Strawberry Latent Ringspot Virus Associated with a New Disease of Olive in Central Italy

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ABSTRACT

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Strawberry latent ringspot virus (SLRV) was repeatedly isolated from flowers, leaves, and roots of olive (*Olea europea* L.) plants (cultivar Ascolana tenera) in central Italy. All infected plants showed symptoms of leaf narrowing and twisting, shortening of internodes, and fruit deformations. SLRV and the associated disease were both transmitted to healthy Ascolana tenera scions by grafting to naturally affected trees. Infectivity to differential hosts, serological properties, and sedimentation behavior of purified viral preparations indicate that our isolate is similar to the SLRV previously obtained from symptomless olive trees. This is the first report of a viral infection on olive associated with clear-cut symptoms.

Olive (*Olea europea* L.) diseases, supposedly of viral origin, have been reported from Europe (1,2,6), the United States (14,15), South America (5), and Israel. Some have been graft-transmitted to *Ligustrum* spp. or *Olea* spp., but in no instance have the causal agents been identified (see reference 4 for a review).

More recently, the following viruses have been isolated from symptomless olive trees in central and southern Italy: arabis mosaic (ArMV), strawberry latent ringspot (SLRV) (9), cherry leafroll (CLRV) (10), olive latent ringspot (OLRV) (12), cucumber mosaic (CMV) (11), olive latent-1 (3), and olive latent-2 (13).

In 1980, we observed a severe disorder on some table-olive trees (cultivar Ascolana tenera) near Ascoli Piceno (central Italy). Main symptoms consisted of narrow and twisted leaves, bunchy growth, reduced crop, and deformed fruit (Figs. 1 and 2) especially detectable during spring and summer. Infected plants were randomly distributed in the field.

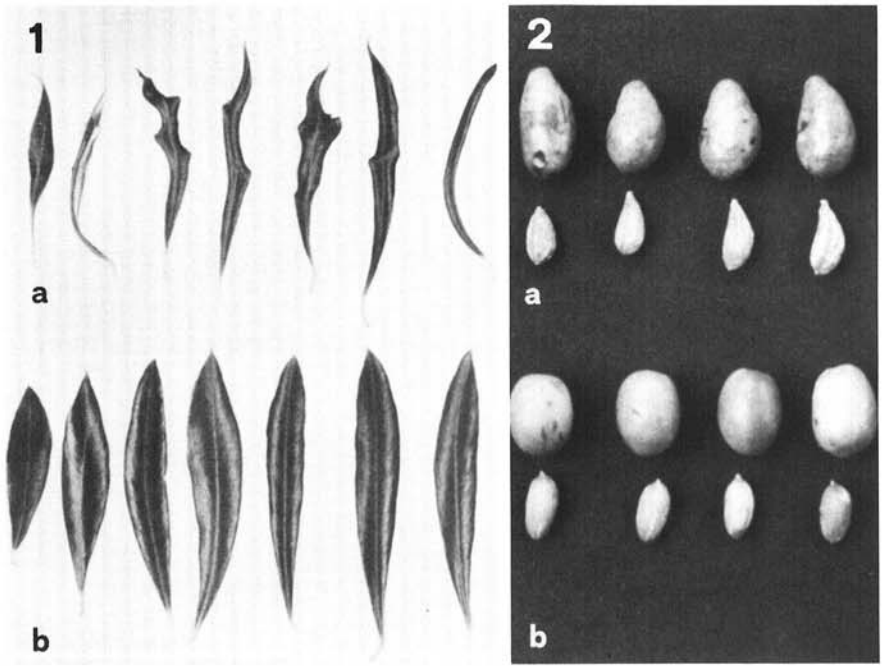
From these plants, a strain of SLRV was isolated by inoculation of sap to herbaceous hosts. This paper reports the results of investigations on the characterization and pathogenicity of this virus strain.

MATERIALS AND METHODS

SLRV was isolated from flowers of affected plants and occasionally from young leaves and roots. The inoculum was extracted in the presence of 0.01 M phosphate buffer, pH 7.0, to which 0.005 M sodium thioglycollate, 0.005 M sodium diethyldithiocarbamate (DIECA), and 0.01 M ethylenediaminetetraacetate (EDTA) were added. A 2.5% nicotine solution was used as alternative extraction medium. The inoculation to indicator plants was carried out in a greenhouse at 18–24 C and 70–85% relative humidity with a 14-hr photoperiod (natural illumination plus artificial light). The following herbaceous indicator species were inoculated: *Chenopodium quinoa* Willd.; *C. amaranticolor* Coste & Reyn.; *Nicotiana tabacum* L. cvs. Bright, White Burley, and Maryland; *N. glutinosa* L.; *N. sylvestris* Speg. & Comes; *N. rustica* L.; *N. clevelandii* Gray; *Petunia hybrida* Hort.; *Datura metel* L.; *D. stramonium* L.; *Gomphrena globosa* L.; and *Fragaria vesca* L.

Attempts to transmit the SLRV isolate to Ascolana tenera plantlets (self-rooted cuttings and seedlings) were made either by approach-grafting or by mechanical inoculation.

In the first experiment, SLRV-infected *C. quinoa* or *C. amaranticolor* were approach-grafted to young olive plants (Ascolana tenera), and the grafted plants were kept in the greenhouse. In mechanical transmission tests, crude sap from leaves of infected *C. quinoa* or purified virus preparations (described later) were used as inocula. The Ascolana tenera binodal seedlings used in the latter tests were obtained by naked embryo culture in agar medium (7,8) and were grown under dark conditions to increase their susceptibility to the virus. Soon after inoculation, the plantlets were transferred to sterile soil in plastic pots under controlled environmental conditions (7).



Figs. 1 and 2. (1A) Misshapen leaves from naturally affected olive plants (cultivar Ascolana tenera) compared with **(1B)** normal leaves. **(2A)** Examples of fruit and stone deformation on affected Ascolana tenera plants compared with **(2B)** normal fruits and stones.

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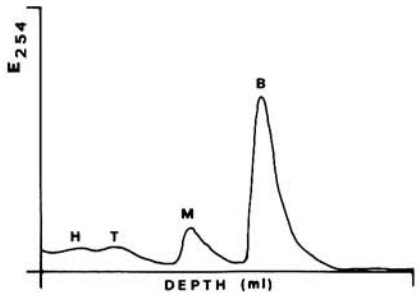

Fig. 3. Sedimentation profile of a partially purified preparation of strawberry latent ringspot virus from olive cultivar Ascolana tenera after centrifugation in sucrose density gradient. T, M, and B represent top, middle, and bottom components, respectively, and H represents healthy plant material.

Finally, graft-transmissibility of the disease from olive to olive was tested in field experiments. Healthy olive scions from five cultivars (Carboncella, Leccino, Pendolino, Ascolana semitenera, and Ascolana tenera) were bark-grafted to branches of field-grown plants showing typical symptoms of the disease.

Partially purified preparations were obtained from systemically infected *C. quinoa* leaves after extraction in 0.5 M citrate buffer, pH 6.5, containing 0.1% thioglycollic acid. The partial purification schedule included 1) clarification with an equal volume of chloroform, 2) two cycles of differential centrifugation (7,500 rpm for 10 min and 36,000 rpm for 90 min), and 3) fractionation by centrifugation in 10–40% sucrose density-gradient tubes; virus components were fractionated with an ISCO 640 density-gradient fractionator equipped with a UA-5 UV analyzer.

Serological tests were performed in gel double-diffusion using crude or, occasionally, concentrated clarified sap from infected *C. quinoa*; the virus was tested against antisera to the following isometric viruses: tomato black ring (TBRV), CLRV, ArMV, two isolates of grapevine fanleaf (GFV), grapevine Bulgarian latent (GBLV), grapevine chrome mosaic (GCMV), artichoke Italian latent (AILV), artichoke yellow ringspot (AYRSV), raspberry ringspot (RRV), cocoa necrosis (CNV), myrobalan latent ringspot (MLRV), sowbane mosaic (SoMV), OLRV, and four isolates of SLRV (SLRV-P, SLRV-Lo, SLRV-GB, and SLRV-Ol 11d).

RESULTS

SLRV has been repeatedly isolated only from symptomatic Ascolana tenera olive trees. Attempts to isolate the virus from healthy-looking plants of the same cultivar in the same orchard consistently failed.

SLRV was sap-transmitted to herbaceous plants from flowers of diseased trees; transmission of the virus was also obtained from roots and young leaves of plants vegetatively propagated from naturally infected olives. Only *C. quinoa* and *C. amaranticolor* proved susceptible and developed chlorotic local lesions followed by systemic mottle and top necrosis. Other indicator plants were not infected by the virus.

Symptoms similar to those observed in the field (narrow leaf and shortening of internodes) developed on some Ascolana tenera scions 1–2 yr after grafting on naturally affected plants. SLRV was recoverable from such scions, thus demonstrating that it had been transmitted along with the symptoms. The virus was also recovered from one symptomless Carboncella scion, whereas SLRV was not transmitted to scions of other cultivars, and they remained symptomless.

Negative results have been obtained from mechanical transmission and approach-grafting trials. To date, no symptoms have appeared on olive plantlets inoculated 2 yr ago by both methods, and no viral infectivity has been recovered from them.

In density-gradient centrifugation, partially purified preparations of the virus sedimented as three components (T, M, and B). The ISCO tracing (sedimentation profile) is shown in Figure 3, where a clear prevalence of the heaviest B component can be seen.

In gel double-diffusion tests, crude sap of infected *C. quinoa* or purified preparations reacted only with antisera to SLRV isolates: SLRV-P and SLRV-Lo (from peach), SLRV-Ol 11 d (from olive), and SLRV-GB (English isolate).

Preliminary ultrastructural observations on infected *C. quinoa* leaves revealed cytological changes typical for nepovirus infections, i.e., occurrence of cytoplasmic inclusion bodies containing abundant profiles of endoplasmic reticulum, membraneous vesicles, and ribosomes.

DISCUSSION

Our findings confirm that olive is a natural host of SLRV, which has been found in this species in various Italian localities (9). Infectivity to herbaceous hosts, sedimentation profile of purified preparations, and serological behavior indicate that the virus from Ascolana tenera is quite similar to the SLRV isolates previously obtained from olive (9).

In contrast to previous studies, where no symptoms were observed on olive trees naturally infected by nepoviruses (9,10,12), the SLRV infection in Ascolana tenera appears closely associated with a clear-cut syndrome. The infectious nature of the disease is suggested by its graft-transmissibility, especially to Ascolana tenera scions; it is noteworthy that the virus too is graft-transmissible from olive to olive. If SLRV were responsible for the symptoms, it may be concluded that Ascolana tenera is particularly sensitive to this virus. This assumption needs further investigation, however, because the syndrome observed in field-infected plants has not yet been reproduced by mechanical or approach-graft inoculation of SLRV to olive. Alternatively, it can be hypothesized that a second unknown virus or viruslike agent not infectious to *C. quinoa* was present with SLRV in affected Ascolana tenera plants. In this case, the disease would result from a synergistic infection of two pathogens, a phenomenon not uncommon in fruit virology.

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