Infectivity of Potato Spindle Tuber Viroid in Potato Plant Parts

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ABSTRACT

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The infectivity of potato spindle tuber viroid (PSTV) was followed at weekly intervals up to 14 weeks after inoculation into potato (*Solanum tuberosum* 'Netted Gem'). The viroid was assayed on *Scopolia sinensis* leaves from extracts of roots, lower and upper stems, and middle and apical leaves of potato plants. The PSTV was detected from the middle leaves and roots as early as 1 week after inoculation, although infectivity was very low. The highest infectivity in most plant parts was reached 4-8 weeks after inoculation and then declined sharply. The infectivity was highest in extracts from the mature leaves in the middle of the plant and lowest in roots and tuber extracts.

Additional key words: virus-free potatoes, infectious ribonucleic acid, indexing.

Often, virus infectivity in a source plant reaches a peak 1 week after inoculation and then falls; it varies greatly with different virus-host combinations, but commonly peak infectivity corresponds with the appearance of symptoms (13). The spindle tuber disease of potatoes (*Solanum tuberosum* L.) is caused by a viroid (1, 9) and various soil and environmental factors that influence viroid symptom expression have been studied extensively in potatoes (3, 12). When potato spindle tuber viroid PSTV) infectivity was studied in tomato (*Lycopersicon esculentum Mill.*) plants, a high infectivity was observed for a few days after the first appearance of symptoms, followed by a decrease throughout the disease development (5). There is no such information for PSTV-potato combinations. This would be useful for indexing potato plants suspected of PSTV infection. The objective of this study, therefore, was to follow the infectivity of PSTV in various parts of potato plants during disease development and estimate the suitability of those parts for PSTV indexing.

MATERIALS AND METHODS

A severe strain of PSTV that had been maintained in *Scopolia sinensis* Hemsl. (7) plants since 1971 was used for inoculum. The inoculum was in the form of partially purified low-molecular-weight ribonucleic acid as described earlier (9). It was used at the concentration 0.5 mg/ml in 0.1 K_2HPO_4 buffer, pH 7.0.

The potato plants were obtained from virus-free stock (11) maintained at this research station, and were free from obvious fungal, bacterial, and virus diseases. The plants were grown from rooted cuttings about 14-18 cm tall, with four to seven leaves. Three fully expanded leaves were mechanically inoculated with PSTV after the leaves were dusted with 0.22 μm (600-mesh) Carborundum. The inoculated potato plants were maintained in a greenhouse with supplemental light of 6,456 lux (600 ft-c) for 15 hours; temperature ranged from 23 to 27 C.

Beginning 1 week after inoculation, samples of different plant parts were collected at weekly intervals for 14 weeks. Samples consisted of roots; lower stem (stem near the ground level below the inoculated leaves); upper stem (stem portion 5-6 cm below the top growth) middle leaf (fully developed mature leaves at the middle of the stem); and the apical leaves (the terminal growth consisting of unexpanded leaves). Tissue from three different potato plants was combined to form a sample. Each sample (5 g) was frozen at -22 C, then freeze-dried at a pressure of 0.05-0.1 mm of Hg. The freeze-dried samples (500 mg each) were stored in coarse powder form at room temperature until use.

For testing infectivity, 50 μg of powder from each sample was removed from storage and ground in 3 ml 0.1 M K_2H PO_4 buffer, pH 7.0, with a mortar and pestle. A drop of this inoculum (50 μliters) was used to inoculate each half-leaf of *S. sinensis* plants with a glass spatula, and each sample was inoculated to two top half-leaves of five uniformly sized plants. Only one leaf per plant was used. All plant-part samples were tested within 5 days on one batch of *S. sinensis* seedlings grown under identical conditions of light and temperature. After inoculation, the *S. sinensis* seedlings were maintained as described earlier (8). The resulting local lesions were counted 15 days after inoculation.

RESULTS

Symptoms of potato spindle tuber viroid in potato cultivar Netted Gem.—When potato cuttings obtained from disease-indexed virus-free stock of cultivar Netted

Gem were inoculated with PSTV nucleic acid and maintained under the environmental conditions described above, very distinct symptoms appeared within 3-4 weeks after inoculation. Severe stunting of the entire plant, severely reduced apical leaves, and numerous axillary shoots were evident by the 4th week. Later on, leaves became pale dull in color and necrotic streaks appeared on the petioles and the main stems. The symptoms in the disease-indexed Netted Gem cuttings were similar to those observed previously in virus-free plants. (10). Therefore, they appeared suitable for such infection studies with severe PSTV in the greenhouse.

The relative infectivity of potato spindle tuber viroid during current year of infection.—The number of local lesions produced by the 14 weekly samples of roots, lower stems, upper stems, middle leaves, and apical leaves are presented graphically in Fig. 1. The PSTV was detected on *S. sinensis* as early as 1 week after inoculation in samples taken from roots and middle leaves, but infectivity was very low for the first 3 weeks. Thereafter, the infectivity increased rapidly in the middle leaves, reached a peak 7 weeks after inoculation, and then declined sharply. The next highest infectivity occurred in the upper stem; it peaked at the end of the 5th week and then declined. No PSTV was detected in apical leaves until the end of the 3rd week and even then the infectivity remained only 30% of that in the middle leaves. The peak infectivity in the apical leaves was reached 5 to 6 weeks after inoculation. Although PSTV was detected in the roots 1 week after infection and peaked at 5-7 weeks, its pattern was erratic. In the lower stems the PSTV infectivity remained low throughout the growing season, with very little fluctuation.

Infectivity comparison of different plant parts for the total period.—In order to compare plant parts on the basis of the total growth period, samples of each plant part representing 1-14 weeks were removed from storage and were combined. The combined samples of each plant part were thoroughly milled in a Wiley mill (Arthur H. Thomas, Laboratory Apparatus, Philadelphia) fitted with a 0.5-mm (40-mesh) screen. About 300 mg of milled samples of each plant part were homogenized in 6 ml of 0.1 M K_2HPO_4 buffer, pH 7.0, and each sample was inoculated to 20 half-leaves. A tuber sample also was included in this experiment. A 3-gram sample of fresh tuber tissue was taken from each of six different tubers and combined before being freeze-dried as the other samples had been. The middle leaves and the upper stem contained the highest infectivity on the basis of the whole growing season. The lower stem and apical leaves had about half the infectivity of the leaf from the middle of the plant or upper stem, and the roots about half again as much. The tubers had less than 10% of the viroid contained in the middle leaves.

Comparison of relative infectivity from tissue extract vs. nucleic acid.—The above experiment shows that differences in PSTV infectivity from various plant parts may be due to their differential release from the tissues, or to varying content of ribonucleases; it may not reflect the true PSTV content of the plant parts. To eliminate these possibilities, the following tests were made: 2 g of freeze-dried tissue representing each plant part was removed

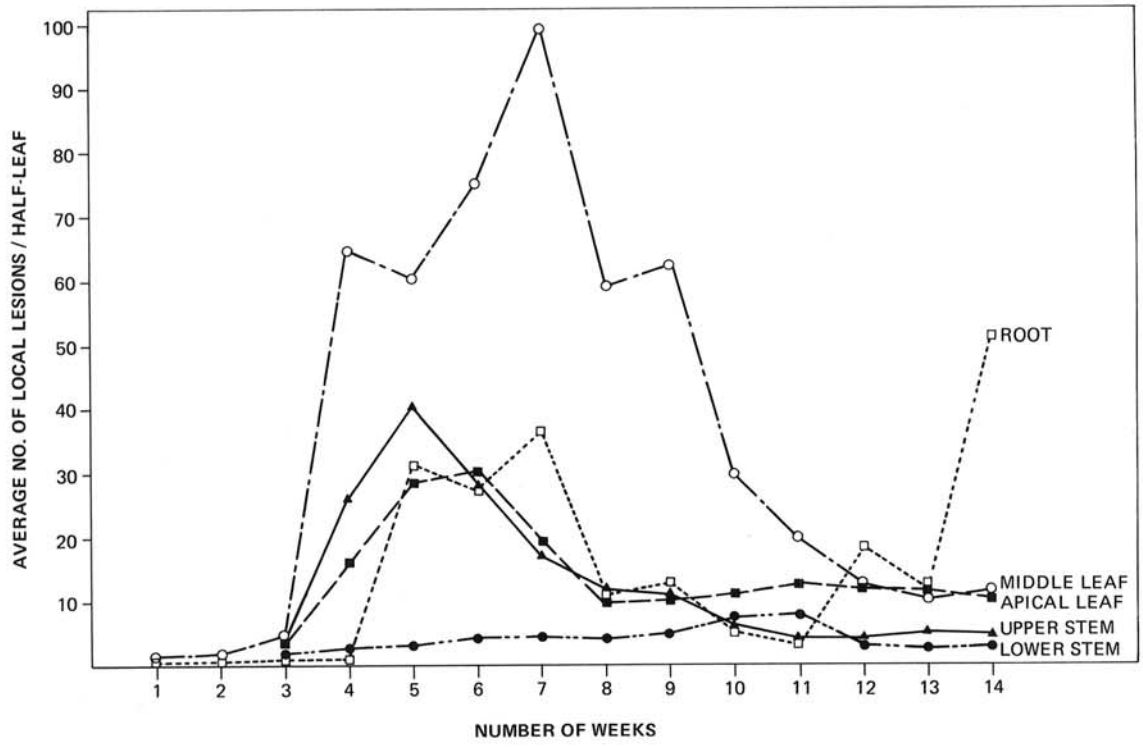

Fig. 1. The infectivity of potato spindle tuber viroid in different parts of potato plants at various periods after inoculation.

TABLE 1. Comparison of infectivity of tissue extract and nucleic acid preparations from various potato plant parts of potato spindle tuber viroid-infected plants of potato cultivar Netted Gem

Plant parts	Local lesions[a](no.)	
	Tissue extract	Nucleic acid
Apical leaves	25	22
Upper stem	64	55
Middle leaves	65	92
Lower stem	34	25
Root	16	30

[a]Average of 10 half-leaves of *Scopolia sinensis*; each tissue extract and respective nucleic acids were paired on each plant and they occurred on left and right half-leaves five times. Only two half-leaves were inoculated on each plant.

from storage, 1 g was extracted with buffer (0.1 M $K_2H PO_4$, pH 7.0) and nucleic acid was extracted from the other one by the procedure of Diener and Smith (2). Finally, both tissue extract and nucleic acid were adjusted to the same volume and used to inoculate 10 half-leaves of *S. sinensis*. The buffer extract and nucleic acid preparations of each plant part had similar infectivity; i.e., either both were high or both were low (Table 1). There was no inconsistency within two preparations of any one part, indicating that the results with tissue extracts or their respective nucleic acids were true reflections of PSTV content. Again, as observed in other experiments, the viroid in the middle leaves and the upper stem was highly infectious and that in the apical leaves, the lower stem, and the roots had low infectivity.

DISCUSSION

The different infectivity of the various parts of a potato plant observed in this study is in agreement with other host-virus combinations (14). Infectivity in the shoot tips have low infectivity in some virus-host combinations (4), whereas the mature leaves are more infectious in cassava infected with cassava brown streak virus (6). The observation that mature leaves and upper stems from potato plants (grown under suitable environmental conditions for PSTV symptom development) were more infectious than other plant parts, should be considered in selecting samples for PSTV indexing.

The differences in the infectivity of various parts probably reflects viroid content of the tissues involved and not just extraction facility, because both 'extract' or 'nucleic acids' from each part produced a similar number of local lesions.

The pattern of infectivity fluctuation in most plant parts appears typical of any virus-host combination in that the infectivity peaked when symptom development was most severe and declined thereafter. In this regard the PSTV-potato combination is similar to the PSTV-tomato combination studied earlier (5).

LITERATURE CITED

1. DIENER, T. O. 1971. Potato spindle tuber "virus". IV. A replicating, low molecular weight RNA. Virology 45:411-428.
2. DIENER, T. O., and D. R. SMITH. 1975. Potato spindle tuber viroid. XIII. Inhibition of replication by actinomycin D. Virology 63:421-427.
3. GOSS, R. W. 1931. Infection experiments with spindle tuber and unmottled curly dwarf of the potato. Neb. Agric. Exp. Stn. Res. Bull. 53. 36 p.
4. HOLMES, F. O. 1955. Elimination of spotted wilt from Dahlias by propagation of tip cuttings. Phytopathology 45:224-226.
5. HUNTER, J. E. 1964. Studies on potato spindle tuber virus. Ph.D. Dissertation, University of New Hampshire, Durham. 97 p.
6. LISTER, R. M. 1959. Mechanical transmission of cassava brown streak virus. Nature 183:1588-1589.
7. SINGH, R. P. 1971. A local lesion host for potato spindle tuber virus. Phytopathology 61:1034-1035.
8. SINGH, R. P. 1973. Experimental host range of the potato spindle tuber virus. Am. Potato J. 50:111-123.
9. SINGH, R. P., and M. C. CLARK. 1971. Infectious low-molecular weight ribonucleic acid from tomato. Biochem. Biophys. Res. Commun. 44:1077-1083.
10. SINGH, R. P., and M. C. CLARK. 1973. Similarity of host response to both potato spindle tuber and citrus excortis viruses. FOA Plant Prot. Bull. 21:121-125.
11. STACE-SMITH, R., and F. C. MELLOR. 1968. Eradication of potato viruses X and S by thermotherapy and axillary bud culture. Phytopathology 58:199-203.
12. WERNER, H. O. 1924. Relation of environment to spindle tuber symptoms. Proc. Potato Assoc. Am. 11:102-106.
13. YARWOOD, C. E. 1964. Assay of infectivity. Pages 79-110 *in* R. J. C. Harris, ed. Techniques in experimental virology. Academic Press, New York. 450 p.
14. YARWOOD, C. E., and R. W. FULTON. 1967. Mechanical transmission of plant viruses. Pages 237-266 *in* K. Maramorosch and H. Koprowski, eds. Methods in virology, Vol. 1. Academic Press, New York. 640 p.