Viroids and their potential danger to potatoes in hot climates[1]

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Viroids are the smallest agents known to be pathogenic to plants. Unlike viruses, they are devoid of a protective protein coat and are composed entirely of circular ribonucleic acid of low molecular weight (ca. 85,000-130,000 *d*). In spite of their small size, viroids cause serious diseases of avocado, chrysanthemum, citrus, coconut, cucumber, hop, potato and tomato. Although viroid diseases have been reported in both tropical and temperate regions of the world, they induce more severe symptoms at high temperatures. They have wide host ranges and at least six different viroids can induce symptoms in potato similar to those caused by the potato spindle tuber viroid. This creates difficulties in making positive identifications. Viroids spread readily by contaminated knives, sickles, and hilling and cultivating equipment. Viroids are detected by bioassays, by gel electrophoresis on polyacrylamide gels or by nucleic acid hybridization tests. Viroids are not a potential danger to potato crop in hot climates.

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Les viroides sont les plus petits agents phytopathogbnes connus. Contrairement au virus, ils sont composbs entibrement d'acide ribonuclbique ciruclaire à bas poid moléculaire (ca. 85,000-130.00 *d*) et ne possèdent pas d'enveloppe protbinique protectrice. En dépit de leur petite taille, les viroides causent de graves maladies chez l'avocado, le chrysanthbme. les citrus, la noix de coco, le concombre, le houblon, la pomme de terre et la tomate. Quoique les maladies causbes par les viroides aient été signalées dans las régions tempérées et tropicales du monde, les symptbmes induits sont plus sévères en climat chaud. Ils peuvent parasiter de nombreuses plantes et au moins six diffbrents viroides peuvent induire chez la pomme de terre des symptbmes semblables à ceux causés par le viroide de la filosité des tubercules, ce qui rend son identification difficile. Les viroides sont propagés facilement par les couteaux, faucilles, buteuse et bquipement aratoire contaminbs. Ils peuvent être détectés à l'aide detests biologiques, d'blectrophorbse sur gel de polyacrylamide ou de tests d'hybridation de l'acide nucléique. Les viroides ne posent pas une grande menace pour la culture de la pomme de terre en climat chaud.

Introduction

A major new development in plant disease research in recent years has been the discovery of the viroid nature of several serious plant diseases. The first viroid was discovered simultaneously but independently in 1971 by Diener (6) and Singh and Clark (49) who were working on the spindle tuber disease of potatoes (9, 48). Since then, viroid-like agents have been demonstrated for ten additional plant diseases. These diseases and their viroids are: avocado sunblotch, ASBV (3, 55); chrysanthemum chlorotic mottle, ChMV (38); chrysanthemum stunt, CSV (8); citrus exocortis, CEV (40, 45); coconut cadang-cadang, CCCV (35, 36); cucumber palefruit, CPFV (42, 56); hop stunt, HSV (43, 44); tomato bunchy top, TBTV (58); tomato planta macho, TPMV (11); and a viroid carried symptomlessly in *Columnea erythrophae* (30).

There are several reviews available that discuss various aspects of viroid research (5, 7, 16). Therefore, an attempt will be made here to point out the biological similarities of viroids, or to speculate on the impact certain ones may have on potato crops in hot climates.

Occurrence and importance of viroid diseases

Although the first viroid was discovered in the temperate regions of North America, viroid diseases are of worldwide occurrence (Table 1) and have caused serious economic losses in tropical climates. For example, cadang-cadang disease of coconut has caused an estimated loss of 12 million palm trees and is considered to be the major threat to coconut production in the Philippines (35). Hop stunt disease was detected in 17% of the total acreage of hops in Japan's Fukushima Prefecture in 1968 where some garden had up to 60% of the plants infected (59). Potato gothic (= spindle tuber) has been widespread and has researched infection rates of as high as 54% in some provinces of the Ukraine S.S.R. (15). Although spindle tuber is now a minor disease in North America, infection rates of 25-90% were observed in the 1920s (7) and PSTV can cause as much as 65% reduction in the yield of infected plants (7, 52).

Potato spindle tuber viroid in Cahada has become rare even in the processing and table-stack field as compared to late sixties (52). The major seed-producing provinces of Canada (New Brunswick and Prince Edward Island) require planting of certified seed for processing and table-stock fields and Canadian potato certification service practices "zero-tolerance" for PSTV in the field. These two measures have reduced the incidence of PSTV in the seed field sharply and in last decade no fields have been rejected because of

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Table 1. Occurence of viroid diseases throuahout the world.

Disease	Countries
Avocado sunblotch	Australia, Israel, Peru, South Africa, U.S.A., Venezuela
Citrus exocortis	Argentina, Australia, Brazil, Corsica, Israel, Japan, Spain, South Africa, U.S.A.
Chrysanthemum stunt	Australia, Canada, India, Japan, The Netherlands, United Kingdom, U.S.A.
Chrysanthemum chlorotic mottle	U.S.A.
Columnea viroid	U.S.A.
Coconut cadang-cadang	Philippines
Cucumber pale fruit	The Netherlands
Hop stunt	Japan
Potato spindle tuber	Argentina, Brazil, Canada, China, Chile, Peru, Scotland, U.S.A., U.S.S.R., Venezuela
Tomato bunchy top	Ivory Coast, South Africa
Tomato planta macho	Mexico

PSTV. The only possible occurrence of PSTV at present is potato breeding institutions, where germplasms from different countries are maintained and whose diversity of morphological characteristics makes diagnosis of PSTV difficult. Even these institutions in Canada are testing parental material for PSTV on a large scale which has further minimized the risk of PSTV in potato production.

The nature of viroids

Viroids are the smallest agents known to be pathogenic to higher plants. Their molecular weight is 85,000 to 130,000 *d,* and their nucleotide chain is only 243-359 nucleotides long (Table 2). Electron microscopy shows native viroids as rod-like molecules and denatured viroids as covalently closed single-stranded RNA species (18, 42). In a proposed model of their secondary structure, the circles have been shown to elongate and form a defective double-helix, in which double helical segments are separated by short unpaired stretches (18, 37). Two of the sequenced viroids, i.e., PSTV and CSV have 69% sequence homology, but the third viroid ASBV which was sequenced recently shows a much lower (18%) sequence homology with PSTV and CSV (54). This suggests divergence in their evolution and subsequent adaptation.

Table 2. Molecular weights and chain length of viroids.

Viroid	Molecular weight	Chain length
Potato spindle tuber	123,325 (18)*	359 (18)
Chrysanthemum stunt	106-127,000 (17)	356 (54)
Avocado sunblotch	85-100,000 (31)	247 (54)
Coconut cadang-cadang	105,000 (36)	243 (54)
Cucumber pale fruit	110,000 (42)	330 (42)
Hop stunt	99,000 (28)	296 (28)
Citrus exocortis	119,000 (42)	357 (42)

*References

Apparently viroids do not have tertiary structures, because the same binding sites are available to dyes at low and high ionic strength (16, 18). Also, the transfer RNA has free access to anticodon binding loops all over the molecules (16, 37).

Unlike viruses, viroids have no protein coat and they are merely a short strand of RNA. They do not carry enough of the genetic code to accomplish their own replication. Therefore, the question arises as to how the viroids are able to multiply. A complete answer cannot be given, but it is certain that viroid replication occurs without the assistance of helper virus (6) and the viroid itself does not act as messenger RNA (4, 19). This suggests that viroids rely upon enzymes already present in the host plant for their replication. In fact, it has been observed that DNA-directed RNA polymerase II from several plant sources will transcribe full length viroid RNA in *vitro* (34). Combined with earlier evidence that ξ-amanitin inhibits viroid replication, there is a strong possibility of this enzyme alone being involved in replication in vivo (34). Another line of indirect evidence supporting a role for RNA polymerase is nutritional studies (53) where manganese stimulated the PSTV symptoms and concentration in infected plants. Since manganese and not magnesium stimulates PSTV concentration, this effect could be similar to that observed in the synthesis of fragmented $Q\upsilon$ RNA or foreign RNA in the presence of $Q\upsilon$ replicase (23).

Viroids are hot climate pathogens

Several studies have been done, using potato and tomato as host plants, to determine the effect of various environmental factors on PSTV. It is generally agreed that air temperature is by far the most important factor because of the profound effect it has on foliage symptoms (13, 21, 27, 41).

In potato, the foliage symptoms of PSTV are more severe if the plants are started in the field, and especially if they are planted late in the season when weather is warm. Under such conditions the symptoms persist throughout the growing season. However, if the potatoes are started when the weather is cooler, the symptoms may never appear (13). High air temparature not only exaggerates the symptoms on aerial parts, but it has been shown to double the amount of viroid synthesized in potato tissues at 30°C as compared to 25°C (27). A similar response to lhigh air temperature has also been observed in PSTV-tomato combinations (21, 27, 41).

High temperatures have also been used to develop symptoms and aid in indexing several viroid diseases. Cucumber pale fruit was detected more reliably when night and day temperatures

remained 27 to 32°C (56), and the incubation period was reduced from 76 days at 20°C to 12-21 days at 30°C (56). In chrysanthemum, the highest infectivity with ChMV was obtained when plants were maintained at 21-32°C (22). Hop stunt viroid in cucumber showed marked symptoms at 33°C and only faint symptoms at 21"C. The incubation period was also reduced to 17 days at 33°C. as compared with 38 days at 21°C (44).The remarkable effect of temperature on ASBV in avocado was also noted recently (2). The incubation period of this viroid was reduced to 90-158 days at 28-30°C and all plants developed symptoms; whereas it took 350 days to complete incubation at 18-20°C and only 2 plants developed symptoms. Like PSTV, the avocado plants developed symptoms rapidly when they were subjected to a high temperature soon after inoculation with ASBV and then transferred to cool temperatures. Those subjected to cooler temperature at the time of inoculation and then transferred to high temperature required a much longer time to develop symptoms (2).

Not only do viroids like PSTV multiply very rapidly at high temperatures and cause more severe symptoms in their host, but their rate of multiplication at low temperatures may be so low that the viroid is eliminated from infected plants (24).

Transmission and spread of viroids

Although viroids are naked RNA, they are mechanically transmissible to plants. Some are transmitted readily while others are not transmitted without special conditions. CPFV (56), CEV (12) and ASBV (31) can be transmitted by razor slashing of stems. PSTV (10, 46, 51), and ASBV (57) are transmitted through pollen and seeds, in addition to normal sap transmission.

Viroids are often spread in the field by contaminated tools and cultivating machinery. For example, PSTV was shown to be transmitted by cutting healthy seed with a knife previously used to cut infected tubers (14). In one study 80-100% infection with PSTV was achieved by brushing actively growing healthy plants with diseased foliage (26); in another study, one hundred per cent of the plants were infected when excessive contact of large vines was made with PSTV contaminated cultivating and hilling equipment (25). The citrus exocortis viroid has been difficult to transmit by conventionel sap inoculation of leaves, yet it can readily be transmitted with contaminated budding knives (12). Contaminated knives, tools, and bare hands used during cultural operations are considered to be the chief sources of spread of chrysanthemum stunt (1). Cucumber pale fruit viroid has been spread by pruning operations in the greenhouse (56), and spread of hop stunt in the garden has also been demonstrated when contaminated sickles or bare hands were used to dress or pull shoots (59).

Symptoms and host range

The range of symptoms exhibited by viroid diseases is similar to that of viruses, except that most viroid infections induce stunting of some kind. Stunting of entire plants is common, and there are usually other symptoms such as smaller upper leaves, shortened internodes and an exaggerated upright appearance in plants infected with PSTV, CEV, CSV, CCMV, CPFV, HSV, TBTV, and TPMV. Malformed, dwarfed flowers are often observed on plants infected with CPFV and HSV. There is also a tendency of viroid-infected plants to produce fruits, tubers and cones which are pointed or more elongated than normal. Fruits infected with CCCV and tubers infected with PSTV usually carry longitudinal scarification or growth cracks. ASBV and CEV are known to cause streaking and splitting of bark in avocado and citrus. Discoloration of leaves, tubers, fruits, and stems have also been observed in other viroid-infected plants.

There is a wide host range for some viroids, whereas others are limited to infecting only one family of host plants (Table 3). Since host range can be affected by host adaptation or serial passaging as noted for CPFV (39), it is difficult to generalize on viroid host ranges. Several viroids may induce similar symptoms. For example, PSTV, CEV, CSV, CPFV, TBTV, TPMV, and *Columnea* viroids all induce epinasty of leaves and stunting of tomato plants. Viroids such as PSTV, CEV, CSV, CPFV, TBTV, TPMV and *Columnea* can infect and also cause similar symptoms in potato plants. PSTV, CEV, TBTV and *Columnea*[3] can induce similar local necrotic lesions in *Scopolia sinensis* plants (47, 50, 58).

Table 3. Host range of viroids.

Viroids	Families in which susceptible plants have been found
Avocado sunblotch	Lauraceae
Citrus exocortis	Compositae, Cucurbitaceae, Papilionaceae, Rutaceae, Solanaceae, Umbelliferae
Chrysanthemum stunt	Compositae, Cucurbitaceae, Solanaceae
Chrysanthemum chlorotic mottle	Compositae
Coconut cadang-cadang	Palmaceae
Columnea viroid	Gesneriaceae, Solanaceae
Cucumber pale fruit	Compositae, cucurbitaceae, Solanaceae
Hop stunt	Cucurbitaceae, Solanaceae
Potato spindle tuber	Boraginaceae, Campanulaceae, Caryophyllaceae, Compositae, Convolvulaceae, Dispaceae, Sapindaceae, Scrophulariaceae, Solanaceae, Valerianaceae
Tomato bunchy top	Compositae, Scrophulariaceae, Solanaceae
Tomato Planta Macho	Compositae, Solanaceae

Table 4. Detection of viroids.

Viroids	Bioassay	Page*	C-DNA**
Avocado sunblotch	*Persea americana* cvs. 'Hass', 'Collinson'	+	+
Citrus exocortis	Gynura aurantiaca	+	+
Chrysanthemum stunt	*Chrysanthemum morifolium* cv. 'Mistletoe'	+	+
Chrysanthemum chlorotic mottle	*Gynura aurentica* *Chrysanthemum morifolium* cv. 'Deep Ridge'	+	-
Columnea viroid	-	+	+
Coconutcadang-cadang	-	+	+
Cucumber pale fruit	*Cucumis sativus* cv. 'Sporu' *Lycopersicon esculentum* cv. 'Rentita'	+	-
Hop stunt	*Cumumis sativus* cv. 'Suuyou'	t	-
Potato spindle tuber	*Lycopersicon esculentum* cv. 'Sheyenne', 'Rutgers' *Scopolia sinensis*	+	+
Tomato bunchy top	*Lycopersicon esculentum*	+	-
Tomato planta macho	*Lycopersicon esculentum* cv. 'Rutgers'	+	-

* Polyacrylamide gel electrophoresis.
** Complementary DNA test so far reported.

Some viroids, such as PSTV, CEV, CSV, CPFV, and TPMV can infect *Gynura aurantiaca* plants, but induce different symptoms. While PSTV and CEV induce characteristic symptoms in this species (47, 50) CSV, CPFV and TPMV are carried symptomlessly (11, 39).

Detection of viroids

Various methods for detecting viroids are summarized in Table 4. Except for *Columnea* and coconut viroids, all of them can be tested with indicator plants. All viroids can also be tested by the polyacrylamide gel electrophoresis (PAGE) (27, 33), and several of them can be assayed by complementary DNA techniques (29, 30, 32) (Table 4). Environmental requirements differ with each viroid-host combination, but high temperature is generally a prerequisite for all test plants. *Scopolia sinensis* is an exception to this rule, because it is a better indicator within a temperature range of 18-21°C (47).

Various modifications of the PAGE procedure have been successfully used in viroid testing, and a recent modification (33) enables the entire test to be completed within a day. However, PAGE procedures have not been satisfactory in testing dormant tuber tissues. In one of our experiments only 24 of 36 PSTV-infected tubers were detected by PAGE procedures. On the other hand, we have used the recently developed nucleic acid hybridization test (29), to detect PSTV in individual dormant tubers, and in composite samples of twenty tubers in which only one was infected with PSTV.[3] In addition, we have used this technique to detect PSTV in true seeds obtained from an infected parent. Our studies have thus confirmed that the nucleic acid hybridization tests to be more sensitive than the PAGE tests, as has also been observed for other viroids (32).

Potential danger to potatoes in hot climates

From the foregoing discussion of environmental effects on replication, spread, and host range of viroids, one may conclude that potatoes are susceptible to viroids in hot climates, and that these organisms may pose a significant threat to successful production. However, when one considers the history of some of the more common viroid diseases, a different picture emerges. Several viroids such as avocado sunblotch, citrus exocortis, coconut cadang-cadang and tomato bunchy top have been known to exist for more than 40 years in countries such as Australia, Israel and South Africa and yet there have been no reports of significant losses in their potato crops because of viroid diseases. This suggests that viroids are like viruses (20) in that they can adapt to a wide range of climates and host plants. Certainly, a trend toward host adaptation was observed with CPFV (39).

Interactions between plants, viruses and vectors are greatly affected by environmental conditions (20). The climate imposes restrictions on survival systems by influencing the number of vectors that are present, and a major difference between tropical and temperate regions is the greater range of vectors in the former (20). As shown earlier, viroids are greatly influenced by high temperatures and, therefore, the

tropical climate will favour their synthesis. This, in turn, results in more obvious symptoms and weaker plants. Diseased plants are easier to detect and remove, and thus they are not permitted to remain and perpetuate the disease through successive crops as they do in temperate regions. Thus, there would be less of an effect on potato crop in hot climates.

Unlike viruses, there are no known efficient insect vectors of viroid diseases. Therefore, even with the greater number and variety of vectors in tropical climates (20) they should not be considered a factor in considering the potential for viroid diseases in the potato crop.

Literature cited

1. Brierly, P. and F.F. Smith. 1951. Chrysantheum stunt. Flor. Rev., 107(2778):27-30.
2. DaGraca, J.V. and S.P. van Vuuren. 1981. Use of high temperature to increase the rate of avocado sunblotch symptom development in indicator seedlings. Plant Dis. 65:46-47.
3. Dale, J.L. and R.N. Allen. 1979. Avocado affected by sunblotch disease contains low molecular weight ribonucleic acid. Australasian Plant Pathol. 8:3-4.
4. Davies, J.W., P. Kaesberg and T.O. Diener. 1974. Potato spindle tuber viroid. XII. An investigation of viroid RNA as a messenger for protein synthesis. Virology 61:281-286.
5. Dickson, E. 1979. Viroids: Infectious RNA in plants. In Nucleic Acids in Plants. Ed. T.C. Hall and J.W. Davies. CRC Press Inc. Boca Raton, Florida.
6. Diener, T.O. 1971. Potato spindle tuber "virus". IV. A replicating, low molecular weight RNA. Virology 45:411-428.
7. Diener, T.O. 1979. Viroids and Viroid Diseases. Wiley Interscience, New York. 252 pp.
8. Diener, T.O. and R.H. Lawson. 1973. Chrysanthemum stunt: A viroid disease. Virology 51:94-101.
9. Diener, T.O. and W.B. Raymer. 1967. Potato spindle tuber virus: A plant virus with properties of a free nucleic acid. Science 158:378-381.
10. Fernow, K.H., L.C. Peterson and R.L. Plaisted. 1970. Spindle tuber virus in seeds and pollen of infected potato plants. Am. Pot. J. 47:75-100.
11. Galindo, J., D.R. Smith and T.O. Diener. 1982. Etiology of planta macho, a viroid disease of tomato. Phytopathology 72:49-54.
12. Garnsey, S.M. and J.W. Jones. 1967. Mechnical transmission of exocortis virus with contaminated budding tools. Pl. Dis. Reptr. 51:410-413.
13. Goss, R.W. 1924. Effect of environment on potato degeneration diseases. Univ. Nebraska Agr. Exp. Sta. Res. Bull. 26, 40 pp.
14. Goss, R.W. 1926. Transmission of potato spindle tuber disease by cutting knives and seed piece contact. Phytopathology 16:233.
15. Grama, D.P. 1969. Distribution and infectivity of potato gothic in the Ukraine. Microbiol. J. 31:42-45 (translated from Ukrainian).
16. Gross, H.J. and D. Riesner. 1980. Viroids: a class of subviral pathogens. Angewandte Chemie 19:231-243.
17. Gross, H.J., H. Domdey and H.L. Sanger. 1977. Comparative oligonucleotide fingerprints of three plant viroids. Nucleic Acid Res. 4:2021-2028.
18. Gross, H.J., H. Domdey, C. Lossow, P. Jank, M. Raba, H. Alberty and H.L. Sanger. 1978. Nucleotide sequence and secondary structure of potato spindle tuber viroid. Nature 273:203-208.
19. Hall, T.C., R.K. Wepprich, J.W. Davies, L.G. Weathers and J.S. Semancik. 1974. Functional distinctions between the ribonucleic acids from citrus exocortis viroid and plant viruses: cell-free translation and aminoacylation reactions. Virology 61:486-492.
20. Harrison, B.D. 1981. Plant virus ecology: ingredients, interactions and environmental influences. Ann. Appl. Biol. 99:195-209.
21. Harris, P.S. and I.A. Browning. 1980. The effect of temperature and light on the symptom expression and viroid concentration in tomato of a severe strain of potato spindle tuber viroid. Potato Res. 23:85-93.
22. Horst, R.K. 1975. Detection of a latent infectious agent that protects against infection by chrysanthemum chlorotic mottle viroid. Phytopathology 65: 1000-1003.
23. Haruna, I. and S. Spiegelman. 1965. Recognition of size and sequence by an RNA replicase. Proc. Nat. Acad. Sci., U.S.A. 54: 1189-1193.
24. Lizarraga, R.E., L.F. Salazar, W.M. Roca and L. Schilde-Rentschler. 1980. Elimination of potato spindle tuber viroid by low temperature and meristem culture. Phytopathology 70:754-755.
25. Manzer, F.E. and D. Merriam. 1961. Field transmission of the potato spindle tuber virus and virus X by cultivating and hilling equipment. Am. Pot. J. 38:346-352.
26. Merriam, D. and R. Bonde. 1954. Dissemination of spindle tuber by contaminated tractor wheels and by foliage contact with diseased potato plants. Phytopathology 44:111.
27. Morris, T.J. and E.M. Smith. 1977. Potato spindle tuber disease: procedures for the detection of viroid RNA and certification of disease-free potato tubers. Phytopathology 67: 145-150.
28. Ohno, T., J. Akiya, M. Higuchi, Y. Okada, N. Yoshikawa, T. Takahashi and J. Hashimoto. 1982. Purification and characterization of hop stunt viroid. Virology 118:54-63.
29. Owens, R.A. and T.O. Diener. 1981. Sensitive and rapid diagnosis of potato spindle tuber viroid disease by nucleic acid hybridization. Science 213:670-672.
30. Owens, R.A., D.R. Smith and T.O. Diener. 1978. Measurement of viroid sequence homology by hybridization with complementary DNA prepared in *vitro*. Virology 89:388-394.
31. Palukaitis, P., T. Hatta, D. McE Alexander and R.N. Symons. 1979. Characterization of a viroid associated with avocado sunblotch disease. Virology 99:145-151.
32. Palukaitis, P., A.G. Rakowski, D. McE Alexander and R.N. Symons. 1981. Rapid indexing of the sunblotch disease of avocados using a complementary DNA probe to avocado sunblotch viroid. Ann. Appl. Biol. 98:439-449.
33. Pfannenstiel, M.A., S.A. Slack and L.C. Lane. 1980. Detection of potato spindle tuber viroid in field-grown potatoes by improved electrophoretic assay. Phytopathology 70:1015-1018.
34. Rackwitz, H.R., W. Rhode and H.L. Sanger. 1981. DNA-dependent RNA polymerase II of plant origin transcribes viroid RNA into full length copies. Nature 291:297-301.
35. Randles, J.W. 1975. Association of two ribonucleic acid species with cadang-cadang disease of coconut palm. Phytopathology 65: 163-167.
36. Randles, J.W. and T. Hatta. 1979. Circularity of the ribonucleic acids associated with cadang-cadang disease. Virology 96:47-53.
37. Riesner, D., K. Henco. U. Rokohl, G. Klotz, A.K. Kleinschonidt, H. Domdey, P. Jank, H.J. Gross and H.L. Sanger. 1979. Structure and structure formations of viroids. J. Mol. Biol. 133:85-115.
38. Romaine, C.P. and R.K. Horst. 1975. Suggested viroid etiology for chrysanthemum chlorotic mottle disease. Virology 64:86-95.
39. Runia, W. Th. and D. Peters. 1980. The response of plant species used in agriculture and horticulture to viroid infections. Neth. J. Pl. Path. 86:135-146.
40. Sanger, H.L. 1972. An infectious and replicating RNA of low molecular weight: the agent of the exocortis disease of citrus. Adv. Bioscience 8: 103-116.
41. Sanger, H.L. and K. Ramm. 1975. Radioactive labelling of viroid RNA. Pages 230-253. In Modification of the Information Content of Plant Cells. Eds. R. Markham, D.R. Davies, D.A. Hapwood, and R.W. Horne, Amsterdam, North Holland.
42. Sanger, H.L., G. Klotz, D. Riesner, H.J. Gross and A.K. Kleinschmidt. 1976. Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures. Proc. Natl. Acad. Sci., U.S.A. 73:3852-3856.

43. Sasaki, M. and E. Shikata. 1977. On some properties of hop stunt disease agent, a viroid. Proc. Japan Acad. 53B:109-112.
44. Sasaki, M. and E. Shikata. 1978. Studies on hop stunt disease 1 host range. Rept. Res. Lab. Kirin Brewery Co., Ltd. 21:27-39.
45. Semancik, J.S. and L.G. Weathers. 1972. Exocortis disease: Evidence for a new species of "infectious" low molecular weight RNA in plants. Nature New Biol. 237:242-244.
46. Singh, R.P. 1970. Seed transmission of potato spindle tuber virus in tomato and potato. Am. Pot. J. 47:225-227.
47. Singh, R.P. 1973. Experimental host range of the potato spindle tuber "virus". Am. Potato J. 111-123.
48. Singh, R.P. and R.H. Bagnall. 1968. Infectious nucleic acid from host tissues infected with potato spindle tuber virus. Phytopathology 58:696-699.
49. Singh, R.P. and M.C. Clark. 1971. Infectious low-molecular weight ribonucleic acid. Biochem. Biophys. Res. Commun. 44:1077-1083.
50. Singh, R.P. and M.C. Clark. 1973. Similarity of host response to both potato spindle tuber and citrus exocortis viruses. FAO Plant Prot. Bull. 21:121-125.
51 Singh, R.P. and R.E. Finnie. 1973. Seed transmission of potato spindle tuber metavirus through the ovule of *Scopolia sinensis.* Can. Plant Dis. Surv. 53: 153-154.
52. Singh, R.P., R.E. Finnie and R.H. Bagnall. 1971. Losses due to the potato spindle tuber virus. Am. Pot. J. 48:262-267.
53. Singh, R.P., C.R. Lee and M.C. Clark. 1974. Manganese effect on the local lesion symptom of potato spindle tuber 'virus' in *Scopolia sinensis.* Phytopathology 64: 1015-1018.
54. Symons, R.H. 1981. Avocado sunblotch viroid: primary sequence and proposed secondary structure. Nucleic Acid Res. 9:6527-6537.
55. Thomas, W. and N.A. Mohamed. 1979. Avocado sunblotch - A viroid disease. Australasian Plant Pathol. 8:1-3.
56. Van Dorst, H.J.M. and D. Peters. 1974. Some biological observations on pale fruit, a viroid-incited disease of cucumber. Neth. J. Pl. Path. 80:85-96.
57. Wallace, J.M. and R.J. Drake. 1962. A high rate of seed transmission of avocado sunblotch virus from symptomless trees and the origin of such trees. Phytopathology 52:237-241.
58. Walter, B. 1981. Un viroide de la tomate en Afrique de l'Ouest: Identité avec le viroide du "Potato Spindle Tuber"? C.R. Acad. Sc., Paris 292:537-542.
59. Yamamoto, H., Y. Kagami, M. Jurokawa, S. Nishimura, S. Ukawa and S. Kubo. 1973. Studies on hop stunt disease in Japan. Rept. Res. Lab. Kirin Brewery Co., Ltd. 16:49-62.