

# DETECTION OF VIRAL PARTICLES ON *QUERCUS SUBER* L. LEAVES: PRELIMINARY RESULTS

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## ABSTRACT

Cork oak (*Quercus suber* L.), being a quite long-lived evergreen tree, has been strongly affected by human exploitation. Their leaves are persistent, usually remaining on the tree for up to three years, with shape roughly ovate, although variable among trees, and colour bright dark green in the upper face and greyish in lower one. It has been reported for a long time that several species of fungi damage cork oak trees, although the causes of the decline of these trees remain still unknown. However, being also plausible that a viral infection may contribute for the cork tree decline, no virus have yet been detected.

In an effort to elucidate this probability, we have initiated a screening programme for detection of viral particles in the leaves of cork oak trees, by using transmission electron microscopy (TEM). We have observed a ringspot virus in the cytoplasm of the cells. All the viral particles were essentially of the same length, with about 20 nm in diameter approximately. The outer layer was less dense to the electrons, while the inner one was darker and with a white central canal.

In the present work, we provide evidence for the existence of virus-like particles that have been detected on leaves of one cork oak tree with severe necrotic symptomatology.

These results, that are here reported for the first time to our knowledge, although preliminaries, may be the basis for further studies in Portugal, concerning either the overall sanitary state of the cork oak trees or the quality of the produced cork as well as the protection against damages that may be caused by virus.

## METHODS

The *Quercus suber* L. leaves (Fig. 1) were collected in the Grândola region, in Portugal.

## SCANNING (SEM) AND TRANSMISSION (TEM) ELECTRON MICROSCOPY

For SEM observations, dried leaves were coated with gold and examined with a JEOL scanning electron microscope (JSM-5220 LV, 15 kV).

For TEM observations, 5 g of fresh leaves were sterilized, macerated and filtrated with a series of funnels (Duran Schott) of successive pore sizes, 40-100 µm, 16-40 µm and 10-16 µm. The extract was centrifugated for 15 min. at 14000 g. The supernatant is discarded and the pellet was stained with standard TEM techniques and observed.

## ISOLATION OF NUCLEIC ACID

The DNA extracts of the leaves were prepared by the method described by Doyle and Doyle (1987).

## CONCLUSIONS

The isometric viral particles detected on *Quercus suber* L. have an icosahedral structure, 20 nm in diameter approximately, linear configuration and 90 kbp of (ds) DNA.

Further investigation has to be carried out, but these results allow us to identify some characteristics that are similar to the algal virus group, in the family *Phycodnaviridae*.

To study the correlation of cork oak decline with the presence of these viruses, it would be advantageous to develop a reliable method based on identification and molecular characterization of the viruses and their control, to combat that problem.

## REFERENCES

Doyle, J. J. and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin*, 19: 11-15.

## INTRODUCTION

*Quercus suber* L. has a high economic and environmental importance to Portugal. The detection of a viral infection in leaves of cork oak is of great interest in order to establish and understand the causes of their decline.



Fig. 1 - *Quercus suber* L. leaves with chlorotic ringspots.

## RESULTS

The SEM observation (Fig. 2) of the lower surface of the cork oak leaves showed the epidermis completely covered by trichomes. They are attached to the plant by a foot embedded in the epidermis. These trichomes consist of several cells, fused together towards the base and separated at the margin.

The TEM observation (Fig. 3) of cork oak leaf cells showed isometric virus particles with an icosahedral structure.

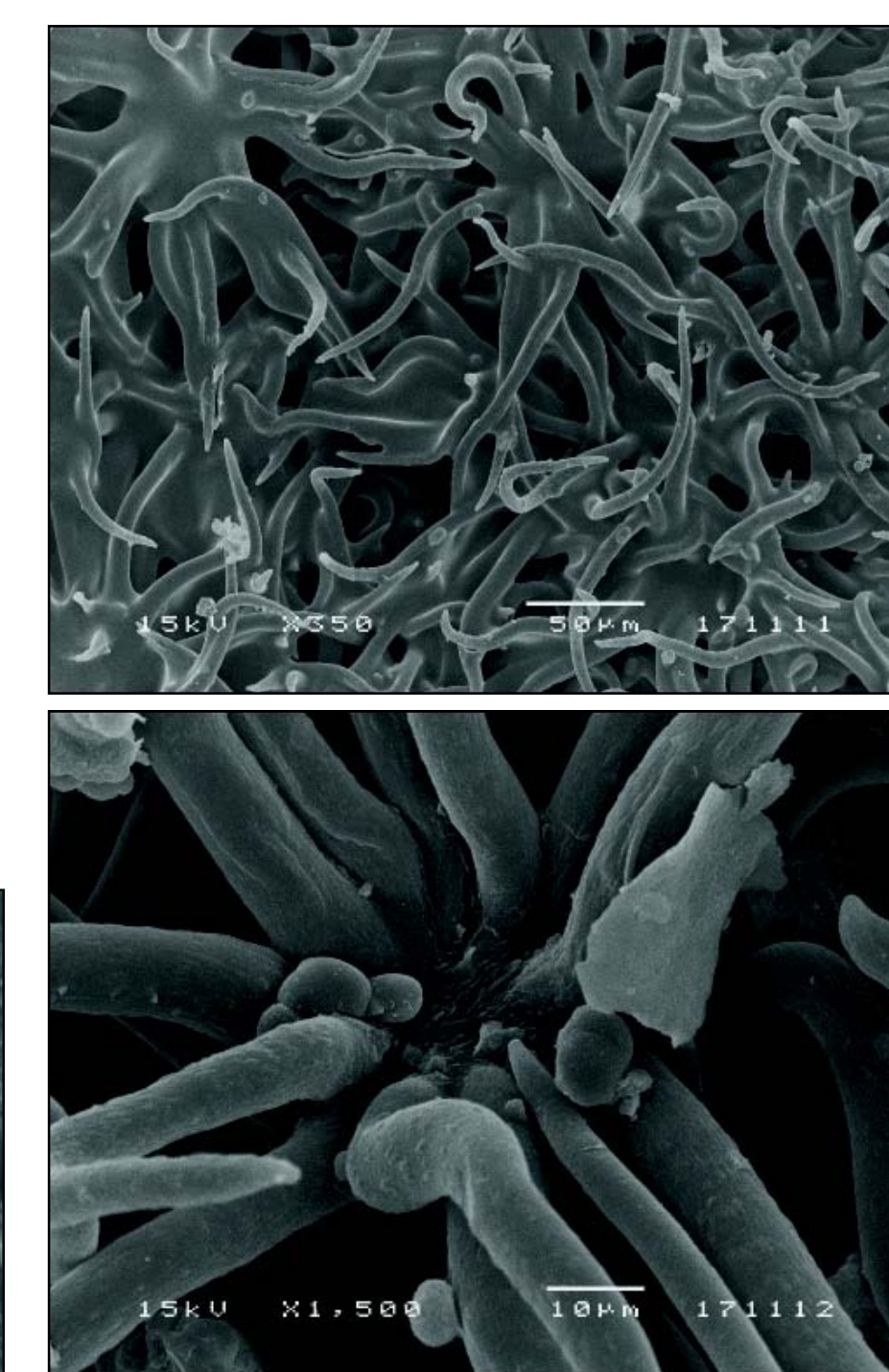
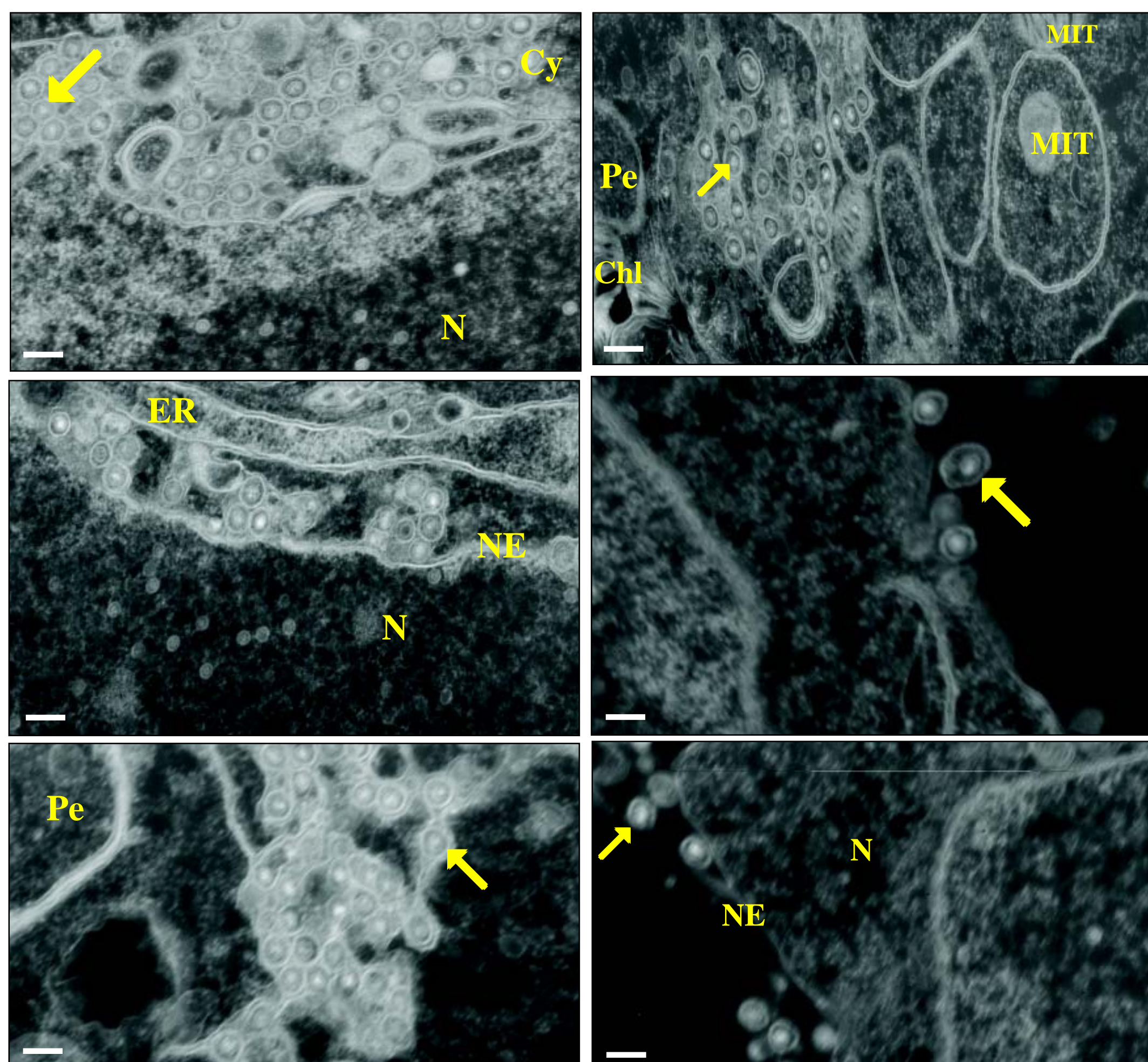


Fig. 2 - SEM micrographs of the epidermic surface of cork oak leaf. Stellate trichomes can be seen.

Fig. 3 - TEM micrographs of cork oak leaf cells. Virus particles (arrows) can be seen in both cytoplasm (Cy) and near the nuclear envelope (NE). Mitochondria (Mit), Peroxisomes (Pe), Chloroplast (Chl), Endoplasmic Reticulum (ER), and Nucleus (N), can also be seen. Bar represents 0.05 µm.

The DNA extract fractionated by bidimensional agarose gel electrophoresis (AGE) in 0.5x TBE buffer (90 mM Tris.Borate, 2mM EDTA, pH 8.3) and incorporated of ethidium bromide (0.5 µg/ml) (Fig. 4), has revealed that the viral DNA was linear. The restriction enzyme *EcoRI*, digested this DNA, which suggests a double stranded (ds) DNA that contains approximately 90 kbp (data not shown).

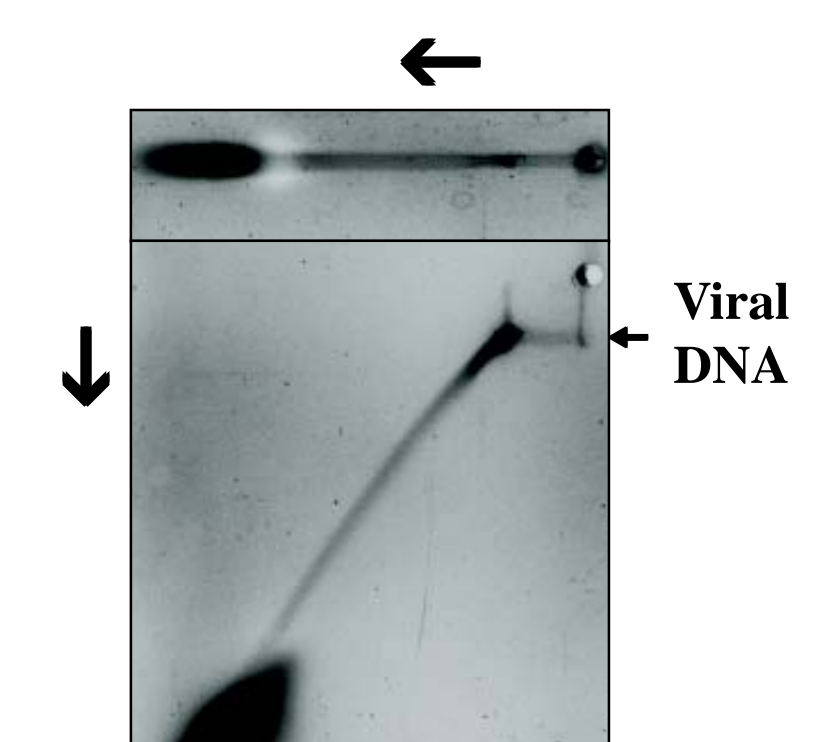


Fig. 4 - Bidimensional AGE (0.8% wt/vol) of the DNA extract.