Etiology

Evidence for Viruslike Agents in Avocado

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ABSTRACT

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Double-stranded RNA (dsRNA), an indicator for the presence of viruses in plants, has been detected in all except 22 of >280 individual avocado plants tested. Three different dsRNA patterns have been detected. DsRNA pattern 1 has three segments. The smallest segment has a molecular weight (MW) of 0.55×10^6 daltons. The larger segments are resolved as a "doublet" by gel electrophoresis with a MW of $6.0–6.5 \times 10^6$. DsRNA pattern 2 has one major segment with a MW of 3.0×10^6. DsRNA pattern 3 has three major segments with MWs of 2.0, 1.9, and 1.7×10^6. The three dsRNA patterns have been detected both singly and in all possible combinations in different individual plants. Three different viruses (avocado viruses 1, 2, and 3) are proposed to explain the variation in the dsRNA patterns detected. All three putative avocado viruses appear to be latent in some cultivars (symptomless carriers). Avocado viruses 1, 2, and 3 are all seed transmitted at a high rate. Avocado viruses 1 and 2 are graft-transmitted across the bud union. Avocado virus 3 is present in all plants of the Hass cultivar tested, both healthy and avocado blackstreak-affected (ABS). Virus 3 alone appears to cause no symptoms. Avocado virus 2 has been detected in tissues from ABS-affected cultivar Hass trees, but has not been detected in healthy Hass trees in the field. It is possible, but not proven, that avocado virus 2, either alone or in combination with avocado virus 3, is involved in the ABS disease. Several avocado cultivars had no detectable dsRNA, including the *Phytophthora* root rot resistant clonal rootstocks Duke 6 and Duke 7. This is the first report of the presence of viruslike agents other than tobacco mosaic virus in avocado.

Avocado blackstreak (ABS) is a newly recognized major disease of avocado (*Persea americana* Miller) in California for which a viral etiology has been proposed based on lack of association of other microorganisms, nonrandom spread of the disease in the field, symptoms, and lack of remission of symptoms after antibiotic treatment (14, and R. L. Jordan, *unpublished*). The objective of this study was to detect viruses in avocado. The detection of double-stranded RNA (dsRNA) (23) is commonly used to study plant diseases believed to be of viral etiology, but for which no viruslike particles have been purified. This approach is based on the observation that high-molecular-weight dsRNA has been detected only in virus-infected plants (12,24).

In this report, we describe three viruslike agents of avocado and

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provide evidence for the presence of viruses other than tobacco mosaic virus (1,14). A portion of this work has appeared in abstract form (15). Evidence of detection of virus particles and disease-specific proteins and efforts to mechanically transmit avocado viruses will be described elsewhere.

MATERIALS AND METHODS

Avocado samples were obtained from 5- to 20-yr-old grafted field-grown trees, and 1- to 2-yr-old grafted and ungrafted seedlings grown in the greenhouse or lathhouse. Tissue from apparently healthy trees, registered sunblotch-free, was obtained from the University of California at Riverside (UCR) Registration Block, and from the Mother Block at nursery 'B' in Ventura County, CA. Other healthy sources of tissue were obtained from various locations at UCR, and from nurseries in San Diego, Riverside, and Ventura counties. Tissue from healthy and avocado blackstreak diseased (ABS) trees were obtained from avocado groves in San Diego and Ventura counties. For graft transmission studies conducted in the greenhouse, scion material was obtained from the UCR Registration Block and bud- or wedge-grafted to 2- to 3-mo-old seedlings. Before grafting, rootstock seedlings and scion sources were analyzed for dsRNA content.

Double-stranded RNA purification. Extraction and purification of double-stranded RNA (dsRNA) was as performed by Dodds (6) and Morris and Dodds (23) with minor modifications. Ten grams of tissue, usually from mature leaves, was ground to a powder in the presence of liquid nitrogen in a chilled mortar and pestle. The frozen powder was extracted with 40 ml of double-strength STE buffer (single strength = 0.1 M NaCl, 0.05 M tris-HCl, 1 mM EDTA, pH 7.0), 6 ml of 10% sodium dodecylsulfate, 1 ml of 2-mercaptoethanol, and 25 mg of bentonite (9). The extract was shaken on ice for 30 min with an equal volume of both STE-saturated phenol and chloroform. The emulsion was broken by centrifugation and the aqueous phase was adjusted to 15% ethanol (v/v). The dsRNA was purified by one or two cycles of CF-11 cellulose chromatography on small columns (23, method 1). Prior to electrophoresis some samples were treated with deoxyribonuclease (DNase I; Worthington Biochemical Corp., Worthington, OH 43085) at 10 μg/ml in 5 mM $MgCl_2$.

Gel electrophoresis. The purified dsRNA from 1 to 5 g of tissue was electrophoresed on 6 × 950 mm 5% polyacrylamide tube gels (acrylamide:bisacrylamide [40:1, v/v]) for 10–12 hr at 5 mA per tube at room temperature. The electrophoresis buffer was 0.04 M tris, 0.02 M sodium acetate, 1 mM EDTA, pH 7.8. Gels were stained in 10–50 ng of ethidium bromide per milliliter of 1 mM EDTA for 2–5 hr at room temperature and destained in several changes of distilled water or 1 mM EDTA for 2–5 hr. Gels were photographed on Polaroid Type 52 film, and the nature of the fluorescent bands was identified by poststaining digestion of the gel with ribonuclease at 50 μg/ml (RNase 1A; Sigma Chemical Co., St. Louis, MO 63178) in the presence of water or 0.3 M NaCl, or DNase I (50 μg/ml) in the presence of 5 mM $MgCl_2$ (6,23).

The dsRNAs of a VLP from *Penicillium chrysogenum* (PcV, MW = 2.15, 1.85×10^6), a VLP from *P. stoloniferum* (PsV, MW = 0.89, 0.42×10^6), and a VLP from *Helminthosporium maydis* (Hm9, MW = 5.7×10^6), (2–4), were used as molecular weight standards (kindly supplied by R. F. Bozarth, Indiana State University, Terre Haute). Tobacco mosaic virus (TMV) dsRNA from TMV-infected tobacco, and cucumber mosaic virus (CMV) dsRNA from CMV-infected *Vinca rosea* were purified as above and also used as molecular weight markers. Molecular weights were calculated by using the graphical method of Bozarth and Harley (3) to obtain corrected values for the dsRNA standards (7,16).

RESULTS

Detection of double-stranded RNA in avocado. The method permitted rapid, efficient isolation and analysis of dsRNA from small amounts of virus-infected (TMV and CMV) tissue of various hosts and of avocado tissue being assayed. For example, the electrophoretic pattern of the dsRNA fraction from 1.0 g of TMV-infected tobacco showed the presence of one major dsRNA species with an estimated molecular weight of 4.1×10^6 (Fig. 1J), consistent with that expected for the replicative form (RF) of TMV with a single RNA genome of 2.05×10^6 daltons (13,27,28). The minor dsRNA species reported by Zelcher et al (28) also were observed after longer film exposure (*unpublished*). The electrophoretic profile of the dsRNA fraction isolated from CMV-infected periwinkle showed four major dsRNA species (not shown) with MWs consistent with the RFs of the four CMV genomic ssRNAs (16).

Three major dsRNA patterns were detected in avocado by polyacrylamide gel electrophoresis of samples from as little as 1 g of tissue. The combinations of the observed dsRNA patterns and the marker dsRNAs are shown in Fig. 1. The fluorescent bands illustrated in Fig. 1 were identified as stained dsRNA segments because fluorescence was not lost after incubation in ribonuclease in 0.3 M NaCl (after 4 hr), but it was lost after incubation in ribonuclease in water (Fig. 2A and B). Partial digestion occurred after 3 hr of incubation in RNase in water, leaving only the core of the gel bands stained (Fig. 2D). No stained dsRNA bands remained after 4–5 hr. The standards were resistant to RNase in 0.3 M NaCl and were digested in water (*unpublished*). Because 0.3 M NaCl both reduces the fluorescence of the ethidium bromide-stained bands and increases the background fluorescence, longer than normal film exposure was required to detect the dsRNA bands shown in Fig. 2A and B. All dsRNA bands were resistant to deoxyribonuclease digestion in digestion experiments performed either on samples prior to electrophoresis or on gels after electrophoresis (*unpublished*).

DsRNA patterns and cultivar distribution. Several avocado cultivar selections had no detectable dsRNA (Fig. 1A; see also Table 1). Among those were the avocado root rot (caused by *Phytophthora cinnamomi* Rands)-resistant clonal rootstocks Duke 6 and Duke 7, and the newly introduced Guatemalan selection G755. All sources of Duke 6 and 7 were consistently found to contain no detectable dsRNA. A total of 28 individual plants

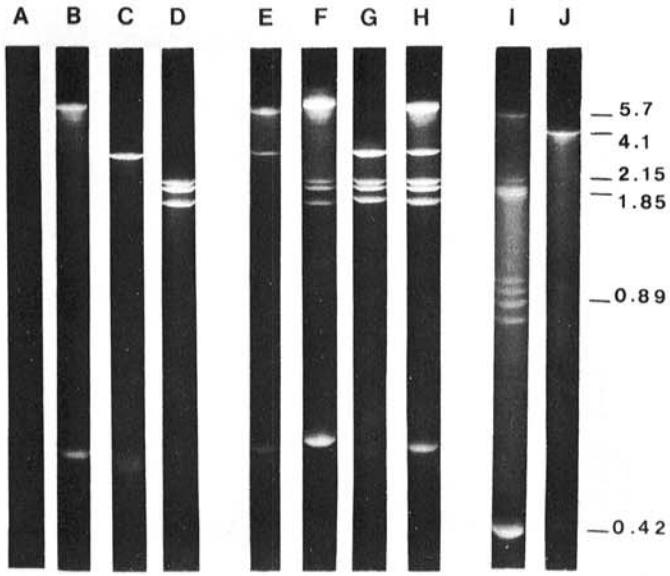

Fig. 1. Polyacrylamide gels showing the different dsRNA patterns detected in avocado tissue (**A–H**), compared with the dsRNAs of: Hm9 VLP (MW = 5.7×10^6), PcV (2.15 and 1.85×10^6), PsV (0.89 and 0.42×10^6), and TMV (4.1×10^6). **A,** DsRNA pattern 0 (no detectable dsRNA). **B,** DsRNA pattern 1. **C,** DsRNA pattern 2. **D,** DsRNA pattern 3. **E,** DsRNA patterns 1 and 2. **F,** DsRNA patterns 1 and 3. **G,** DsRNA patterns 2 and 3. **H,** DsRNA patterns 1, 2, and 3. **I,** DsRNAs from Hm9 VLPs, PcV, and PsV (MWs noted at right). **J,** TMV dsRNA from TMV-infected tobacco. DsRNAs from 3.0 g (**A–H**) or 1.0 g (**J**) of tissue. Electrophoresis was in 5% polyacrylamide gels at 5 mA/gel for 10 hr. Gels were stained in ethidium bromide and destained in water.

from three different sources representing eight different cultivars contained no detectable dsRNA.

DsRNA pattern 1 was resolved as three bands on polyacrylamide gels (Fig. 1B) and was detected in eight plants representing three different cultivars (Table 1). The fastest migrating minor segment had a molecular weight of 0.55×10^6 daltons. The slower migrating major segments consistently migrated as a pair of bands (a "doublet") just above Hm9 VLP dsRNA (MW = 5.7×10^6, Fig. 1I). The doublet migrated faster than the dsRNAs of citrus tristeza and beet yellows viruses (13×10^6 and 8.4×10^6, respectively) (8). The estimated molecular weight of the doublet was calculated to be $6.0–6.5 \times 10^6$ daltons by the graphical method of Bozarth and Harley (3). Several minor bands migrating just below the major species were always present in dsRNA pattern 1 (Fig. 1B); the intensity of these varied directly with the intensity of the major segment.

DsRNA pattern 2, which has one major segment with a molecular weight of 3.0×10^6 daltons (Fig. 1C), was detected in 68 individual plants representing 14 different cultivars (Table 1).

DsRNA pattern 3 had three major segments with molecular weights of 2.0, 1.9, and 1.7×10^6 daltons (Fig. 1D) and was detected in 36 plants representing five different cultivars (Table 1).

A fourth dsRNA pattern (*unpublished*) had a major segment with an estimated MW of $12–14 \times 10^6$, and was detected in only four field-grown cultivar Fuerte trees.

One, two, or all three dsRNA patterns were detected in different samples (Fig. 1B–H). The dsRNA pattern that was detected depended on the avocado cultivar, individual plant, and/or tissue source being analyzed. A portion of the results of analyzing over 30 different cultivars from 12 different sources, including symptomless (presumed healthy), registered sunblotch viroid-free trees, healthy field and nursery stock, and field trees affected with avocado blackstreak disease, are shown in Table 1. Details of selections, sources, and registration numbers of the more than 280 individual plants that were examined are described elsewhere (14). Most of the plants containing no detectable dsRNA (Fig. 1A) or only one dsRNA pattern (Fig. 1B–D), or a combination of dsRNA patterns 1 and 2 (Fig. 1E) or patterns 1 and 3 (Fig. 1F) were symptomless and apparently healthy. Most of the plants containing dsRNA patterns 2 and 3 (Fig. 1G) and all plants containing all three dsRNA patterns (Fig. 1H) had typical symptoms of viral infection. These symptoms included stunting and general decline of the trees, and chlorosis and interveinal rugosity or necrotic spots on the leaves. Four adjacent field-grown cultivar Fuerte trees exhibiting these symptoms contained dsRNA patterns 1 and 3 and the fourth dsRNA pattern.

All plants of cultivar Hass that were tested exhibited dsRNA pattern 3 (Fig. 1D), including the original parent Hass, from which all Hass cultivar budwood is derived. In some Hass plants, dsRNA patterns 2 and 3 were detected (Fig. 1G, Table 1). These plants showed symptoms of avocado blackstreak disease (14; and R. L. Jordan, *unpublished*). Not all avocado blackstreak-affected Hass scions yielded dsRNA pattern 2, however.

Graft transmission. Several observations suggest the possibility of transmission across the bud union of the agents (viruses) responsible for dsRNA patterns 1 and 2 (Table 2). A selection of cultivar Bacon, which consisted of scion budwood derived from the original parent Bacon (unavailable for testing) propagated on Everett rootstock (also unavailable for testing), contained no

TABLE 1. Double-stranded RNA (dsRNA) patterns detected in selected examples of certain avocado (*Persea americana*) cultivars

Cultivar[a]	Source[b]	dsRNA pattern[c]
Bacon, parent	A	0
Duke 6	A,B	0
Duke 7	A,B	0
G6	A	1
Bacon	B	2
Reed	A,B,C	2
Topa Topa, ABS	C	2
Zutano	A	2
Hass, original	A,C	3
Hass, ABS	C	3
Topa Topa	A	3
G6	A	1,2
Fuerte	A,C	1,3
Hass, ABS	C	2,3
Topa Topa, ABS	C	2,3
Ganter, VLSym	A,B	1,2,3
Teague, VLSym	A,B	1,2,3

[a] All individuals tested are from symptomless, healthy trees unless noted: ABS = avocado blackstreak-affected; VLSym = designated healthy in the Registration block, but with viruslike symptoms (see text). Other notations: original = original parent stock on own rootstock; parent = parent stock budded to, and propagated on, different cultivar as rootstock.

[b] A = University of California, Riverside registration block and greenhouses; B = avocado nurseries, Mother stock; and C = avocado field grove sources.

[c] 0 = no detectable dsRNA; for description of dsRNA patterns 1, 2, and 3 see text and Fig. 1.

Fig. 2. Poststaining ribonuclease (RNase) treatment of electrophoresed gels of dsRNA patterns 1, 2, and 3 in the presence of 0.3 M NaCl or water. DsRNA was electrophoresed in 5% polyacrylamide gels, stained and destained as described in Fig. 1. Gels were incubated in 0.3 M NaCl (**A,B**) or water (**C,D**). **A,** Four hours after incubation in 0.3 M NaCl only; **B,** 4 hr after incubation in 0.3 M NaCl with 50 μg/ml RNase 1A (no digestions; reduced band fluorescence and increased background fluorescence due to 0.3 M NaCl); **C,** 4 hr after incubation in water only; **D,** 3 hr after incubation in water and 50 μg/ml RNase 1A (partial digestion; the core of the gel bands was digested in the 4th hr).

detectable dsRNA. This source of Bacon was grafted on a Zutano rootstock (which was analyzed and found to contain dsRNA pattern 2). Upon dsRNA analysis, the resultant Bacon scions had dsRNA pattern 2, indicating transmission across the bud union of the virus responsible for dsRNA pattern 2 (Table 2). A selection of G6 (containing only dsRNA pattern 1) was grafted on a Topa Topa selection (which contained dsRNA patterns 2 and 3). The resultant G6 scion contained dsRNA patterns 1 and 2 (Table 2), again indicating graft transmission of the virus responsible for dsRNA pattern 2. When this same selection of cultivar G6 was propagated on Duke 6 (no dsRNA) the resultant G6 scion still had only dsRNA pattern 1. Other examples of the graft transmission of the viruses responsible for dsRNA patterns 1 and 2 are given in Table 2.

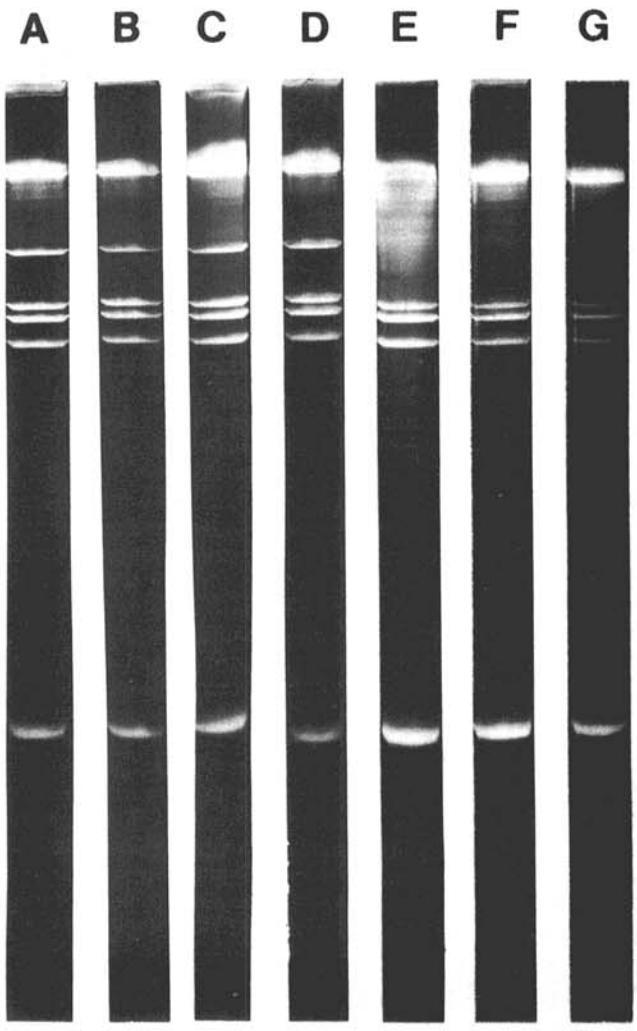

Fig. 3. Polyacrylamide gels showing the dsRNA distribution in an avocado source containing dsRNA patterns 1, 2, and 3. All three dsRNA patterns were detected in: **A,** young, terminal leaves; **B,** mature leaves; **C,** leaf blades of mature leaves with petioles and midribs removed; and **D,** in the excised petioles and midribs. **E,** Mature bark, and **F and G,** large and small roots, respectively, contained only dsRNA patterns 1 and 3. Conditions for electrophoresis are as in Fig. 1. All gels were loaded with the respective dsRNA fractions isolated from 3 g of tissue. Gels **E–G** represent longer film exposure than that for gels **A–D**.

Evidence for the lack of transmission across the bud union has also been observed. In the above example of G6 propagated on Topa Topa, dsRNA pattern 3 (present in the rootstock) was not detected in the G6 scion (Table 2). DsRNA pattern 3 also has not been detected in all analyzed samples of Bacon, Reed, and G6 propagated on a selection of Topa Topa containing dsRNA patterns 2 and 3. The lack of movement of the virus responsible for dsRNA pattern 3 across the bud union might be explained by assuming that Bacon, Reed, and G6 are resistant to the virus. Analysis of Duke 6 and 7 has revealed two other examples of possible cultivar resistance to the viruses responsible for dsRNA patterns 2 and 3. Both cultivars continue to contain no detectable dsRNA after propagation on a selection of cultivar Topa Topa, which contains dsRNA patterns 2 and 3.

DsRNA distribution within an individual plant. Leaves, bark, and roots from a source containing dsRNA patterns 1, 2, and 3 (Fig. 1H) were analyzed to determine the distribution of the dsRNA patterns within a given individual plant. The tissue was taken from a 10-yr-old field-grown Teague seedling exhibiting interveinal pinpoint necrotic lesions on the young terminal leaves and rugosity of the older leaves. DsRNA patterns 1, 2, and 3 were detected in all portions of all leaf tissue tested (Fig. 3A–D). Both older, mature "hardened off" leaves and young terminal succulent leaves contained all three dsRNA patterns (Fig. 3A and B). All three patterns were also detected in mature leaf blades with petioles and midribs removed (Fig. 3C), as well as in the excised petioles and midribs (Fig. 3D). The bark tissue sampled consisted of the cambium and first centimeter of functional phloem adjacent to the woody cylinder from mature bark pieces from the trunk approximately 30 cm above ground level. The large root pieces were approximately 5–7 mm in diameter and the small feeder roots were 1–2 mm in diameter. Only dsRNA patterns 1 and 3 were detected in the bark and root samples (Fig. 3E–G).

Less dsRNA was recovered from bark and roots than from leaves (Fig. 3). DsRNA pattern 2 was not present in bark and roots, although not clearly demonstrated in Fig. 3E (bark) because of the overexposure of the minor bands of dsRNA pattern 1, which migrate in the region of dsRNA pattern 2. In another experiment (not shown), the rootstock sucker leaves from an ABS-infected Hass tree propagated on Topa Topa contained dsRNA patterns 2 and 3, yet the rootstock mature bark contained only dsRNA pattern 3. These results suggest that dsRNA pattern 2 is only found in green plant tissue, but that the virus responsible for dsRNA pattern 2 can indeed reside in nongreen tissue and move to new growth with subsequent production of dsRNA pattern 2.

Seed transmission. DsRNA patterns 2 and 3 were detected in each of 10 greenhouse-grown 6- to 18-mo-old Topa Topa seedlings as well as in the parent Topa Topa tree, from which the seeds were derived. In some individuals, dsRNA pattern 2 was barely

TABLE 2. Graft transmission across the bud union of the agents (viruses) responsible for dsRNA patterns 1 and 2[a]

Scion source		Rootstock		Resultant scion[b] dsRNA pattern	Agent	
Cultivar	dsRNA pattern	Cultivar	dsRNA pattern		Transmitted	Not transmitted
Bacon	0	Zutano	2	2	2	0
G6	1	Topa Topa	2,3	1,2	2	3
Hass	3	Topa Topa	2,3	2,3	2	0
Hx48	3	Ganter	1,2,3	1,2,3	1,2	0
Whitsell	3	Ganter	1,2,3	1,3	1	2
T225	2,3	Ganter	1,2,3	1,2,3	1	0
Duke 7	0	Topa Topa	2,3	0	0	2,3

[a]Scion material was bud or wedge grafted to 2- to 3-mo-old rootstock seedlings. Scion source and rootstocks (before grafting) were analyzed for dsRNA content.

[b]Shoots developing from bud or wedge grafts were analyzed for dsRNA pattern content.

detectable, and longer than normal exposure times were necessary to demonstrate its presence. In other individuals, dsRNA patterns 2 and 3 fluoresced with approximately equal intensity. The dsRNA isolated from the leaves of 15 greenhouse-grown Hass seedlings and from the parent Hass, from which the seeds were derived, all showed the presence of dsRNA pattern 3. In a third experiment, dsRNA patterns 1, 2, and 3 were detected in 6 of 10 Ganter seedlings as well as in the parent Ganter seed source. DsRNA patterns 2 and 3 were detected in the remaining four Ganter seedlings. These observations suggest a high rate of seed transmission of the viruses of dsRNA patterns 1, 2, and 3.

DISCUSSION

The results presented demonstrate the presence of up to three major double-stranded RNA patterns in avocado. These dsRNAs are presumed to be viral in origin, either the RFs of ssRNA viruses or the genomes of dsRNA viruses. This is believed to be the first report of the detection of viruslike agents other than TMV (1,14) in avocado.

It is unlikely that we have discovered three new dsRNA plant viruses with simple genomes in one host plant species. If the dsRNAs detected are not the genome of dsRNA viruses, but indeed the RFs of single-stranded RNA viruses, then from the molecular weights of the respective dsRNAs we can predict the expected viral genomic ssRNA molecular weights. Information of this kind may indicate the viral group (22) in which the putative avocado viruses might be classified. For example, host range studies and purification procedures most appropriate to a specific virus group could then be used to attempt to purify and characterize a particular putative avocado virus.

A virus with an RF of about $6.0–6.5 \times 10^6$ daltons would be expected to have a ssRNA genome of about $3.0–3.5 \times 10^6$ daltons. We could therefore predict that the putative virus of dsRNA pattern 1 (designated avocado virus 1) could be a long flexuous rod belonging to the poty- or closterovirus groups. The faster migrating dsRNA (0.55×10^6) segment, and the multiple minor segments could represent subgenomic dsRNAs of the type described for other viruses (5,8,10,23,28) but as yet not described for potyviruses.

DsRNA pattern 2 (MW = 3.0×10^6) could represent the RF of a virus with an ssRNA of $1.4–1.5 \times 10^6$. The putative virus represented by dsRNA pattern 2 (avocado virus 2) could belong to the groups of small spherical, single component, RNA viruses(11) that replicate via a single major dsRNA species of about 3.0×10^6 daltons (10). The results presented also indicate that avocado virus 2 is latent in many avocado cultivars, is graft transmitted, and is seed transmitted at a high rate.

The expected ssRNA for the putative avocado virus 3 from dsRNA pattern 3 (MW = 2.0, 1.9, and 1.7×10^6) is a multipartite genome of about 1.0, 0.95, and 0.85×10^6 daltons. However, the electrophoretic pattern of dsRNA 3 does not resemble the electrophoretic patterns of the dsRNAs isolated from tripartite genome (22,25) virus-infected plants such as brome mosaic virus, cucumber mosaic virus, cowpea chlorotic mottle virus, or alfalfa mosaic virus (R. L. Jordan, *unpublished*).

It is also possible that avocado virus 3 is a "cryptic" virus in avocado similar to beet cryptic virus (17,26), carnation cryptic virus (20,21), poinsettia cryptic virus (19), and vicia cryptic virus (18). To date the viral genome of only one cryptic virus has been characterized (20), and it is difficult to make comparisons to putative avocado virus 3. Carnation cryptic virus (CCV) has been reported (20) to have a dsRNA genome consisting of three major segments (MW = 1.04, 0.95, and 0.84×10^6) and one minor segment (MW = 0.88×10^6) and, like the other cryptic viruses, is seed transmitted at a high rate, occurs in low concentration in plants, usually without causing symptoms, and appears not to be mechanically or graft transmissible. As described above, dsRNA pattern 3 has three segments with molecular weights of 2.0, 1.9, and 1.7×10^6 (approximately twice that of the dsRNA of CCV), is seed transmitted at a high rate, alone appears to cause no symptoms in Hass, and does not appear to be graft transmissible.

The virus groups in which each of the putative avocado viruses should be placed will not be determined until the viruslike agents have been purified and their particle morphology, serological relationships, and genome size have been characterized. This research is in progress.

Putative avocado virus 2 appears to be latent in several cultivars, and putative avocado virus 3 is present in all Hass individuals tested, healthy and ABS, and alone appears to cause no symptoms in Hass avocado. Putative avocado virus 2 has been detected in many Hass trees affected with avocado blackstreak, but not in ABS-free Hass. Putative avocado virus 2 has not been detected in rootstock suckers from field-grown healthy Hass propagated on Topa Topa, whereas all rootstock suckers from ABS-affected Hass on Topa Topa tested have contained avocado virus 2. It is, therefore, possible that the putative avocado virus 2, either alone or in combination with putative avocado virus 3, is (or are) involved in the avocado blackstreak disease. Heat therapy of avocado selections for graft-transmission experiments is now in progress to test this hypothesis.

The use of dsRNA analysis as a new tool to detect the presence of viruses in avocados has been substantiated. The procedure is useful for the study of plant diseases presumed to be of viral etiology, if no previous physical evidence has been presented to confirm that belief.

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