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## OP 44 - Transmission studies of the *Grapevine leafroll-associated virus 1* and the *Grapevine virus A* by the mealybug *Phenacoccus aceris*.

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### INTRODUCTION

Grape production faces many severe viral, bacterial and fungal diseases, among which leafroll is one of the most devastating worldwide. Leafroll disease can be caused by several viruses belonging to the *Closteroviridae* family (GLRaV-1, -2, -3, -4 and -4-like). While GLRaV2 (genus *Closterovirus*) has no known vector and to our knowledge does not spread naturally in vineyards, the other three ones (*Ampelovirus*) are specifically transmitted by mealybugs (*Pseudococcidae*) and soft scales (*Coccidae*) and thus prone to be efficiently dispersed within and between vineyards (Sforza *et al.*, 2003 ; Le Maguet *et al.*, 2013). GLRaV-1 and GVA have flexuous virions of ca. 2000 nm and 800 nm composed of positive ssRNAs of 18,7 Kb and 7,5 Kb, respectively.

GLRaV-1 and GVA are restricted to vascular tissues of host plants and are frequently present in co-infection in grapevine. Their transmission by mealybugs has been shown to comply the criteria of a “semi-persistent and non-circulative” mode (Tsai *et al.*, 2008). The mealybug *Phenacoccus aceris* has been shown to transmit very efficiently ampeloviruses and vitiviruses (Le Maguet *et al.*, 2012), but neither the biological parameters of the transmission, nor the precise location of virions specific retention within the vector are known so far.

### MATERIALS AND METHODS

To address these questions, mouthparts anatomy of the vector *P. aceris* was described using scanning electron microscopy (SEM) and transmission electronic microscopy (TEM) techniques. Transmission tests from infected to healthy plants were developed to determine the biological parameters of the transmission, such as the minimum acquisition and inoculation access periods of the viruses by mealybugs, and the maximum retention time within the vector. Fluorescent labelling by successive membrane feedings were used to locate the precise zone of particles retention. Virions were purified following the protocol described by Gugerli *et al.* (1984) and hybridization with specific primary (BioReba) then secondary (ALEXA Fluor 488 & 568) antibodies was used to label the virions particles within the mealybug.

### RESULTS AND DISCUSSION

*Phenacoccus aceris* mouthparts anatomy appears to be close to those of other sap-sucking insects.

Mouthparts are composed of a clypeus, a labium and the stylet fascicle. The three-segmented labium bearing some mechanosensory hair-like sensilla, show a labial groove on its anterior surface, enclosing the stylet fascicle. The stylet bundle is needle-like and composed of two distinct mandibular and maxillary stylets.

Experiments to determine the biological parameters of the transmission showed that *P. aceris* is able to acquire and transmit both viruses in a very short time (1 h acquisition and inoculation time). However, the transmission efficiency increase in time as previously shown for the GLRaV-3 transmission by *Planococcus ficus* (Tsai *et al.*, 2008).

The “semi persistent and non circulative” transmission mode of the GLRaV-1 and the GVA implies a virion retention on ectodermic structures, such as the stylet fascicle and/or the foregut of the insect.

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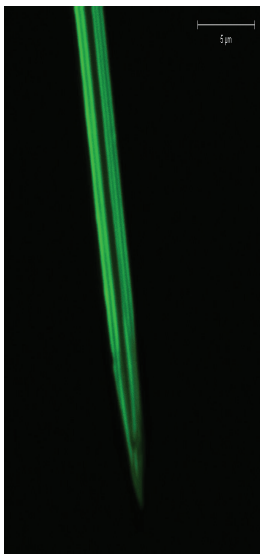
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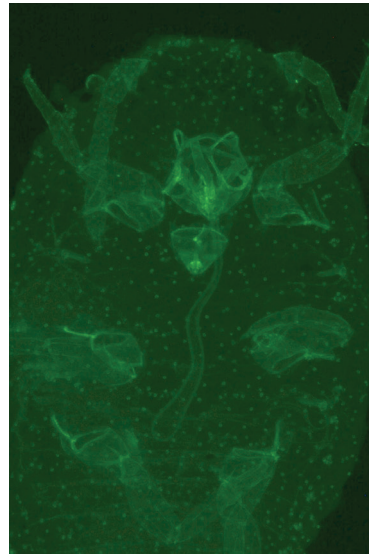
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**Figure 1.** Tip of a *P. aceris* maxillary stylet in confocal microscopy



**Figure 2.** Ventral view of a *P. aceris* nymph in epifluorescence microscopy