Association of Double-Stranded Ribonucleic Acids with Lettuce Big Vein Disease

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ABSTRACT

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Healthy roots and leaves of lettuce (*Lactuca sativa*) plants lacked detectable double-stranded ribonucleic acid (dsRNA). Disease-specific dsRNAs were readily detected in lettuce plants experimentally infected with each of four isolates of *Olpidium brassicae* known to transmit the lettuce big vein (LBV) agent. No dsRNAs were detected when an isolate of *O. brassicae* that does not transmit the LBV agent was used. The highest concentration of dsRNA was in the roots of plants maintained at 18 C for 30–40 days after inoculation, but dsRNA could be detected as early as 6 days after inoculation. No dsRNA has been detected before or after symptom development in extracts from up to 100 g of leaves of plants infected by the LBV agent. The dsRNAs were detected in roots, but not leaves, of lettuce plants vegetatively propagated from shoots from plants infected by the LBV agent. These roots were no longer infected with *O. brassicae*, and an isolate of *O. brassicae* known to be free of the LBV agent acquired the dsRNAs from these roots and transmitted them to healthy plants. These plants developed LBV symptoms 3–4 wk later, and dsRNA was detected in their roots. The dsRNAs of tobacco necrosis virus are distinct from, and do not hybridize to, the dsRNAs associated with LBV disease. The number (between one and six) and the relative amounts of dsRNAs associated with LBV disease varied with the experiment, the isolate, and the time of harvest. Three dsRNAs (MW = 2.3, 2.1, and 0.72×10^6) have been detected more consistently than the others. The results suggest that an RNA virus causes LBV disease.

Additional key words: soilborne viruses, viroid.

Lettuce big vein (LBV) disease is a widespread plant disease without an identified causal agent (2). The disease has viruslike symptoms that include stunting and severe vein clearing. It is graft transmissible from shoots (4) and is naturally vectored from and to roots by the chytrid fungus, *Olpidium brassicae* (Wor.) Dang (3,17). For these reasons, a virus or viroid cause for this disease has been considered (19). Prior to this study, the best evidence for an LBV agent is the report of rod-shaped viruslike particles, found only in diseased plants (12). These particles resemble those of tobacco stunt virus, which is also vectored by *O. brassicae*.

The objective of this research was to examine the LBV disease by searching infected tissues for viroid or viruslike nucleic acids, in addition to virus particles, and to confirm that the tissue was not contaminated with tobacco necrosis virus (TNV), another virus transmitted by *O. brassicae* (10).

MATERIALS AND METHODS

Viruses and hosts. The isolate (I-1) of the LBV agent that was used was initially transmitted to lettuce (*Lactuca sativa* L.) seedlings by *O. brassicae* recovered from roots of an infected field plant collected in the Imperial Valley, CA, in 1981. Other isolates of *O. brassicae* that were used, which also had the LBV agent associated with them, included an isolate (I-2) used by R. N. Campbell, Department of Plant Pathology, University of California, Davis, and an isolate (I-3) provided by E. Ryder, USDA, Salinas, CA. Campbell also provided an isolate from Holland (I-4) and an LBV-free isolate (I-5). Two-week-old seedlings of a susceptible lettuce cultivar (*Lactuca sativa* L. 'Great Lakes') grown in sterile sand were infected with the LBV agent by exposing their roots to a suspension of zoospores of *O. brassicae* released from the roots of plants that had shown LBV symptoms for at least 2 wk (3). Plants were maintained in a growth chamber at 18 C with 16 hr of light (400 μEinsteins/m^2/sec) a day.

TNV was maintained in leaves in Turkish tobacco (*Nicotiana tabacum* L.) (7) grown in a greenhouse and was used to show that experimental plants were free of TNV and that the LBV agent is not related to TNV. Buffered (0.1 M potassium phosphate, pH 7.0, 1.0% Celite) extracts from LBV-affected lettuce roots, containing readily detectable amounts of dsRNA, were rubbed on leaves of bean (*Phaseolus vulgaris* 'Top Crop') or tobacco. TNV in potassium phosphate-buffered tobacco sap was mechanically transmitted to these hosts in the same manner (10).

Vegetative propagation of LBV-affected plants. Lettuce plants with LBV symptoms but free of *O. brassicae* were obtained by vegetative propagation of LBV-affected shoots. Two-week-old lettuce seedlings were inoculated with a suspension of zoospores carrying the LBV agent. The growing tips of these plants were excised 4 wk after inoculation and transferred to KRM rooting medium (11). Roots began to regenerate within 5 days and were well developed within 4 wk. At this point, the plantlets were transplanted to sterile sand and maintained as above.

Nucleic acid purification and analysis. Two nucleic acid purification procedures were used: fractionation in 2 M LiCl (15) (a standard approach for isolation of viroids) and selective purification of dsRNA by cellulose chromatography of buffered extracts in 15% ethanol (8,13).

In the first scheme, healthy or infected tissues were homogenized in two volumes of GPS buffer (0.2 M glycine, 0.1 M $Na_2HPO_4 \cdot 7H_2O$, 0.6 M NaCl adjusted to pH 9.6) containing 1.0% polyvinylpyrrolidone-10, 1.0% sodium dodecyl sulfate (SDS), 1.0% bentonite, 0.2% sodium diethyldithiocarbamate (DIECA), and 0.1% 2-mercaptoethanol. An equal volume of buffer saturated with phenol and a half volume of chloroform:pentanol (25:1) were mixed with this homogenate. The extract was centrifuged at 10,000 *g* for 15 min, and the aqueous phase was adjusted to 2 M LiCl and incubated at 0 C for 4 hr. The solution was centrifuged at 10,000 *g* for 15 min, and the pellet was resuspended in 40 mM tris(hydroxymethyl)aminomethane (tris), 40 mM sodium acetate, 1

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mM (ethylenedinitrilo)-tetraacetic acid (EDTA), disodium salt, containing 15% glycerol, pH 7.8 (electrophoresis buffer). Three volumes of cold ethanol were added to the 2 M LiCl-soluble supernatant. This solution was stored at −20 C for 4 hr, centrifuged at 10,000 *g* for 15 min, and the resulting pellets were resuspended in electrophoresis buffer. The 2 M LiCl-soluble and insoluble nucleic acids were normally electrophoresed for 2.5 hr at 6 mA per gel through 2.5% polyacrylamide gels cast in glass tubes (6 mm × 9 cm). Gels were stained with ethidium bromide (10 ng/ml), placed on an illuminator (260 nm wavelength), and photographed.

In the second scheme (8,13) nucleic acids were extracted as above, except that double-strength STE buffer (0.1 M tris, 0.2 M NaCl, 0.002 M EDTA, pH 6.8) was used in place of GPS buffer. The resulting aqueous phase was adjusted to 15% ethanol and passed over a column of Whatman CF-11 cellulose powder (2.5 g dry weight, 10.0 ml wet volume) equilibrated with STE-buffered 15% ethanol. Unbound deoxyribonucleic acid (DNA) and single-stranded (ss) RNA, were removed from the columns by washing with 200 ml of STE-buffered 15% ethanol. The dsRNA was subsequently eluted with ethanol-free STE buffer. Contaminant DNA and ssRNA were removed by treating with specific nucleases as previously described (16,18). Samples prepared as described above were electrophoresed on 6% polyacrylamide tube gels for 10 hr at 6 mA per gel.

Stained gels were treated with nucleases as follows. Stained, electrophoresed gels were incubated in diluted (1:10) electrophoresis buffer at room temperature for 2–4 hr in the presence or absence of appropriate nucleases. RNase IA (bovine pancrease; Sigma Chemical Company) was added to a final concentration of 0.5 μg/ml (13). Proteinase K-treated (18) DNase I (bovine pancrease; Worthington Biochemicals, Freehold, NJ) was added to a final concentration of 10 μg/ml in the presence of 50 mM $MgCl_2$ (14). Treated gels were incubated in four changes of one-tenth-strength electrophoresis buffer to remove cations and restained in ethidium bromide (10 ng/ml).

Purified TNV dsRNA was end-labeled with ^{32}P as previously described (9). The dsRNA was hydrolyzed by boiling in deionized formamide for 10 min. Single-stranded fragments were 5′-end-labeled with gamma-^{32}P-ATP by using T_4 polynucleotide kinase. Plant tissue (0.5 g) was homogenized in 5.0 ml of 50 mM sodium borate, pH 7.8, 1.5% SDS, and the extract was adjusted to 10 mM CH_3HgOH. Denatured samples (20 μl) were spotted onto nitrocellulose membranes. The 5′-^{32}P-labeled hydrolyzed dsRNAs were hybridized to the spotted nitrocellulose as previously described (9). Kodak X-Ray film was exposed to the washed nitrocellulose for 2 hr at −60 C by using an intensifying screen.

Partial purification of viruslike particles. Purification was from leaves and/or roots of LBV-affected or healthy 8-wk-old lettuce plants 20–30 days after inoculation. Tissue (100 g) was homogenized with a Waring blender in 200 ml of 0.1 M sodium acetate (NaOAc) buffer, pH 5.0, containing 0.4% DIECA. The slurry was expressed through cheesecloth, adjusted to pH 5.0 with glacial acetic acid and left at 4 C for 24 hr. The precipitate was removed by centrifugation at 8,000 *g* for 20 min. The supernatant was decanted and adjusted to 9% polyethylene glycol and stirred for 3 hr at 4 C. The resulting precipitate was pelleted by centrifugation at 12,000 *g* for 30 min. The pellets were resuspended in 10 ml of 0.1 M acetate buffer, pH 5.0, and stirred overnight at 4 C. The precipitate was removed by centrifugation for 20 min at 8,000 *g*. The supernatant was decanted and then centrifuged for 2 hr at 75,000 *g*. The resulting pellets were resuspended in 1 ml of 0.1 M acetate buffer, pH 5.0.

Electron microscopy. Leaf dips of healthy and LBV-affected lettuce tissue were prepared on 100-mesh copper grids that had been coated with Formvar and carbon coated. All preparations were stained in 2% potassium phosphotungstic acid (PTA), pH 7.0. After being stained, the preparations were examined with a Hitachi model HU12 transmission electron microscope.

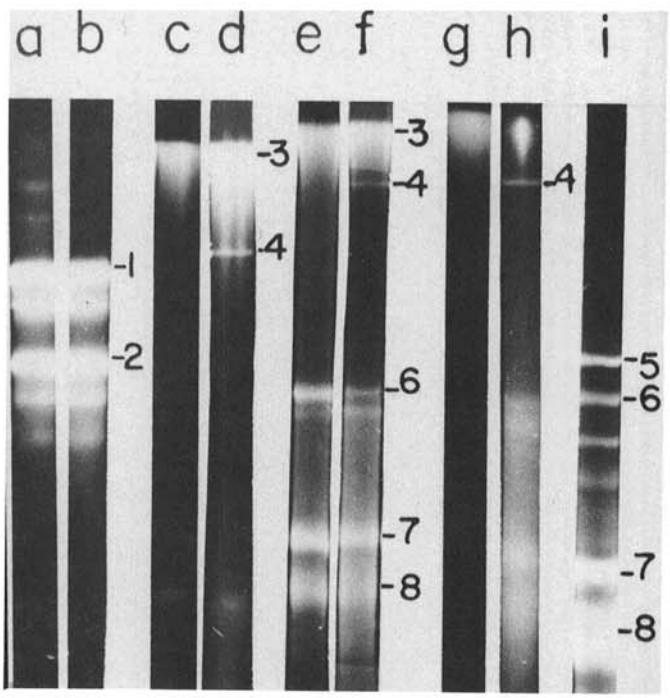

Fig. 1. Fractionation of nucleic acids with 2.0 M LiCl. Samples were from healthy (lanes a, c, and e) and lettuce big vein (LBV)-diseased (lanes b, d, f–h) lettuce or from *Gynura aurantiaca* infected with citrus exocortis viroid (lane i). Insoluble (lanes a and b) and soluble (lanes c–i) nucleic acids were analyzed by electrophoresis for 2.5 hr on 2.5% polyacrylamide gels (lanes a–d) or for 4 hr on 6.0% polyacrylamide gels (lanes e–i). The stained gel shown in lane f was incubated in RNase (lane g) or DNase (lane h), destained, restained, and photographed. Nucleic acids identified in the gels were ribosomal RNA (bands 1 and 2), DNA (band 3), a nucleic acid (dsRNA) diagnostic for the LBV agent (band 4), viroid RNA (band 5), 7S RNA (band 6), 5S RNA (band 7), and 4S RNA (band 8).

RESULTS

Nucleic acid characterization. Ribosomal ssRNAs (Fig. 1, bands 1 and 2), which were insoluble in 2 M LiCl, were the same in preparations from healthy (Fig. 1, lane a) and infected (Fig. 1, lane b) plants. Infected plants did not contain disease-specific ssRNAs. Healthy (Fig. 1, lanes c and e) and infected (Fig. 1, lanes d and f) plants contained similar 2 M LiCl-soluble dsDNA (Fig. 1, band 3), 7S, 5S, and 4S RNAs (Fig. 1, bands 6–8).

No viroidlike molecules were detected in 2.0 M LiCl-soluble nucleic acids from infected plants (Fig. 1, lane f). Such a molecule would be expected to migrate near lettuce 7S RNA (Fig. 1, band 6). The mobility of citrus exocortis viroid (Fig. 1, band 5) is shown for comparison (Fig. 1, lane i).

A diagnostic molecule (Fig. 1, band 4), which was soluble in 2.0 M LiCl, was detected in samples from infected plants, but never in healthy plants, analyzed on 2.5% (Fig. 1, lane d) and 6% (Fig. 1, lane f) polyacrylamide gels. It had migrated faster than lettuce DNA and a dsRNA molecular weight standard ($MW = 1.0 \times 10^6$, not shown), but slower than another dsRNA molecular weight standard ($MW = 0.4 \times 10^6$, not shown). Presumably, it was soluble in 2 M LiCl because it did not appear among 2 M LiCl-insoluble nucleic acids (Fig. 1, lane b). The diagnostic molecule was resistant to digestion by deoxyribonuclease (Fig. 1, lane h) and to digestion by ribonuclease in one-tenth-strength electrophoresis buffer in the presence of 0.3 M NaCl, but was digested by ribonuclease in one-tenth-strength electrophoresis buffer in the absence of NaCl (Fig. 1, lane g). Its solubility in 2 M LiCl and its nuclease digestion properties suggest that the diagnostic molecule is a double-stranded ribonucleic acid.

Purification of dsRNA by cellulose chromatography. The detection of disease-specific dsRNA was confirmed by cellulose chromatography, a technique designed to selectively purify dsRNA (13). Up to six (Fig. 2) dsRNAs ($MW = 3.4, 2.3, 2.1, 0.82, 0.72$, and 0.54×10^6) were detected after prolonged (10 hr) electrophoresis of samples from infected root tissues (Fig. 2, lane j; and Fig. 4, lane a).

Three of the dsRNAs (MW = 2.3, 2.1, and 0.72×10^6) were more abundant than the others and, of these, the 0.72×10^6 was most abundant. This may explain why it was the only dsRNA detected in the 2 M LiCl-soluble nucleic acids extracted from infected plants. All six dsRNAs resisted RNase in 0.3 M NaCl and were digested in one-tenth-strength electrophoresis buffer.

Distribution of dsRNAs. A broad band of DNA 1 cm from the top of the gels (9 cm long) in Fig. 2 was a consistent contaminant in dsRNA samples prepared from roots of healthy and diseased lettuce plants by cellulose chromatography (Fig. 2, lanes a–h). It was routinely removed by incubation of samples or gels in DNAse as described above (Fig. 2, lanes i and j; Fig. 4. Compare predigestion, Fig. 2, lanes g and h, with postdigestion, Fig. 2, lanes i and j). Diagnostic dsRNAs were readily detected in samples prepared from 3.5 g of root tissue from LBV-affected plants (Fig. 2, lanes b, d, f, h, and j) but were not detected in equivalent samples prepared from leaf tissue (Fig. 2, lanes a, c, e, g, and i). Contaminating DNA was not detected as readily in extracts from leaves as in those from roots when an equal amount (fresh weight) of tissue was used. The dry weight of 10 g of roots and leaves was 1.0 g and 0.1 g, respectively, and this difference could account, in part, for the difference in DNA recovery. A large fresh weight of leaf tissue was examined to be sure that lack of detection of dsRNAs in leaves compared to roots was not simply a failure to examine an equivalent sample. DsRNA was not detected in samples isolated from as much as 100 g of leaves with strong symptoms.

Results were the same in two susceptible lettuce cultivars, Great Lakes (Fig. 2, lanes a, b, e, and f) and Iceberg (Fig. 2, lanes c, d, g, and h). Recovery of dsRNA was higher from plants maintained at 18 C (Fig. 2, lanes e–h) than from those maintained at 28 C (Fig. 2, lanes a–d). Symptom development was stronger at 18 C as previously reported (19). Disease-specific dsRNAs were not detected in extracts from up to 100 g of leaves or roots of uninoculated plants or in roots infested with an isolate of *O. brassicae* (I-5) known to be free of the LBV agent (Fig. 3, lane a). Disease-specific dsRNAs were detected in samples from roots of plants inoculated with isolate I-1 as early as 6 days after inoculation and in samples taken at 7-day intervals up to 65 days after inoculation. The concentration of dsRNA in roots increased over this period and was maximal at 30–40 days after inoculation. Disease symptoms appeared in leaves 30–40 days after inoculation. Three other isolates of LBV-agent-transmitting *O. brassicae* (I-2, 3, and 4) have been tested, and dsRNAs were found in the roots of infected plants.

Specific association of dsRNAs with LBV disease. Diagnostic dsRNAs were detected in roots, but not in leaves, of symptomatic

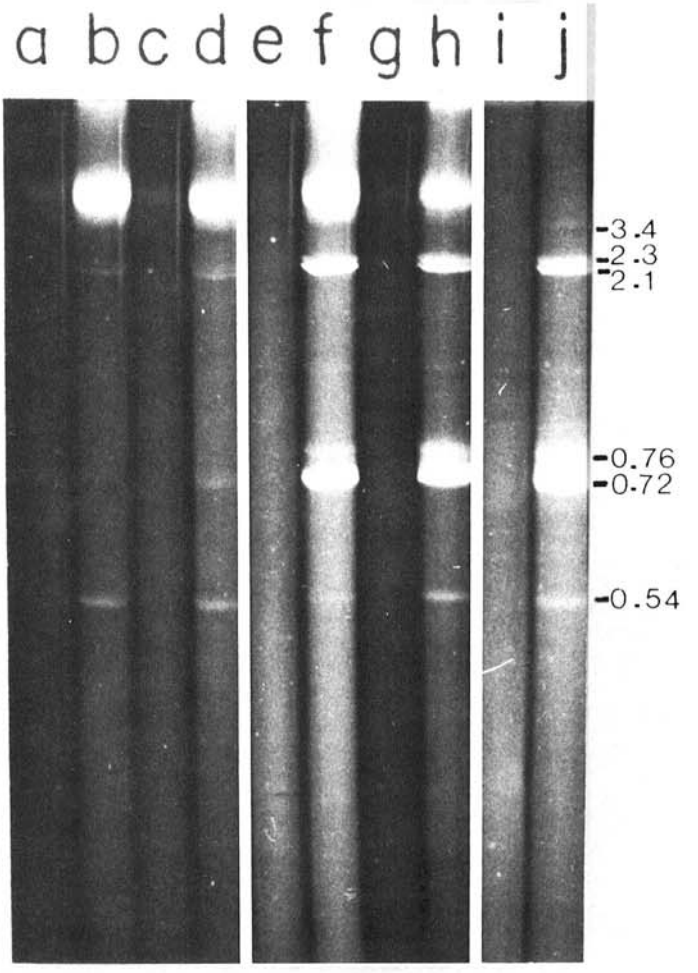

Fig. 2. Effect of growth conditions, lettuce cultivar, tissue source, and DNase treatment on viruslike dsRNA associated with lettuce big vein (LBV) disease. The nucleic acids recovered from 3.5 g of tissue by two cycles of cellulose chromatography were electrophoresed for 9 hr on 6.0%, 9-cm-long, polyacrylamide gels. Plants were maintained after inoculation either in a greenhouse at 28 C (a–d) or an 18 C growth chamber (e–h). Two lettuce cultivars, Iceberg (a, b, e, f, i, and j) and Great Lakes (c, d, g, and h), were compared. Roots (b, d, f, h, and j) or leaves (a, c, e, g, and i) were used. Harvesttime was 60 days after inoculation with zoospores of *Olpidium brassicae* obtained from roots of LBV-affected plants. All results are from inoculated plants. Gels in lanes i and j were, and gels in lanes a–h were not, treated with DNase after staining. Lanes i and j show the effect of DNase treatment on the gels in lanes g and h, respectively (molecular weights [$\times 10^6$] are indicated to the right of lane j).

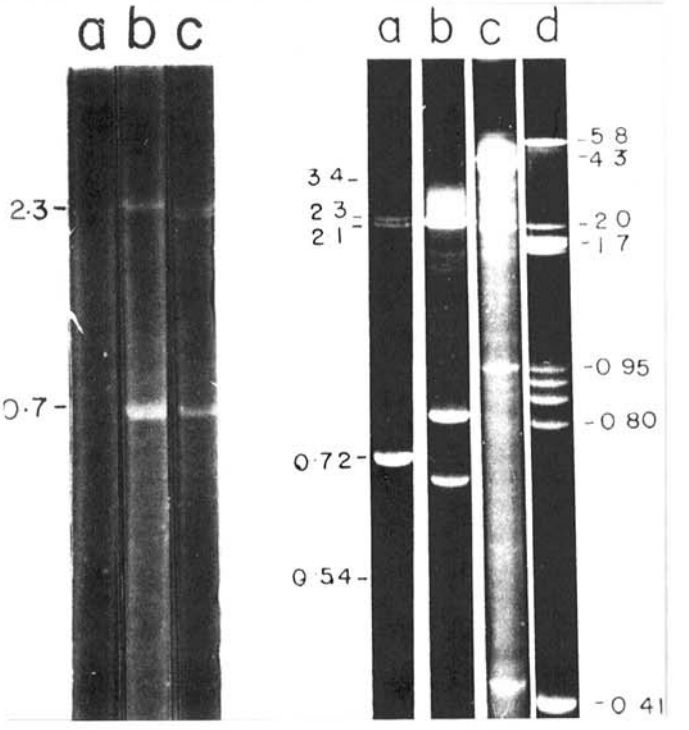

Fig. 3 and 4. 3 (left), Comparison of the dsRNAs isolated from roots of lettuce plants infested with an isolate of *Olpidium brassicae* (I-5) known to be free of the lettuce big vein (LBV) agent (lane a) roots of LBV-affected lettuce plants freed of the LBV-agent-transmitting isolate (I-1) of *O. brassicae* via sterile regeneration of roots from shoots showing LBV symptoms (lane b); and roots of lettuce plants that had been exposed to zoospores of isolate I-5 after the vector had fed on the roots of the plants analyzed in lane b (lane c). **4 (right),** Comparison of the dsRNAs isolated from lettuce roots infected with the LBV agent (lane a), tobacco leaves infected with tobacco necrosis virus (lane b), and tobacco leaves infected with tobacco mosaic virus (lane c), and a series of dsRNAs isolated from three mycoviruses (1). Molecular weights ($\times 10^6$) of the mycovirus dsRNAs, TMV genome RF dsRNA (6) (to the right), and the dsRNAs associated with LBV disease (to the left) were estimated by the graphical method of Bozarth and Harley (1). Two minor dsRNAs (MW = 3.4 and 0.54×10^6) were visible in the original gel (channel a). Their position is indicated but they are not visible in the figure.

LBV-affected plants freed of *O. brassicae* by regeneration of whole plants via tissue culture of aboveground parts (Fig. 3, lane b). Symptoms were observed in newly formed leaves no sooner than 6 wk after roots had been regenerated, and dsRNA could not be detected in extracts from roots until foliar symptoms had developed. Transmission of the LBV agent was not possible with root washings from these plants or from the plants used to test transmissibility, and infections by *O. brassicae* were not detected in the roots of the transplants. Zoospores of isolate I-5 were poured onto roots of LBV-affected plants freed of *O. brassicae* via regeneration through tissue culture, and 2–3 wk later newly released zoospores were transferred to the roots of healthy lettuce seedlings. The diagnostic dsRNAs of LBV were detected 14 days after inoculation in the roots of the final assay plants (Fig. 3, lane c), which developed symptoms within 30–40 days. This experiment, which requires 20 wk to complete, was performed twice with similar results.

Comparison with tobacco necrosis virus. DsRNAs from LBV-affected lettuce tissue, TNV-infected bean tissue, tobacco mosaic virus (TMV)-infected tobacco tissue, and dsRNA molecular weight standards were compared (Fig. 4, lanes a–d, respectively). The dsRNAs isolated from LBV-diseased plants and TNV-infected plants were distinctly different. When 5′-^{32}P-labeled hydrolyzed dsRNAs of TNV were hybridized to nitrocellulose spotted with extracts from TNV-infected beans, healthy beans, LBV-affected lettuce roots, LBV-affected lettuce leaves, healthy lettuce roots, and healthy lettuce leaves, the TNV RNAs hybridized to only nucleic acids from the sap of TNV-infected beans (Fig. 5-e). Reciprocal hybridization experiments have not been performed because recoveries of LBV dsRNAs were 100-fold less than recoveries of TNV dsRNAs from the same amounts of tissue, and labeled dsRNA probe specific for detection of the LBV agent has not been obtained from LBV dsRNAs.

Buffered extracts from LBV-infected lettuce roots containing readily detectable amounts of dsRNA did not induce local lesions when rubbed on bean or tobacco leaves. A typical strain of TNV did induce local lesions on these hosts.

Attempts to purify virus particles. Rigid rod-shaped particles approximately 250–350 nm long and approximately 8–12 nm wide were observed in electron micrographs of partially purified preparations and in dip preparations from both healthy (Fig. 6) and infected lettuce leaves and roots. Preparations containing the rods isolated from infected plants had an $A_{260\ nm}/A_{280\ nm} = 1.1–1.2$. A sample of this material was diluted to 1.0 mg/ml ($E^{0.1\%}_{260\ nm} = 2.0$ was assumed), and when an equal volume of 0.04 M tris, 0.002 M EDTA, 2.0% SDS, pH 9.0, was added, the solution became transparent. No evidence for RNA was observed in 2.5% polyacrylamide gels stained with ethidium bromide after electrophoresis of 200 μl of the dissociated sample. The RNA of TMV was readily detected when purified virus at 1.0 mg/ml was similarly treated and analyzed.

DISCUSSION

The conclusion from these studies is that roots of lettuce plants that develop leaf symptoms of LBV after inoculation with zoospores released from other plants affected by LBV contain dsRNAs. Leaves and roots of healthy lettuce plants and, surprisingly, leaves of infected lettuce plants contain no detectable dsRNA. We interpret these results to mean that an RNA virus or viruslike agent may be associated with LBV disease. We have examined three possible problems that complicate the interpretation.

The first problem is that we could have been detecting a contaminant of TNV, a common plant virus, which is also vectored by *O. brassicae* (10). This cannot be the case for two reasons. First, infective TNV could not be recovered from LBV-affected plants. Secondly, the dsRNAs of TNV are distinct from (Fig. 4), and do not hybridize to (Fig. 5-a), the dsRNAs from LBV-affected lettuce roots.

The second problem is that the disease-specific dsRNAs may be the genome of a mycovirus present only in cells of *O. brassicae* and not in lettuce cells. Two of our results indicate that this is not the case. First, roots infested with isolate I-5 of *O. brassicae* do not contain dsRNA. Second, disease-specific dsRNAs have been detected in LBV-affected plants freed of *O. brassicae* by regeneration of whole plants by tissue culture of aboveground parts. These plants slowly redeveloped symptoms 6–8 wk after

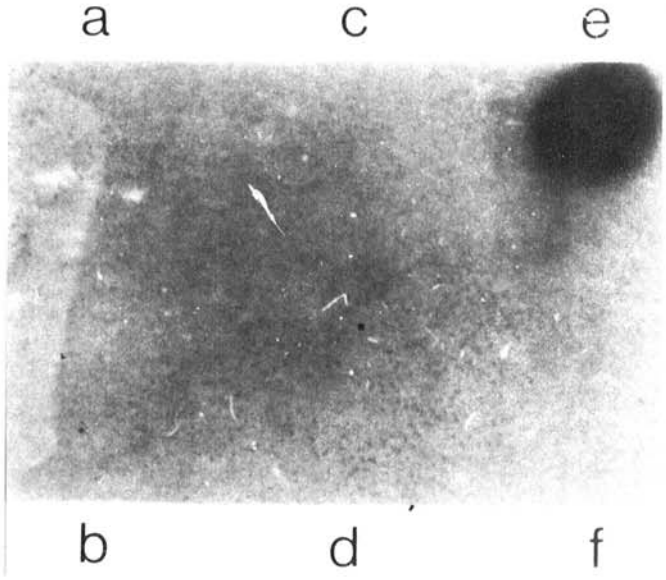

Fig. 5. Homology between tobacco necrosis virus nucleic acids and nucleic acids from lettuce big vein (LBV)-affected lettuce. Total nucleic acids in CH_3HgOH-treated sap from LBV-affected lettuce roots (a) or healthy lettuce roots (b), LBV-affected lettuce leaves (c) or healthy lettuce leaves (d), and tobacco necrosis-infected (e) or healthy beans (f) were spotted onto nitrocellulose and hybridized with 5′-^{32}P-labeled hydrolyzed tobacco necrosis virus dsRNAs.

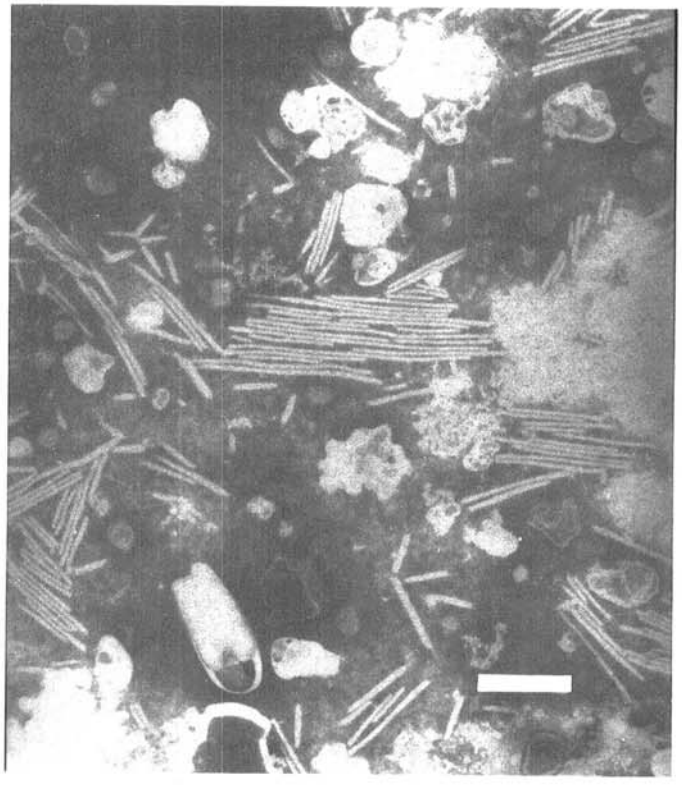

Fig. 6. Leaf dip from healthy lettuce leaves. These rod-shaped particles are present in both healthy and lettuce big vein (LBV)-affected lettuce leaves and roots. The white bar represents 200 nm.

roots had been regenerated and did not contain *O. brassicae*. Furthermore, isolate I-5 of *O. brassicae* can acquire the LBV agent from these plants and transmit it to healthy lettuce seedlings which develop typical symptoms of LBV 30–40 days later. These plants contain detectable diagnostic dsRNAs 2 wk after inoculation. These experiments confirm association of the disease with the diagnostic dsRNAs.

The final problem is that even though the agent is graft transmissible from shoots, and rod-shaped viruslike particles have been reported in diseased, but not healthy, leaves as well as roots (12), dsRNA cannot be detected in the leaves. This could reflect insufficient accumulation or lack of replication in the leaves. Inefficient replication of the agent in leaf tissue would explain the unusually long delay before new symptoms appeared in plants propagated from shoot apices. The delay could be explained if efficient replication of the LBV agent, and subsequent symptom development requires the formation of new roots.

This study indicates that viruslike rod-shaped particles can be detected in healthy as well as infected lettuce plants. These particles presumably have nothing to do with the LBV disease and appear not to be virus particles. A low $A_{260\ nm}/A_{280\ nm}$ ratio, and an inability to detect associated RNA, suggests they are not nucleoproteins. These rods may be the same as particles described elsewhere (5). Rods of the type described by Kuwata et al (12) were not detected in this study, but we did not follow their purification scheme.

This report confirms association of dsRNA with the LBV disease. It suggests that root tissues should be a good source of LBV virions. It should also be possible to test for homology between LBV-specific virion RNAs (12) and dsRNAs.

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