Detection of Potato Spindle Tuber Viroid in Avocado Growing in Peru

M. QUERCI, The International Potato Center (CIP), P.O. Box 1558, Lima, Peru; R. A. OWENS, USDA-ARS, Molecular Plant Pathology Laboratory, Plant Sciences Institute, Beltsville, MD 20705; C. VARGAS, Estación Exp. Agric. Inst. Nac. Inv. Agrop. La Molina, Lima, Peru; and L. F. SALAZAR, The International Potato Center (CIP)

ABSTRACT

Querci, M., Owens, R. A., Vargas, C., and Salazar, L. F. 1995. Detection of potato spindle tuber viroid in avocado growing in Peru. Plant Dis. 79:196-202.

Potato spindle tuber viroid (PSTVd) was detected in avocado trees growing at several locations in Peru by using nucleic acid spot hybridization assays. A viroidlike nucleic acid with a mobility similar to that of PSTVd was also found by return polyacrylamide gel electrophoresis analysis of RNA extracted from several trees, and a mild strain of PSTVd was recovered from *Nicandra physalodes* inoculated with leaf sap from affected avocados. Sequence analysis of cDNAs obtained using the polymerase chain reaction with primers to PSTVd indicated the presence of three different sequence variants of PSTVd in the avocado trees. The PSTVd infections were often latent, but some trees coinfected with avocado sunblotch viroid showed symptoms that included bunchiness of the inflorescence, decrease in both fruit size and number, and eventual decline and death. This is the first report of the isolation of PSTVd from a natural host other than potato.

Avocado (*Persea americana* Miller) is an important crop in tropical areas, yet viroid and viruslike diseases of avocado have been little studied. In 1978, Alper et al (1) isolated a tobamovirus from avocado. Analysis of double-stranded RNAs isolated from avocados, some of which exhibited symptoms of avocado black streak, indicated the presence of as many as three "viruslike agents" in avocado (11,12). More recently, Vargas et al (26) used a nucleic acid spot hybridization (NASH) assay to determine the distribution of avocado sunblotch viroid (ASBVd) in Peru. In the course of those studies, some trees were found to contain a nucleic acid species apparently related to potato spindle tuber viroid (PSTVd). We now report the results of further molecular studies with this viroid as well as those from a survey to determine its distribution within Peru. PSTVd strains capable of inducing either mild or intermediate symptoms in tomato (*Lycopersicon esculentum* Mill.) cv. Rutgers were recovered from diseased avocado trees growing in nurseries and commercial groves.

MATERIALS AND METHODS

Viroid isolates and cDNAs. The nucleotide sequences and biological properties of various field isolates of PSTVd (including mild, intermediate, and severe strains) were described by Schnölzer et al (21). Details of the construction of dimeric viroid cDNAs derived from ASBVd (9) and the intermediate strain of PSTVd (3) have also been described.

Avocado cultivars. Most of the trees tested were grafted onto avocado variety Duke or Topa Topa rootstocks. A total of 12 different scion varieties were examined for the presence of PSTVd or ASBVd: Campong, Collinred, Criollo, Duke, Fuerte, Hass, Mexicano, Nabal, Super Fuerte, Topa Topa, Villacampa, and Zutano. All cultivars were derived from plant materials introduced to the La Molina Experiment Station in 1940, 1958, and 1966. Most were obtained from the University of California, Riverside, but a few introductions may have come from Mexico.

Survey procedures. Symptomatic and, when practical, asymptomatic trees in nurseries and commercial groves were tested for the presence of both PSTVd and ASBVd (Table 1). When a complete survey was impractical, asymptomatic trees were randomly sampled. Samples of leaf tissue were collected from young sprouts in January–March (coastal locations) or June–September (inland locations on the eastern side of the Andes). The exact times of sampling varied somewhat from year to year according to the appearance of sprouts and the possibility of sampling.

For NASH analysis, approximately 1 g of fresh leaf tissue collected from either asymptomatic or diseased plants was homogenized with 2 ml of extraction buffer (37% formaldehyde-10× SSC; 1:1 [v/v]) (1× SSC contains 0.15M NaCl, 0.015M trisodium citrate [pH 7.0]) as described by Salazar et al (17). The mixture was clarified by extraction with an equal volume of phenol/chloroform (1:1[v/v]) and, after low-speed centrifugation, 3–5 µl aliquots of the aqueous phase were spotted onto nitrocellulose membranes (Schleicher & Schuell, Keene, NH) equilibrated with 20× SSC and air dried. Membranes were baked under vacuum at 80 C for 2 hr and stored at room temperature until hybridization.

Preparation of RNA probes and hybridization analysis. ^{32}P-labeled RNA probes complementary to either PSTVd or ASBVd were prepared by transcription of dimeric cDNAs cloned in the BamHI site of plasmid pSP65 using the Riboprobe System (Promega, Madison, WI) according to the manufacturer's instructions. Prior to transcription, plasmid DNAs were linearized by digestion with either *Pst*I (PSTVd cRNA probe) or *Hind*III (ASBVd cRNA probe). Following digestion with DNase to remove template DNA and phenol/chloroform extraction, cRNA probes were recovered by ethanol precipitation, dissolved in 10mM Tris-HCl-1mM EDTA buffer (pH 7.5) containing 1% 2-mercaptoethanol, and stored at −70 C.

Hybridization reactions were carried out in heat sealable plastic bags using ca. 4×10^5 cpm ^{32}P-labeled RNA probe/ml hybridization solution (40% [v/v] formamide, 0.18 M NaCl, 10 mM sodium cacodylate (pH 7.0), 1mM EDTA, 0.1% sodium dodecyl sulphate, 300 µg/ml denatured calf thymus DNA, and 10% [w/v] dextran sulphate). A prehybridization step was found to be unnecessary, and, following overnight incubation at 55 C, membranes were treated with RNAse A and washed as described by Salazar and Querci (19) before autoradiography at −70 C using Kodak X-OMAT AR film and an intensifying screen.

Recovery of PSTVd by inoculation of *Nicandra physalodes*. Leaf tissue from diseased avocados was homogenized in cold 0.1M phosphate buffer (pH 7.2) and immediately inoculated to the carborundum-dusted leaves of *Nicandra physalodes* (L.) Gaertn, *Solanum tuberosum* L. cv. DTO-33, tomato cv. Rutgers, *Cucumis*

Accepted for publication 4 October 1994.

Table 1. Detection of potato spindle tuber and avocado sunblotch viroids in avocado nurseries and commercial groves by nucleic acid spot hybridization

Location[a]	Number of trees tested	Viroid detection					
		Symptomatic			Asymptomatic		
		PSTVd	ASBVd	PSTVd + ASBVd	PSTVd	ASBVd	PSTVd + ASBVd
Nurseries							
EEA La Molina (Lima)	122	25	4	6	6	3	24
Topara (Chincha)	62	0	1	1	4	2	2
EEA Moquegua (Moquegua)	51	...	...	...	1	8	1
EEA Donoso (Huaral)	108	...	...	...	0	16	0
EEA Canchan (Huanuco)	16	3	0	1	12	0	0
Commercial groves							
Fdo. Lurin (Lima)	22	0	4	0	0	0	0
Fdo. Santa Rosa (Moquegua)	17	...	...	...	0	0	0
Fdo. Diana (Satipo)	33	8	0	0	0	0	0
Fdo. Guadalupe (Ica)	16	...	...	...	0	0	0
Fdo. San Juan (Ica)	46	0	5	0	0	4	0
Fdo. JJ (Huaral)	50	4	0	2	41	0	0
Fdo. Paraiso, Pichanaki (Chanchamayo)	25	10	0	2	2	0	0
UNH Valdizan (Huanaco)	13	4	0	2	7	0	0
UNA Selva, Tingo Maria (L. Prado)	9	5	0	4	...	...	...
Total	590 (107)[b]	59	14	18	73	33	27
Total as percent	100	55.1[c]	13.1[c]	15.9[c]	15.1	6.8	5.6

[a] Provincial locations for individual nurseries and commercial groves are shown in parentheses.
[b] Number of symptomatic trees is shown in parentheses.
[c] Note that viroid(s) were not detected in all symptomatic trees growing in coastal locations (see Table 2). Overall viroid incidence: PSTVd, 22.4%; ASBVd, 8.0%; PSTVd + ASBVd, 7.5%.

sativus L. cv. Suyo, *Nicotiana tabacum* L. cv. Samsun, *Nicotiana benthamiana* L., and *Chenopodium murale* L. seedlings (six plants/treatment). The plants were tested for both PSTVd and ASBVd by NASH analysis 4 and 8 wk postinoculation. None of these species is known to support ASBVd replication (4).

RNA extraction and return polyacrylamide gel electrophoresis (R-PAGE). Low molecular weight RNA was extracted from avocado leaves by phenol-chloroform extraction and LiCl fractionation (16). Although removal of DNA (by digestion with DNase) and polysaccharides (by extraction with ethylene glycol monomethyl ether) is not required for R-PAGE analysis, these steps were included to ensure reliable synthesis and amplification of PSTVd cDNAs (see below).

Analysis of low molecular weight RNA by R-PAGE was conducted essentially as described by Singh and Boucher (25) except that the polyacrylamide concentration was increased to 7.5% (39 acrylamide/ 1 N,N'-methylene-bis-acrylamide) and the "low salt" buffer used for the second electrophoresis was heated to 100 C instead of 87–90 C before addition to the upper and lower reservoirs. After the second electrophoresis, RNAs were visualized by silver staining (22). As shown in Fig. 1, R-PAGE analysis under these conditions reproducibly resolves mild, intermediate, and severe-lethal strains of PSTVd.

Synthesis, amplification and sequence analysis of PSTVd-specific cDNAs. Owens et al (15) have previously described a strategy for the amplification and sequence analysis of randomly primed viroid cDNAs synthesized from low molecular weight RNA templates. This strategy was used to amplify PSTVd-specific cDNAs representing the left and right sides of the molecule in PCR reactions containing one of three pairs of primers: RAO33 (116-GCCGGTACCAGTTCGCTCCAGGTTTCCCC-95) plus RAO2 (263-GCGGATCCGGTGGAAACAACTGAAGC-282); RAO34 (342-GCCGGTACCAAGGGCTAAACACCCTCGCCC-320) plus RAO14 (85-AGGGATCCCCGGGGAAACC-103); and T2 (174-CTGTTTCGGCGGGAATTA-157) plus RAO2. Primers RAO33, RAO34, and T2 are complementary to PSTVd, while RAO2 and RAO14 have the same sequence as PSTVd. The numbers that precede and follow the sequence of each primer refer to the corresponding positions in PSTVd-Intermediate strain (21), and the non-PSTVd-related nucleotides (underlined) at the 5'-termini of primers RAO2, 33, and 34 were added to facilitate possible cloning of the respective PCR products by creating recognition sites for either *Bam*HI (GGATCC) or *Kpn*I (GGTACC). In later experiments with RNA extracted from California avocados, cDNA synthesis was specifically primed with primer cPSTV (88-CCCTGAAGCGCTCCTCCGAG-69), and primer PSTV (89-ATCCCCGGGGAAACCTGGAGCGAAC-113) was added for PCR amplification of full-length PSTVd cDNAs as described by Levy et al (13).

Amplification products from 25 μl PCR reactions were purified by elution from Prep-A-Gene DNA purification matrix (Bio-Rad Laboratories, Richmond, CA) as described by the manufacturer. A 2 μl aliquot (i.e., 10%) of the purified dsDNA was used as template for nucleotide sequence analysis using the *fmol* DNA Sequencing System

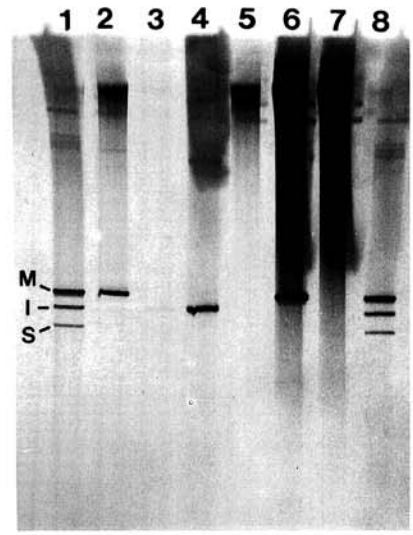

Fig. 1. Return polyacrylamide get electrophoresis analysis of potato spindle tuber viroid (PSTVd) isolates. Lanes 1 and 8, a mixture of mild (M), intermediate (I), and severe (S) PSTVd strains propagated in tomato and used as markers; lanes 2 and 6, PSTVd recovered from *Nicandra physalodes* inoculated with a leaf extract from avocado; lane 3, nucleic acid extract from a second avocado; lane 4, PSTVd- intermediate strain propagated in tomato; and lanes 5 and 7, RNA extracted from uninoculated tomato plants.

Fig. 2. Locations of nurseries and commercial groves containing potato spindle tuber viroid–infected avocado trees.

(Promega, Madison, WI) and 5'-^{32}P-labeled primers.

RESULTS

The presence of PSTVd or a closely-related viroid in diseased avocados was first detected during surveys carried out to determine the incidence of ASBVd in Peru (26). Once it became clear that many trees contained a non-ASBVd viroid, additional surveys were carried out to determine its distribution and to relate its presence to symptom expression.

Incidence of PSTVd infection among avocados growing in Peru. The incidence of PSTVd in nurseries and commercial avocado groves located in different regions of Peru was estimated by NASH. The location of each site is shown in Fig. 2, and the survey results are presented in Table 1. For each site, the numbers of PSTVd-, ASBVd-, and doubly-infected trees (either diseased or asymptomatic) are shown.

PSTVd-infected trees were present at each location, and 132 of 590 trees examined (22.4%) were found to be infected with PSTVd. ASBVd and ASBVd/PSTVd double infections were detected in 8.0 and 7.5% of the trees, respectively. More than half of all symptomatic trees were PSTVd-infected, but latent infections were also common. At three locations (EAA Canchan, Fdo. JJ, and UNH Valdizan), 93–100% of asymptomatic trees tested were found to be PSTVd-infected. Infected trees ranged in age from 1 to 27 yr, and there appeared to be no obvious correlation between PSTVd infection and either tree age or location. At least one tree from each of the 12 cultivars included in our surveys was found to be PSTVd-infected.

Grouping the survey data from symptomatic trees by region rather than individual location revealed the need for a more precise definition of disease in order to assess the potential involvement of PSTVd. As shown in Table 2, neither PSTVd nor ASBVd was detected in 25% of symptomatic trees from coastal locations. Symptomatic trees were nearly three times as common at inland locations as near the coast, and the percentage of trees containing both PSTVd and ASBVd was lower than that containing PSTVd alone. Only six of the 12 avocado varieties included in our survey were present at the inland locations: Collinred, Criollo, Duke, Fuerte, Nabal, and Villacampa.

Table 2. Distribution of symptomatic avocado trees

Region	Symptom incidence	Viroid incidence in symptomatic trees			
		PSTVd	ASBVd	PSTVd + ASBVd	None
Coastal	14.4[a] (68/494)	42.6	20.6	11.8	25
Inland	49.2 (39/96)	76.9	0	23.1	0
Total	18.1 (107/590)	55.1	13.1	15.9	15.9

[a]All values expressed as percentages; fractions in parentheses are number of diseased trees/number of trees tested.

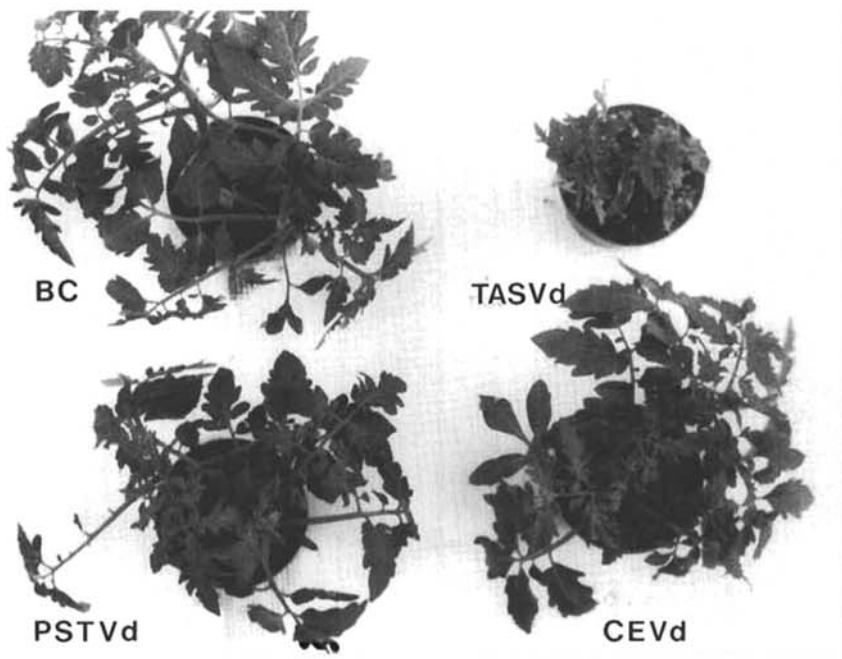

Fig. 3. Symptoms induced on Rutgers tomato by a mild strain of potato spindle tuber viroid (PSTVd) isolated from avocado. Cotyledons of tomato seedlings were mechanically inoculated with low molecular weight RNA extracted from *Nicandra physalodes* infected with a PSTVd isolate from avocado; seedlings were maintained in a greenhouse for 4–5 wk under conditions favoring symptom expression. Mild stunting and epinasty induced by PSTVd isolate from avocado are very similar to those produced by a mild strain of citrus exocortis viroid (CEVd-J(c)). Under same conditions, inoculation with tomato apical stunt viroid induces very severe symptoms (20). BC, buffer control.

Molecular characterization of PSTVd sequence variants isolated from avocado. In certain cases, a viroidlike nucleic acid could be visualized in low molecular weight RNA preparations from diseased avocados. R-PAGE analysis of one such RNA preparation revealed the presence of a viroid whose mobility was very similar or identical to that of the PSTVd-intermediate strain used as a marker (Fig. 1, compare lanes 3 and 4). Samples from another tree appeared to contain a strain similar in mobility to a severe or lethal strain of PSTVd (data not shown).

To confirm the presence of PSTVd in diseased trees, attempts were made to transmit the viroid to *N. physalodes* and Rutgers tomato. Mechanical inoculation of young *N. physalodes* seedlings with crude leaf homogenates from two different PSTVd-infected trees was followed by the appearance of a viroid whose mobility during R-PAGE was similar to that of a previously described mild strain of PSTVd (Fig. 1, lanes 2 and 6). Transfer of this viroid to tomato seedlings produced mild stunting and epinasty symptoms characteristic of infection by a mild strain of PSTVd (Fig. 3). In another experiment, six of eight tomato and three of six potato seedlings inoculated with sap prepared from infected *N. physalodes* plants were found to be infected with PSTVd by NASH. The viroid from *N. physalodes* was also sap inoculated to four young Villacampa

avocado seedlings. Ten weeks postinoculation, samples of newly emerged leaves from three of the four inoculated seedlings were positive by NASH, but the plants were asymptomatic.

PCR-mediated sequence analysis of two PSTVd isolates from avocado (Fig. 1, lanes 2 and 3) indicated that they were identical to the previously described mild and intermediate strains of PSTVd (21). A severe strain of PSTVd (KF440-1) (21) was also detected in one tree from the U.C. Riverside avocado collection (results not shown).

Association of PSTVd with symptom expression. To better characterize the relationship between the presence of viroid(s) and the appearance of specific types of symptoms, 24 viroid-infected trees growing at La Molina were monitored for the presence of PSTVd or ASBVd as well as symptom expression over a 3-yr period (Table 3). Nine of the 13 symptomatic trees were infected with both PSTVd and ASBVd, and four trees were infected with PSTVd alone. Both PSTVd and ASBVd had been detected in the three trees that died during the observation period. Single infections were more common among asymptomatic trees. Of the 11 asymptomatic trees, PSTVd was detected in 5 trees, ASBVd was detected in four trees, and both viroids in two trees. As previously reported for the detection of PSTVd in tuber sprouts (18), NASH analysis was more sensitive and reliable than PAGE for viroid detection.

PSTVd infection of trees growing in coastal locations was associated with two different disease syndromes. Infected Topa Topa and Zutano trees showed an erect branching pattern with weak and slender branches (Fig. 4A), but in Super Fuerte, branches were slender and frequently displayed a horizontal growth pattern (not shown). In severely affected trees, leaf size was reduced to between one- to two-thirds of that typical for healthy trees. Leaves from infected trees were chlorotic in all cultivars examined.

Table 3. Presence of viroids and symptom in avocados tested at La Molina

Variety	NASH analysis[a] 1990	1991	1992	July 93	Dec. 93	PAGE[b] Dec. 93	Symptoms observed during survey period[c]
Fuerte #3	−	+		+	+	−	
		−		+	+	−	None
#4	−	+	+	+	+	+	
	−	−	−	+	+	−	None
#8					−	−	
					+	+	None
Super Fuerte #8[d]	+	+	+	+	+		
	−	−	+	+	+		Hb, Df, Rls, Bi, Rfs, Db
Topa Topa #5[d]	+	+	+				
	−	−	+				Vb, Df, Rls, Bi, Rfs, Db
Zutano #13[d]	−	+	+	+	+		
	−	+	+	+	+		Vb, Df, Rls, Bi, Rfs, Db
#17	−	−	+	+	+	+	
	−	+	−	+		−	Vb, Df
Zutano	−	−	+				
	−	−	−				Vb, Df
Collinred #11	−	−	+	+		+	
	−	−	+	+	+	+	Vb, Df
#16	−	−	+				
	−	−	−				None
#19	−	−	+				
	−	−	−				None
Villacampa	−	+	+				
	−	−	−				Vb, Df
	−	+	+				
	−	−	−				Vb, Df
	−	+	+				
	−	−	−				Vb, Df
Villacampa #12				+	+	−	
				+	+	−	Vb, Df
Duke #1	−	+	+				
	−	−	−				None
#2	−	−	+				
	−	−	−				None
#3	−	−	+				
	−	−	−				None
#11	−						
	+						None
#12	−						
	+						None
#13	−						
	+						None
Campong	−	+	+				
	−	+	+				Vb, Df
P-11		+	+	+			
		−	+	+			Vb, Df
Mexicano		+		+	+	+	
		−		+	+	+	Vb, Df

[a]Nucleic acid spot hybridization. For each entry, results of the potato spindle viroid analysis are shown above those of the avocado sunblotch viroid analysis.
[b]Polyacrylamide gel electrophoresis.
[c]Not all symptoms listed were present at all times. Abbreviations: Df, defoliation; Vb, vertical branch growth; Hb, horizontal branch growth; Rls, reduced leaf size; Bi, bunchy inflorescence; Rfs, reduced fruit size; Db, death of branches.
[d]Tree died during observation period.

Chlorosis was more severe in the older foliage, and chlorotic leaves in Zutano sometimes showed tip necrosis as well as necrotic arcs or rings along their margins. Apical necrosis followed by death of secondary and tertiary branches was also observed in some affected plants. Severe chlorosis and extensive leaf drop preceded the dessication and death of several trees.

The most prominent symptom found in PSTVd-infected avocado was a "bunchiness" of the inflorescence (Fig. 4B). Bunchiness was severe in infected Topa Topa and Duke but less pronounced in Zutano and Super Fuerte. In the latter cultivars, inflorescences also became severely chlorotic. Inflorescences occasionally developed directly upon the lignified branches of infected trees, and fruit peduncles of infected trees were usually shorter than those of healthy trees. Fruits from infected Hall trees were much smaller in overall size than those from healthy plants, but seed size was not reduced (Fig. 5A). Although uninfected avocados often produce parthenocarpic fruit, the proportion of such fruit was much higher on PSTVd-infected trees. PSTVd-infected Super Fuerte trees produced only parthenocarpic fruit, and those fruit were much smaller than fruits from viroid-free trees (Figs. 5B and 5C).

Severe yield reductions were observed at La Molina. A single PSTVd-infected Topa Topa tree growing there produced 28 fruit with a total weight of 1.4 kg, whereas healthy trees in the same field yielded an average of 500 fruit per tree with an average total weight of 150 kg. Similarly, a diseased Zutano tree produced 64 fruit per tree with a total weight of 10.5 kg, whereas a healthy tree produced 220 fruit with a total weight of 80 kg. Data from other sites are not available.

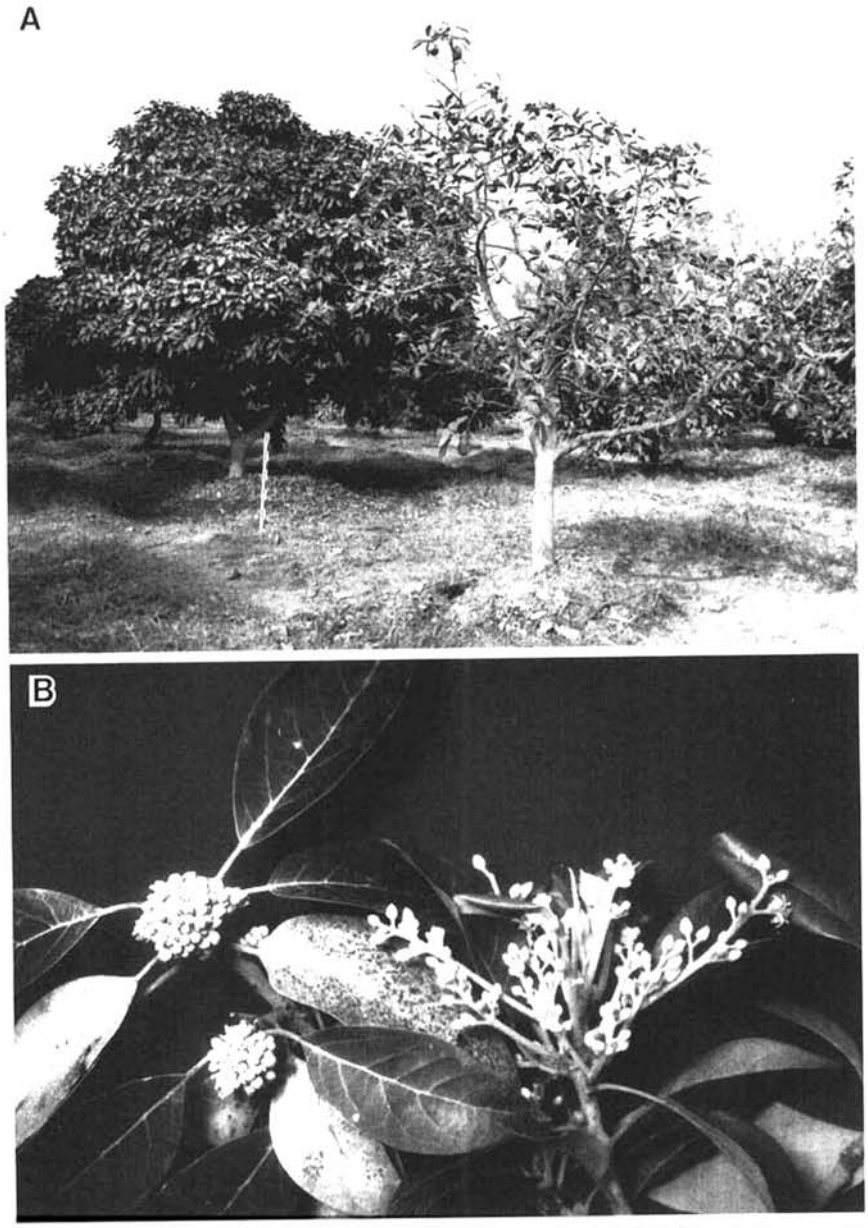

Fig. 4. Symptoms associated with potato spindle tuber viroid (PSTVd) infection in avocado. **(A)** Erect branching habit associated with advanced disease; weak, slender branches and extensive leaf drop on tree in foreground. **(B)** Characteristic bunchiness of inflorescence in Topa Topa. Left, inflorescences from viroid-infected tree; right, uninfected control.

DISCUSSION

Avocado trees growing at several locations in Peru have been shown to be infected with PSTVd. This is the first report of the isolation of PSTVd from a natural host other than potato, and two different PSTVd sequence variants were recovered from infected trees by transfer to *N. physalodes* or PCR-mediated amplification of cDNAs synthesized in vitro. PSTVd and ASBVd were not always detectable by R-PAGE analysis.

Reliance upon a single source of imported germ plasm provides the most likely explanation for widespread occurence of PSTVd in Peruvian avocados; indeed, we were able to demonstrate the presence of PSTVd in at least one tree in the University of California, Riverside collection. Potato is the only other known natural host of PSTVd (24), and how the initial transfer of PSTVd from potato to avocado occurred is unknown. Two scenarios for such a transfer appear plausible.

In Peru (and possibly elsewhere), potatoes and avocados are often intercropped. Both PSTVd and ASBVd are known to be transmitted through the seed or pollen of infected plants (5,7), and insect-mediated transfer of viruses between genetically incompatible species via virus-infected pollen, although rare, is known to occur (23). Other viruses can be mechanically transmitted using pollen from virus-infected plants as inoculum (8). Transmission of PSTVd by nematodes (e.g., *Meloidogyne incognita*) provides yet another possible means of viroid transfer (10). The precise role of these processes in spread of PSTVd in the field remains to be determined.

The role of PSTVd in disease induction remains unclear. The incidence of latent PSTVd infections in moist, inland areas was as high as 100%, and a number of PSTVd/ASBVd coinfections were detected. Certain of the symptoms exhibited by PSTVd-infected trees growing at La Molina resemble those previously attributed to either environmental stress or other pathogens. The leaf chlorosis and tip necrosis observed in several trees are commonly associated with sodium toxicity (28). La Molina is located in the arid coastal region of Peru, and trees growing there must be irrigated. Other symptoms including leaf drop followed by dessication and death resemble those caused by infection with *Phytophthora cinnamomi* (2,29), which characteristically results in girdling cankers in the cambium and phloem tissue near the collar region. None of the viroid-infected trees identified during our

surveys showed any evidence of such cankers, and the foliar symptoms did not include branch dieback.

Several of the symptoms observed have previously been associated with other viroid infections. A sprawling, somewhat stunted appearance and reduced fruit yield have been associated with ASBVd infection (4), but other symptoms (e.g., vertical branch growth and the bunchiness of inflorescences shown in Fig. 2) may reflect hormonal imbalances similar to those associated with PSTVd infection in other hosts (6). Even these symptoms may not be specific to PSTVd infection, however, as similar symptoms were also observed during studies of avocado black streak disease carried out at the University of California, Riverside (11,12,14). The presence of 1–3 distinctive groups of double-stranded RNAs in those trees was taken as evidence for the existence of "viruslike agents" in avocado (12).

Why PSTVd and ASBVd were not always detected symptomatic trees is unclear. ASBVd-infected trees are known to enter a "symptomless carrier state" characterized by a dramatic drop in viroid titer and the complete absence of foliar symptoms (27). Further inoculation studies will be necessary to investigate the possible role of interaction(s) between PSTVd and ASBVd in disease induction.

ACKNOWLEDGEMENTS

We thank I. Bartolini and V. Lazarte for technical assistance, H. D. Ohr for providing leaf samples from the avocado collection at the University of California, Riverside, and L. Levy and E. V. Podleckis for performing the nucleic acid hybridization and initial PCR analyses on these samples. Cloned ASBVd cDNAs were kindly provided by R. H. Symons (Department of Plant Science, Waite Campus, University of Adelaide). Discussions with J. W. Randles, T. O. Diener, and R. L. Jordan were most helpful during preparation of this manuscript for publication.

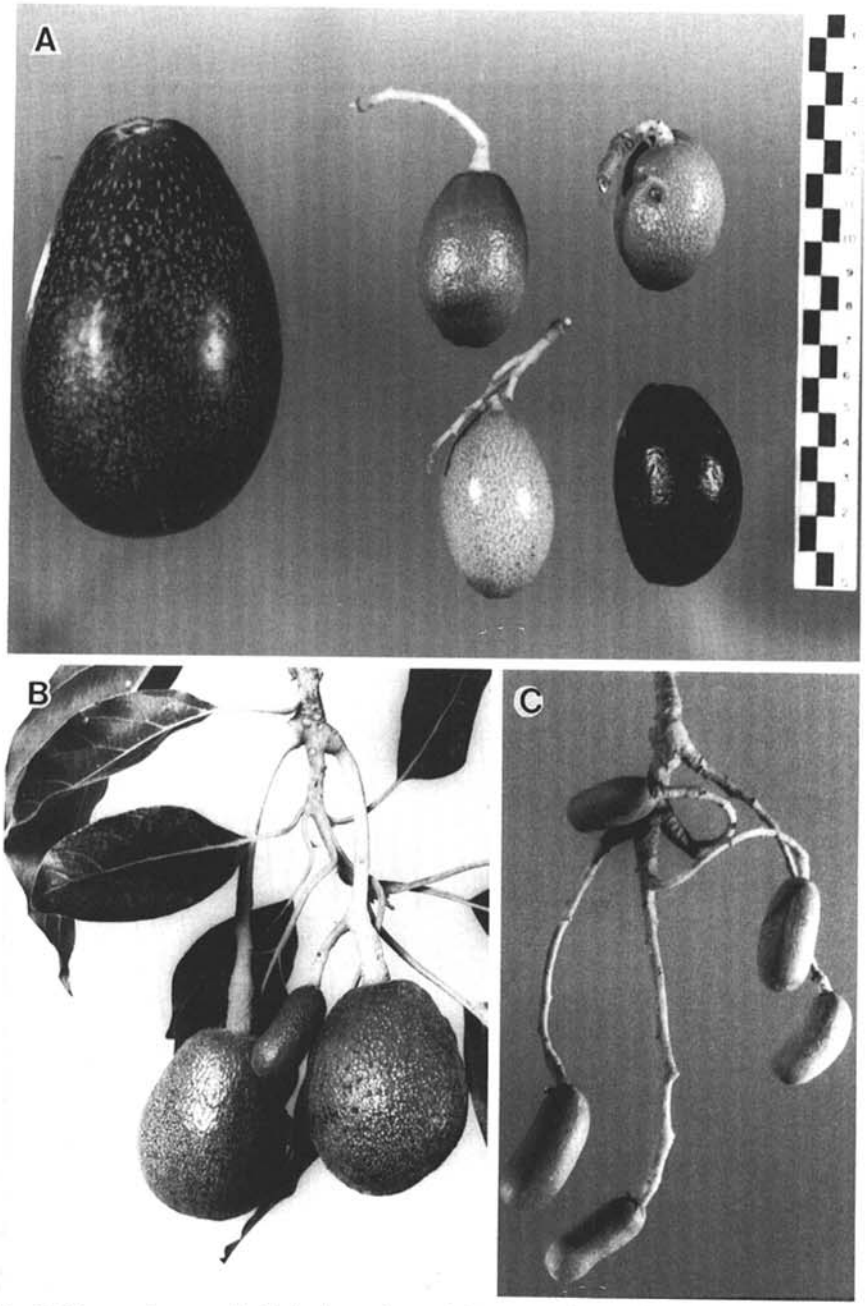

Fig. 5. Changes in avocado fruit size and morphology associated with potato spindle tuber viroid (PSTVd) infection. (**A**) Fruits from healthy (left) and PSTVd-infected Hall trees. (**B, C**) Fruit set on healthy and PSTVd-infected Super Fuerte, respectively.

LITERATURE CITED

1. Alper, M., Bar-Joseph, M., Salomon, R., and Loebenstein, G. 1978. Some characteristics of an avocado strain of tobacco mosaic virus. Phytoparasitica 6:15-20.
2. Crandall, B. S. 1948. *Phytophthora cinnamomi* root rot of avocados under tropical conditions. Phytopathology 38:123-130.
3. Cress, D. E., Kiefer, M. C., and Owens, R. A. 1983. Construction of infectious potato spindle tuber viroid cDNA clones. Nucleic Acids Res. 11:6821-6835.
4. Desjardins, P. R. 1987. Avocado sunblotch. Pages 299-313 in: The Viroids. T. O. Diener, ed. Plenum Press, New York.
5. Desjardins, P. R., Drake, R. J., Sasaki, P. J., Atkins, E. L., and Bergh, B. O. 1984. Pollen transmission of avocado sunblotch viroid and the fate of the pollen recipient tree. Phytopathology 74:845.
6. Diener, T. O. 1987. Biological properties. Pages 9-35 in: The Viroids. T. O. Diener, ed. Plenum Press, New York.
7. Fernow, K. H., Peterson, L. C., and Plaisted, R. L. 1970. Spindle tuber virus in seed and pollen of infected potato plants. Am. Potato J. 47:75-80.
8. Francki, R. I. B., and Miles, R. 1985. Mechanical transmission of sowbane mosaic virus carried on pollen from infected plants. Plant Pathol. 34:11-19.
9. Hutchins, C. J., Rathjen, P. D., Forster, A. C., and Symons, R. H. 1986. Self-cleavage of plus and minus RNA transcripts of avocado sunblotch viroid. Nucleic Acids Res. 14:3627-3640.
10. Jatala, P., Delgado de la Flor, R., Querci, M., Salazar, L., and Lazarte, V. 1991. Possible implication of *Meloidogyne incognita* in the dissemination of potato spindle tuber viroid. Nematropica 21:127.
11. Jordan, R. L. 1981. Avocado blackstreak disease: Symptomatology, epidemiology and attempts to identify the causal agent. Ph.D. diss. University of California, Riverside.
12. Jordan, R. L., Dodds, J. A., and Ohr, H. D. 1983. Evidence for viruslike agents in avocado. Phytopathology 73:1130-1135.
13. Levy, L., Lee, I-M., and Hadidi, A. 1994. Simple and rapid preparation of infected plant tissue extracts for PCR amplification of virus, viroid, and MLO nucleic acids. J. Virol. Methods. 49:295-304.
14. Ohr, H. D., Platt, R. G., and Zentmeyer, G. A. 1976. Black streak disease of avocados. Calif. Avocado Soc. Yearb. 60:177-179.
15. Owens, R. A., Candresse, T., and Diener, T. O. 1990. Construction of novel viroid chimeras containing portions of tomato apical stunt and citrus exocortis viroids. Virology 175:238-246.
16. Owens, R. A., and Diener, T. O. 1984. Spot hybridization for detection of viroids and viruses. Pages 173-187 in: Methods in Virology. Vol. 7. K. Maramorosch and H. Koprowski, eds. Academic Press, New York.
17. Salazar, L. F., Balbo, I., and Owens, R. A. 1988. Comparison of four radioactive probes for the diagnosis of potato spindle tuber viroid by nucleic acid hybridization. Potato Res. 31:431-442.
18. Salazar, L. F., Owens, R. A., Smith, D. R., and Diener, T. O. 1983. Detection of potato spindle tuber viroid by nucleic acid spot hybridization: Evaluation with tuber sprouts and true potato seed. Am. Potato J. 60:587-597.
19. Salazar, L. F., and Querci, M. 1992. Detection of viroids and viruses by nucleic acid probes. Pages 129-144 in: Techniques for the Rapid Detection of Plant Pathogens. J. M. Duncan and L. Torrance, eds. Blackwell Scientific Publications, Oxford.

20. Sano, T., Candresse, T., Hammond, R. W., Diener, T. O., and Owens, R. A. 1992. Identification of multiple structural domains regulating viroid pathogenicity. Proc. Natl. Acad. Sci. U.S.A. 89:10104-10108.
21. Schnölzer, M., Haas, B., Ramm, K., Hoffmann, H., and Sänger, H. L. 1985. Correlation between structure and pathogenicity of potato spindle tuber viroid (PSTV). EMBO J. 4:2181-2190.
22. Schumacher, J., Meyer, N., Wiedemann, H. L., and Riesner, D. 1986. Diagnostic procedure for detection of viroids and viruses with circular RNAs by "return"-gel electrophoresis. J. Phytopathol. (Berl.) 115:332-343.
23. Sdoodee, R., and Teakle, D. S. 1987. Transmission of tobacco streak virus by *Thrips tabaci:* A new method of plant virus transmission. Plant Pathol. (Oxf.) 36:377-380.
24. Singh, R. P. 1973. Experimental host range of the potato spindle tuber "virus". Am. Potato J. 50:111-123.
25. Singh, R. P., and Boucher, A. 1987. Electrophoretic separation of a severe from mild strains of potato spindle tuber viroid. Phytopathology 77:1588-1591.
26. Vargas, C. O., Querci, M., and Salazar, L. F. 1991. Identificación y estado de diseminación del viroide del manchado solar del palto (*Persea americana* L.) en el Perú y la existencia de otro viroide en palto. Fitopatología 26:23-27.
27. Wallace, J. M., and Drake, R. J. 1962. A high rate of seed transmission of avocado sunblotch virus from symptomless trees and the origin of such trees. Phytopathology 52:237-241.
28. Zentmeyer, G. A., Paulus, A. O., Gustafson, C. D., Wallace, J. M., and Burns, R.M. 1965. Avocado diseases. Univ. Calif. Agric. Exp. Stn. Circ. 535.
29. Zentmeyer, G. A., and Richards, S. J. 1952. Pathogenicity of *Phytophthora cinnamomi* to avocado trees, and the effect of irrgation on disease development. Phytopathology 42:35-37.