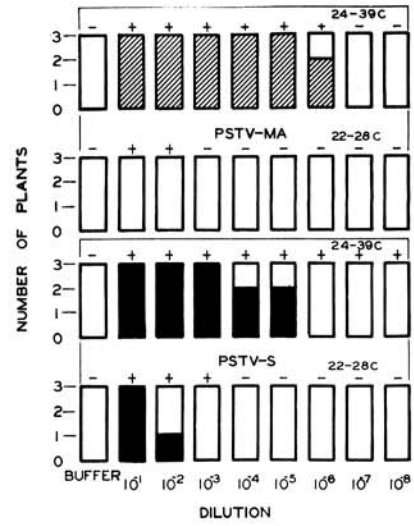

Fig. 3. Effect of temperature on symptoms at various inoculum dilutions and the ability to detect potato spindle tuber viroid (PSTV) with polyacrylamide gel electrophoresis (PAGE) in Rutgers tomato plants inoculated with 10-fold dilutions of crude sap from a mild strain (PSTV-MA) or 10-fold dilutions of a 5.6-μg/ml solution of a severe strain (PSTV-S). Symptoms were recorded 8 wk after inoculation. Plants were rated as follows: □ = no symptoms, ▨ = weak or questionable symptoms, and ■ = distinct symptoms. Positive (+) or negative (−) PAGE results completed 8 wk after inoculation are indicated above each bar.

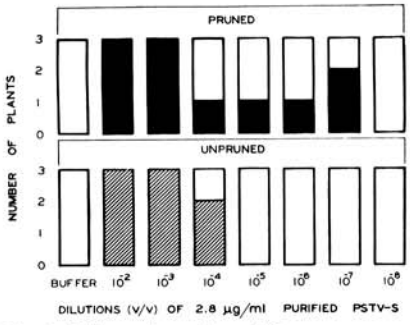

Fig. 2. Effect of pruning of Rutgers tomato plants inoculated with potato spindle tuber viroid (PSTV) at various inoculum dilutions. Symptoms were recorded 8 wk after inoculation. Plants were rated as follows: □ = no symptoms, ▨ = weak or questionable symptoms, and ■ = distinct symptoms.

two 1-g samples were harvested and stored at −20 C until one was used for summer bioassays and the other for winter bioassays. Before inoculation, tissue was thawed on ice and ground (1:10, fresh weight/volume) in 0.1 M phosphate buffer, pH 8.0, with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY).

Each dilution of PSTV-S and PSTV-MA inoculum was applied with a sterile cotton swab to three corundum-dusted (600-mesh) tomatoes when the first true leaves were visible. On the basis of preliminary experiments, the most concentrated PSTV-S inoculum selected was known to produce only marginal symptoms on Rutgers tomato at 4 wk postinoculation. Tomatoes were fertilized with a commercial fertilizer (23-19-17) every 2 wk. Symptoms were rated at 3 and 10 wk postinoculation as symptomless, weak to questionable, or distinct. Plants that had symptoms and indexed positive by PAGE (8) were counted at 3 and 10 wk and used to calculate infectivity indices (9).

RESULTS

Tomato plants inoculated with PSTV-S and pruned 4 wk later developed more severe and distinct symptoms than unpruned plants (Fig. 1). Tomato plants inoculated with higher concentrations of PSTV-S (≥2.8 μg/ml) developed distinct symptoms within 4 wk. Pruned tomato plants developed symptoms at higher dilutions of PSTV-S than unpruned plants (Fig. 2). Symptoms on unpruned plants inoculated with low concentrations of PSTV-S were weak to questionable. The lowest concentration of PSTV-S that produced symptoms was 2.8×10^{-4} μg/ml for unpruned compared with 2.8×10^{-7} μg/ml for pruned tomatoes (Fig. 2). Pruning of tomato plants 4 wk after inoculation was incorporated into all remaining experiments.

Symptoms on tomato plants inoculated with PSTV-MA and grown at high temperatures (24–39 C) could be distinguished but were always less severe than those produced on plants inoculated with PSTV-S (Fig. 3). The dilution end point for symptom development was higher for both strains when inoculated plants were grown at higher temperatures (24–39 C). Detection of PSTV-MA and PSTV-S in tomatoes by PAGE was enhanced 10,000- and 100,000-fold, respectively, for tomatoes grown at 24–39 C compared with 22–28 C (Fig. 3).

Lower concentrations of PSTV-MA and PSTV-S inoculum produced distinct symptoms, and symptoms developed earlier when tomatoes were supplemented with sodium vapor lamps rather than fluorescent lights in summer or winter (Fig. 4). Sodium vapor lighting increased the dilution end point for symptom development at least 1,000-fold for PSTV-MA in summer-grown or winter-grown tomatoes, and the infection threshold as determined by PAGE was increased about 10-fold (Fig. 5). The dilution end point for symptom development on plants inoculated with PSTV-S and supplemented with sodium vapor lighting was not different between the summer and winter bioassays. Viroid was not detected in winter-grown tomatoes inoculated with concentrations lower than 5.6×10^{-4} μg/ml when supplemented with fluorescent lights, but PSTV was detected in plants inoculated with 5.6×10^{-8} μg/ml of PSTV-S when supplemented with sodium vapor lighting.

DISCUSSION

In this study, we have shown the

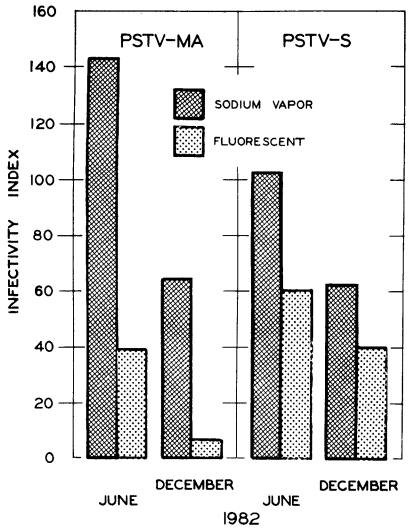

Fig. 4. Infectivity indices of Rutgers tomato plants inoculated with 10-fold dilutions of crude sap from a mild strain of potato spindle tuber viroid (PSTV-MA) or a 5.6-μg/ml solution of a severe strain (PSTV-S). Inoculated plants were grown in summer (June) or winter (December) under fluorescent (135 μE m^{-2} s^{-1}) or sodium vapor (650 μE m^{-2} s^{-1}) supplemental lighting. Infectivity indices calculated by number of plants showing symptoms or indexing positive with polyacrylamide gel electrophoresis multiplied by the negative log of dilution.

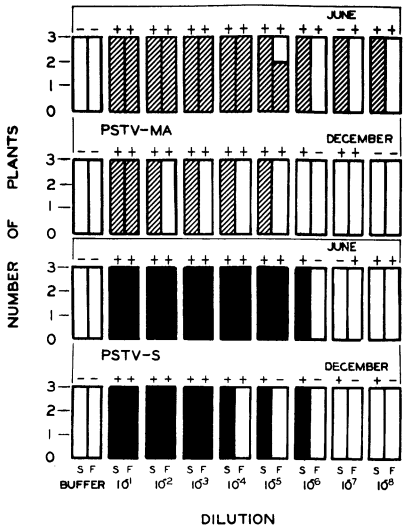

Fig. 5. Effect of season and type of supplemental lighting on symptoms at various inoculum dilutions and the ability to detect potato spindle tuber viroid (PSTV) with polyacrylamide gel electrophoresis (PAGE) in Rutgers tomato plants. Plants were inoculated with 10-fold dilutions of crude sap from a mild strain (PSTV-MA) or a 5.6-μg/ml solution of a severe strain (PSTV-S). Plants were supplemented with sodium vapor (s) or fluorescent (f) lighting for 16-hr photoperiods. Symptoms were recorded 8 wk after inoculation. Plants were rated as follows: □ = no symptoms, ▨ = weak or questionable symptoms, and ■ = distinct symptoms. Positive (+) or negative (−) PAGE results completed 8 wk after inoculation are indicated above each bar.

importance of pruning, high temperature, and high-intensity sodium vapor lighting in optimizing the detection of sub-picogram-per-milliliter concentrations of PSTV inocula in indicator plants. Although other investigators showed an enhancement of symptoms (2,10) and an increased replication (3) of PSTV when indicator plants were grown at elevated temperatures, our study has quantified inoculum concentrations of PSTV-S that can infect and produce symptoms on Rutgers tomato plants when the various parameters are optimized. We demonstrated that high temperatures and high light intensities, either separately (Figs. 3 and 5, respectively) or together (Fig. 4), increased detection sensitivity and provided conditions whereby concentrations of PSTV-S were amplified in plants (10,000- to 100,000-fold) to levels detectable by PAGE. Plants maintained under optimum conditions became infected with inoculum concentrations at levels lower than we previously thought infection would occur. This information may explain why it has been difficult to detect PSTV in true potato seedlings suspected of being PSTV-infected (M. E. Grasmick and S. A. Slack, *unpublished*).

Although Harris and Browning (2) reported no effect of light intensity on the concentration of PSTV in inoculated plants, our study suggests that the light quality and intensity (650 μE m^{-2} s^{-1}) provided by sodium vapor lighting is superior to fluorescent lighting (130 μE m^{-2} s^{-1}) for development of symptoms. Sodium vapor lighting was shown to have a greater influence on the detectability of the mild than of the severe strain tested and is essential for enhanced detection of both strains regardless of ambient greenhouse lighting conditions (Fig. 4). Winter-grown plants inoculated with PSTV-S concentrations lower than 5.6×10^{-3} μg/ml and supplemented with sodium vapor lighting did not develop symptoms, but all concentrations of inoculum, including the lowest concentration 5.6×10^{-8} μg/ml, were amplified in plants to levels detectable by PAGE.

Major disadvantages to PSTV indexing with indicator plants are that a considerable amount of greenhouse space is required, plant growth time can be as long as 8 wk postinoculation, and mild strains have not developed reliable symptoms in earlier studies. We have described conditions that permit reliable detection of a mild PSTV strain on the basis of symptom development. Indicator hosts also permit many samples to be bulked and are advantageous for large-scale testing (3). In addition, many studies require an infectivity assay. Although radioactive complementary DNA (cDNA) is now available for detection of PSTV (5), many laboratories do not have the capacity to conduct such tests and the indexing scheme described in this paper is a feasible alternative. Even in indexing programs where PAGE and cDNA techniques are readily available, the optimum maintenance of inoculated Rutgers tomato plants could be used as a preliminary amplification tool augmented with PAGE or the cDNA hybridization test to confirm infection.

ACKNOWLEDGMENT

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