Transmission of Virus from Oak Leaves Fractionated with Sephadex

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ABSTRACT

By passing crude juice from normal oaks (11 species of *Quercus* or *Lithocarpus*) through a Sephadex gel filtration column, fractions which gave TMV-like infections on a variety of hosts were recovered, whereas the unfiltered juice was noninfectious. This recovery of fractions with infectivity was due to the elimination of several inhibitors in oak by the exclusion chromatography.

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Although virus in oak has been indicated on the basis of symptoms, graft transmission, and aphid transmission (2, 3, 8), and TMV-like rods were seen in oaks showing viruslike symptoms and in oaks without viruslike symptoms, most attempts to mechanically transmit a virus from oak have been unsuccessful. The low level of mechanical transmission by Yarwood & Hecht-Poinar (13) is presumed to be due to the demonstrated high level of virus inhibitors in oak; if these inhibitors could be removed, increased transmission might result.

The development of gel matrices, especially the dextran gels, introduced a valuable technique in separating particles according to their molecular size (7). By exclusion chromatography, also referred to as gel filtration, large molecules can be excluded from the gel matrix according to the different degrees of cross linkage of the gel (1). Stegemann & Loeschke (10) prevented virus inactivation by separating phenols from phenol oxidases by running the extract through a Sephadex-G25 column. Steere (9) described the technique as one of the most satisfactory procedures for the removal of contaminants smaller than the virus. Ebrahim-Nesbat (5) recommended exclusion chromatography for the separation of viruses from inhibitors in sugar beet and spinach.

MATERIALS AND METHODS.—Dry Sephadex (G25 medium, 20 g/100 ml, or G100, 5 g/100 ml) was allowed to swell in a solution of 1% K_2HPO_4 for 24-72 hr at 4 C, or 1-3 hr in boiling water. Separation tubes (12 cm X 2 cm inside) were fitted at the

bottom with fiber glass, cleaned, and sterilized in boiling water for at least 2 hr, packed with the Sephadex gel (10 cm high, about 60-ml gel bed), washed with several volumes of 1% K_2HPO_4, and adjusted to run at about 3 ml/12-15 min.

Samples of buds or young leaves of *Lithocarpus densifolia* (Hook. & Arn.) Rehd. (tan oak), *Quercus agrifolia* Née (coast live oak), *Q. alba* L. (white oak), *Q. douglasii* (Hook. & Arn.) (blue oak), *Q. engelmannii* Greene (mesa oak), *Q. ilex* L. (holly oak), *Q. ithaburensis* Decne, *Q. kellogii* Newb. (Kellog oak), *Q. obtusata* Humb. & Bonpl. (Mexican live oak), *Q. phellos* L. (willow oak), and *Q. robur* L. (English oak) were collected mainly on the Berkeley campus of the University of California, in Alameda County, but a few specimens were also collected in Briones Park, Contra Costa County, at Pt. Reyes in Marin County, and at the Davis campus of the University in Solano County. Except for the single specimen of *Q. ithaburensis* in Solano County, all specimens were from normal-appearing trees. One gram of each was homogenized in a sterilized mortar with 5 ml of 1% K_2HPO_4, and 3 ml of the crude extract was layered on top of the gel bed. When the extract had entered the bed, more K_2HPO_4 was layered on top of the extract. During chromatography, this upper layer was continuously refilled while avoiding disturbing the bed surface. Three-ml fractions were collected, beginning with the first fraction when the extract had entered the gel bed. Fractions 1-8 or 10 were inoculated to *Chenopodium amaranticolor* (4- to 8-leaf stage) and *C. quinoa* (5- to 12-leaf stage) plants grown both at 21 and 27 C. Lesions were counted at 9-12 days after inoculation.

All equipment used for the extraction procedure and the transmission to the test plants was thoroughly cleaned and boiled in water for at least 2 hr.

RESULTS.—*Elimination of inhibitors in healthy oaks.*—The brown ground suspension of young leaves of *Lithocarpus* seedlings was filtered through a column of Sephadex-G25. Each of 80 successive 3-ml fractions was mixed with 0.5 ml of a 1:1,000 suspension of crude tobacco mosaic virus from tobacco (TMV) and inoculated on *Nicotiana tabacum Xanthi nc* by the half-leaf method. Results are presented in Table 1. Fractions 19-25, 32-44, and 51-57 apparently differed from each other in the molecular size of the inhibitors that they contained. When suspensions of TMV were run through the column, infectivity was restricted to fractions 4-6. One can logically expect, therefore, to effect a separation of TMV from virus inhibitors in oak if these are not strongly connected to the virus particle. A test that involved passing a mixture of crude TMV and oak leaf extract through a column confirmed this, as the infectivity of fractions 4-7 was 80-95% of that of phosphate-TMV mixture, whereas that of the unfiltered TMV-oak leaf extract was only 10% or less of the phosphate-TMV mixture.

TABLE 1. Effect of Sephadex filtration fractions of tan oak on the infectivity of tobacco mosaic virus[a]

Fraction no.	Color of fractions	Local lesions as per cent of control
1-3	Colorless	100
4-6	Turbid, yellowish	100
7-13	Colorless	100
14-18	Yellowish	100
19-25	Yellow	10-25[b]
26-31	Yellow-brown	90-100
32-44	Yellowish-brown	25-40[b]
45-50	Gray-brown	80-100
51-57	Gray-brown	40-50[b]
58-64	Yellowish	100
65-80	Faint yellowish	100

[a] Crude tobacco mosaic virus was added separately to each fraction, and the mixture inoculated on *Chenopodium quinoa*.

[b] The reduction in infection indicates that these fractions contain inhibitors.

Virus in normal oaks.—Yarwood & Hecht-Poinar (13) indicated in a noncommittal way that TMV was present in most or all normal-appearing oaks. In the present study, buds and young leaves of a blue oak tree grown in the greenhouse were ground in 1% K_2HPO_4, in K_2HPO_4 plus 1% Bentonite; in borate buffer, pH 9, 0.1 M with and without sodium diethyldithiocarbamate; or in 1% K_2SO_3, and the mixture was then used directly as inoculum on *C. quinoa* or *C. amaranticolor*. No infection resulted. When the suspension prepared in 1% K_2HPO_4 was passed through a Sephadex-G25 column, fractions 4-6 gave 15-90 lesions/plant. Similarly, infectivity was recovered from outdoor trees of six of eight coast live oaks, three of three tan oaks, two of two willow oaks, and from one each of white oak, mesa oak, holly oak, *Q. ithaburensis*, Kellog oak, Mexican oak, and English oak. But 10 of 10 tan oak seedlings grown in the greenhouse from seed showed no virus infectivity.

The number of assay lesions per *Chenopodium* leaf inoculated with filtrates through Sephadex ranged from 0 to 20. Reinoculations from these *Chenopodium* leaves with lesions to additional *Chenopodium* leaves always yielded hundreds of lesions. Surprisingly, when *C. quinoa* leaves inoculated with filtrates from oak, but which developed no lesions, were used as inoculum on additional *C. amaranticolor* leaves, heavy infections sometimes resulted, indicating that infection with the oak virus could be latent in *C. quinoa*. Inoculum from healthy *Chenopodium* was never infectious on *C. quinoa* or *C. amaranticolor*.

Visible zones in Sephadex filtration.—When crude extracts of tan oak or Mexican live oak leaves were passed through Sephadex-G100, a marked zonation of the extracts occurred. Within 15 min, a green zone moved ahead of the brown zone. Within 30 min, a red-brown zone appeared ahead of the brown zone. Sap from this red-brown zone gave a reddish reaction with K_2SO_3, and is presumably the fraction responsible for the K_2SO_3 reaction which Yarwood (11) and Yarwood & Hecht-Poinar (13) associated with virus.

In the present study, this K_2SO_3-staining material was clearly free of virus, as was indicated by the filtration as well as by the fact that virus-free seedlings of *Lithocarpus* gave a strong K_2SO_3 reaction.

Ahead of the green zone was a colorless zone in which most of the virus was located; this zone was collected as fraction 4 from about 45 to 60 min from starting the separation. Fraction 5, which was colorless but usually turbid, also contained much virus.

Symptoms on differential hosts.—The viruses isolated from oak by Sephadex gel filtration were all strains of TMV, as indicated by electron microscopy, heat inactivation, and premunity tests, but distinct differences were apparent in reactions on certain differential hosts. On *Chenopodium quinoa, Nicotiana glutinosa, N. tabacum Xanthi nc, Vigna sinensis* 'Blackeye', *Gomphrena globosa*, and *Cucumis sativus*, all isolates were apparently identical to type TMV and to each other. On *N. tabacum* 'Turkish', most isolates were milder than type TMV; three caused local lesions and a systemic necrosis, which was also observed in *N. clevelandii* in response to the same isolates. On *Phaseolus vulgaris* 'Pinto', most strains caused fewer and smaller lesions than TMV, and some strains caused no lesions, but the number of lesions was greatly increased by predisposition heat (10 sec at 50 C). *Petunia hybrida* was the most useful host in distinguishing oak TMV strains from type TMV in that most oak isolates caused local lesions, whereas type TMV usually caused only systemic infection. On *C. amaranticolor*, several isolates caused systemic yellow spots and leaf malformation in addition to local lesions, whereas the type TMV developed only local lesions.

DISCUSSION.—The main implications from this and four previous studies (6, 8, 12, 13) are that TMV is a common component of normal-appearing oaks of several species, that the virus may be transmitted by *Sphaerotheca lanestris*, and that its isolation from oak may be greatly aided by fractionation of virus suspensions with Sephadex. Other evidence emphasized by Cadman (4) indicates that TMV is soil-borne. These views on vectors of TMV (excluding man, who is generally recognized as an important vector in commercial agriculture) are not incompatible, as a virus can have several vectors, though this is apparently unusual. More importantly, tests not reported here show that *S. lanestris* transmits a virus from oak to oak.

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