

Scottish Horticultural Research Institute, Invergowrie, Dundee, Scotland,
and
Laboratorio di Nematologia Agraria del C.N.R., 70126 Bari, Italia

SPECIFIC ASSOCIATION OF ARTICHOKE ITALIAN LATENT
VIRUS WITH THE ODONTOSTYLE OF ITS VECTOR,
LONGIDORUS ATTENUATUS

by

C. E. TAYLOR, W. M. ROBERTSON and F. ROCA

Longidorus attenuatus Hooper is consistently found in association with outbreaks of Artichoke Italian Latent Virus (AILV) (Majorana and Rana, 1970; Rana and Roca, 1973; Roca, Martelli and Rana, 1975; Roca *et al.*, 1975) occurring in crops of artichokes in Apulia, southern Italy. An isolate of AILV is also the causal agent of chicory chlorotic ringspot and is also transmitted by *L. attenuatus* (Vovlas and Roca, 1975). In laboratory experiments it has been shown that *L. attenuatus* is an efficient vector of the virus with up to 42% infections of tomato bait seedlings occurring in pots to which five nematodes were added, and 67% infections with 20 nematodes added (Roca, Martelli and Rana, 1975). It therefore seemed likely that a specific association between the AILV particles and some cuticular part of the feeding apparatus might occur, as seen in *L. elongatus* with raspberry ringspot and tomato black ring viruses (Taylor and Robertson, 1969), and *L. macrosoma* and raspberry ringspot (Taylor and Robertson, 1975). This paper describes the results of electron microscopy investigations of AILV-infected *L. attenuatus* to find the site of virus retention within the nematode.

Materials and methods

Soil containing *L. attenuatus* was obtained from a crop of artichokes at Mola, near Bari. In the laboratory, pots of the soil were

planted with *Chenopodium quinoa* Willd. seedlings which, about 5 days later, were manually inoculated with AILV; some pots were left with uninoculated *C. quinoa* as controls. After a further 4 weeks nematodes were extracted from the pots by wet sieving. They were then fixed in cold 3% glutaraldehyde and the anterior oesophageal region severed with a transverse cut, made with a scalpel blade, just posterior to the oesophageal bulb. The anterior portion was then post-fixed in 1% osmium tetroxide, dehydrated in a graded ethanol series and embedded in Araldite by the method of Robertson and Taylor (1975). Specimens were sectioned transversely and longitudinally at about 60 nm thick using an LKB Ultratome I. Sections were stained with alcoholic uranyl acetate, followed by lead citrate, using the bulk staining technique of Robertson and Roberts (1972). They were examined in an Hitachi HS-8 electron microscope operated at 50KV at magnifications up to x 40,000.

Results

Transverse and longitudinal sections of *L. attenuatus* from AILV-infected *C. quinoa* showed virus-like particles apparently adsorbed to the inner surfaces of the odontostyle (Figs. 1 and 2). The particles were consistently found in a single layer in the anterior half of the odontostyle in six of the 16 *L. attenuatus* sectioned. No particles were seen in sections of *L. attenuatus* from the *C. quinoa* and it therefore seems likely that the particles observed are those of AILV and that the inner surface of the odontostyle is the site of specific retention for this virus. In one of the specimens cut longitudinally at the junction of the odontostyle with the odontophore, a few virus-like particles were found associated with the cuticle at the most anterior part of the odontophore (Fig. 5), but because this is a single observation it is considered to be a chance effect and not indicative of the normal site of retention within the nematode.

As seen in *L. elongatus* associated with raspberry ringspot and tomato black ring viruses (Taylor and Robertson, 1969), transverse sections of infected *L. attenuatus* also showed virus particles apparently trapped in the longitudinal anterior fold of the guiding sheath (Fig. 4). In longitudinal sections of some *L. attenuatus* AILV

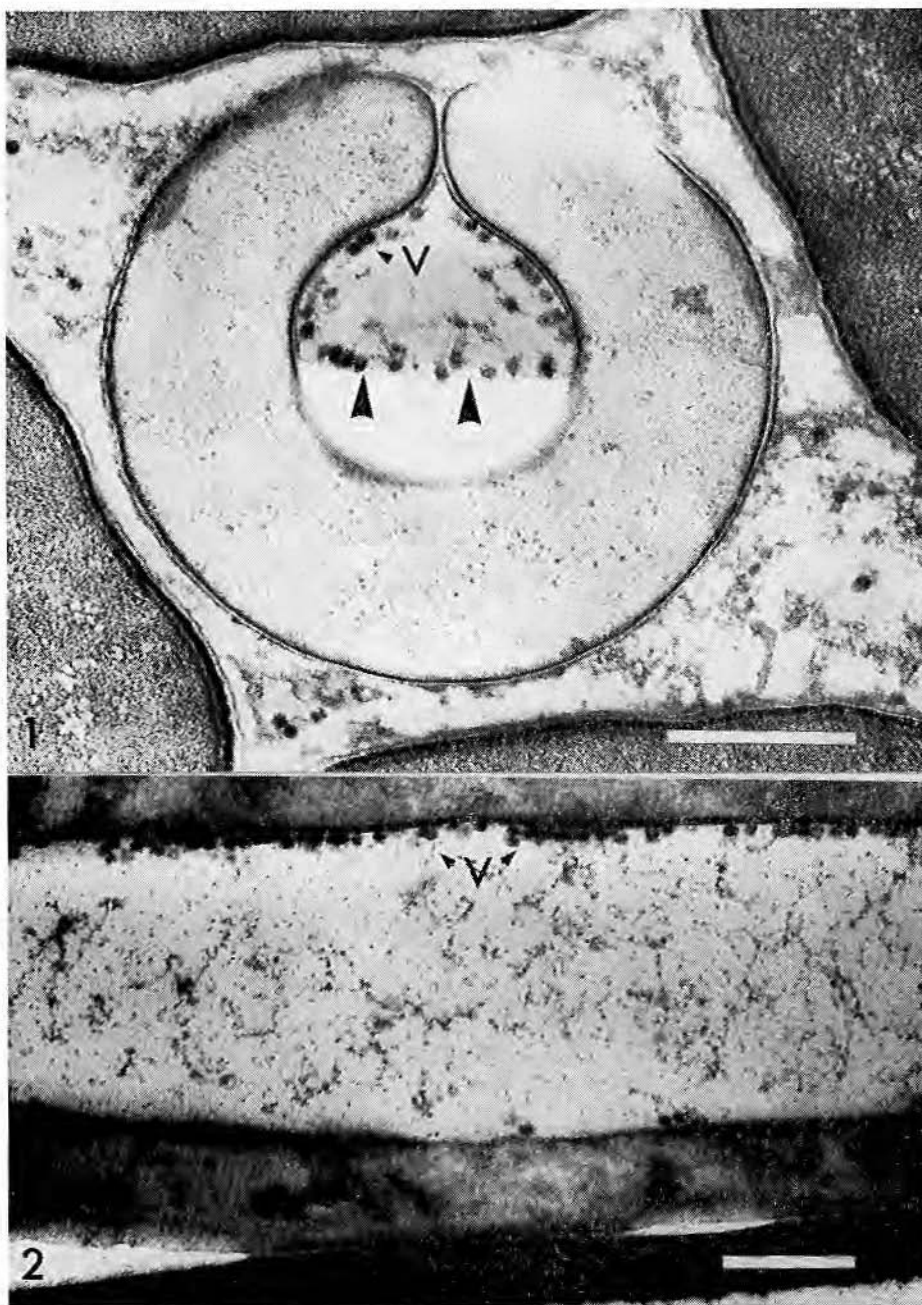


Fig. 1 - Transverse section of the odontostyle of *L. attenuatus* showing particles of AILV (V) adsorbed to the inner surface. Part of the section (arrowed) within the odontostyle has become detached from the ventral surface of the stylet.

Fig. 2 - Longitudinal section through the stylet of *L. attenuatus* with AILV particles (V) adsorbed to the inner surface (bars represent 250 nm).

particles were found in the transverse folds of the guiding sheath (Fig. 3) (Taylor and Robertson, 1971; Robertson and Taylor, 1975) but these were not sufficiently numerous to suggest that the guiding sheath is a site of specific retention as in *L. elongatus* (Taylor and Robertson, 1969).

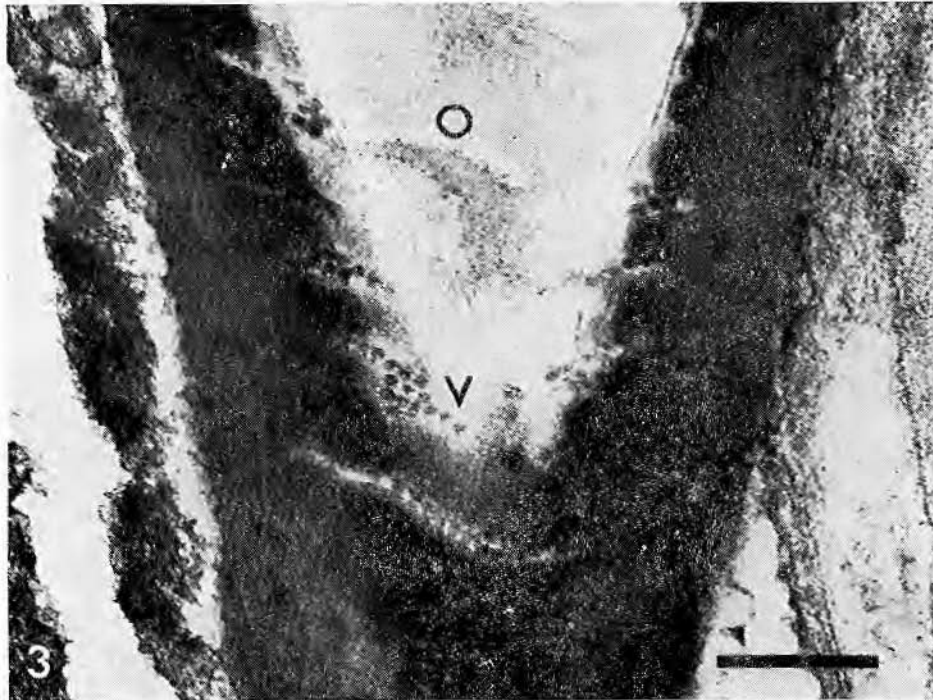


Fig. 3 - An oblique longitudinal section of the anterior end for the guiding sheath showing AILV particles (V) in the transverse folds. Odontostyle (O) (bar represents 250 nm).

In some specimens from infected plants, crystalline aggregates of virus particles were seen in transverse sections of the odontostyle (Fig. 4). In one of the six specimens sectioned longitudinally, flexuous tubules each containing a single row of virus particles were present in the lumen of the odontostyle and the odontophore (Fig. 5). Both types of aggregates probably represent the arrangement of virus particles as ingested by the nematode from the plant cell. Davison (1969) working with root tips of *Phaseolus vulgaris* L. infected with tobacco ringspot virus found that plasmodesmata contained single rows of virus-like particles.

Most observations of cellular inclusions of nepo viruses have been made with infected leaves, but Martelli (1975) concluded that tubular inclusions in plant cells are common to most if not all nepo viruses and our observations indicate that particles of AILV occur

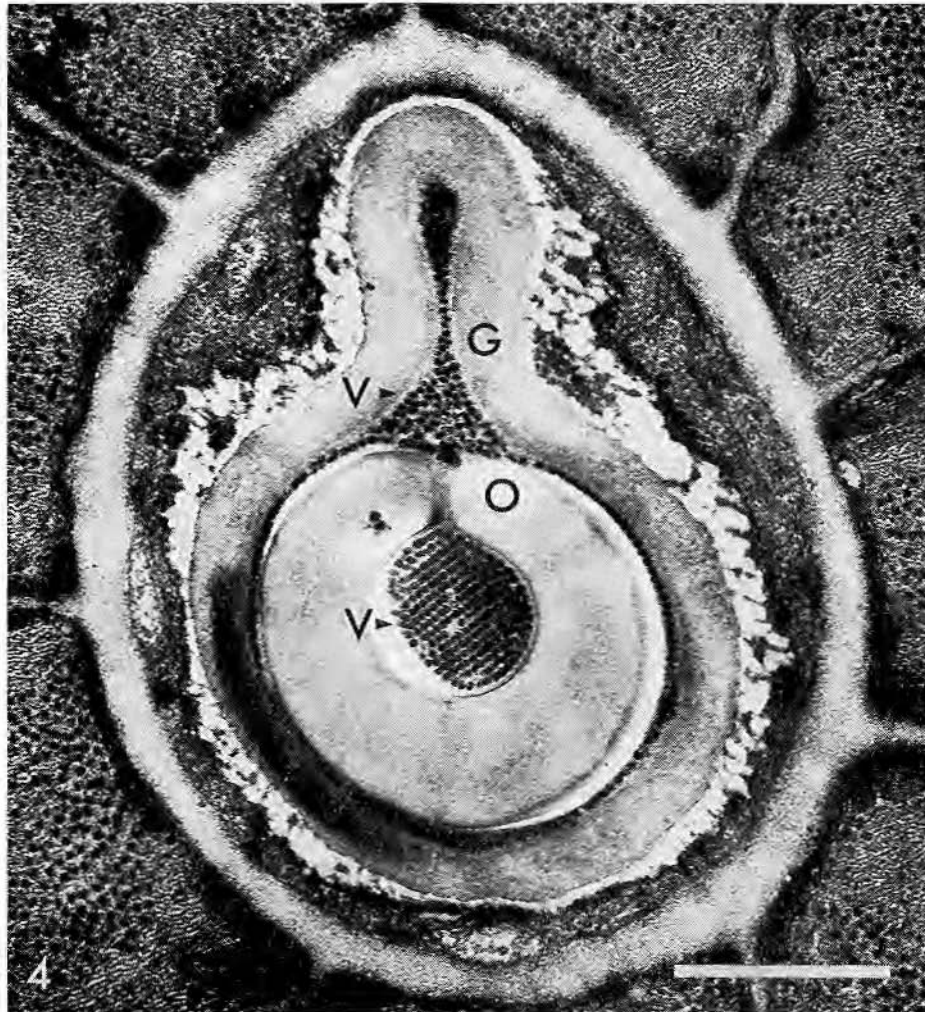


Fig. 4 - Transverse section. Particles of AILV (V) are present between the odontostyle (O) and the guiding sheath (G) and in crystalline array in the lumen of the odontostyle (bar represents 500 nm).

in membranous tubules within the roots of *C. quinoa*. It seems likely that the virus particles cannot be adsorbed at the sites of retention in *L. attenuatus* because of the protection afforded by the membranes forming the tubules and that with other plant material



Fig. 5 - Longitudinal section at the junction of the odontostyle (O) and the odontophore (P). Flexuous tubules (T) containing AILV particles are present in the lumen. A few particles (V) are apparently adsorbed to the most anterior part of the odontophore (bar represents 500 nm).

they pass into the intestine. Virus particles adsorbed to the inner surface of the odontostyle are thus likely to be those that are free within the plant tissues, or those that are « unprotected » at the periphery of the « crystals ». Such « crystals », although not specifically adsorbed to the sites of retention, may nevertheless provide a large inoculum of infection if they are held within the odontostyle until the nematode next feeds on a susceptible plant and thus transmits in a non-specific manner (Taylor and Robertson, 1975 a).

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S U M M A R Y

Particles of Artichoke Italian latent virus are specifically adsorbed to the odontostyle of its vector, *Longidorus attenuatus* Hooper. Flexuous tubules of virus particles present in the plant roots are ingested by the nematode, but the membranous covering forming the tubule apparently prevents the particles from being adsorbed at the site of retention. It is suggested that virus particles at the surface of crystalline aggregates may be unprotected and that these are also adsorbed to the site of retention.

R I A S S U N T O

Associazione specifica del virus latente italiano del Carciofo con l'odontostilo del suo vettore, Longidorus attenuatus.

Particelle del virus latente italiano del Carciofo sono specificamente adsorbite dalla superficie interna dell'odontostilo del suo vettore, *Longidorus attenuatus* Hooper. Tubuli flessuosi di particelle virali presenti nelle radici della pianta sono ingeriti dal nematode, ma non vengono adsorbiti dal sito di ritenzione, probabilmente a causa della superficie membranosa che forma il tubulo. Si pensa, invece, che le particelle virali vengano adsorbite a livello di aggregati cristallini, non essendo protette da membrane.

R É S U M É

Association spécifique du virus latent italien de l'artichaut avec l'odontostyle de son vecteur, Longidorus attenuatus.

Des particules du virus latent italien de l'artichaut sont spécifiquement adsorbées par la surface interne de l'odontostyle de son vecteur, *Longidorus attenuatus* Hooper. Des tubes flexueux des particules virales présents dans les racines des plantes sont ingérés par le nématode, mais la couverture membraneuse des tubes empêche apparemment l'adsorption par le site de rétention. On croit, au contraire, que les particules virales sont adsorbées au niveau des agrégats cristallins, n'ayant pas de protection.

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