

## APPLYING LASER MICRODISSECTION TECHNOLOGY TO STUDY THE MOLECULAR MECHANISMS INVOLVED IN PLANT - PATHOGEN INTERACTIONS IN GRAPE LEAVES

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*Oidium, grape, laser capture microscopy*

Here we present our preliminary results on the application of Laser Capture Microdissection (LCM) technology for the isolation of mRNA from single cells derived from leaves of grape (*Vitis vinifera* L.), to be used for addressing crucial aspects of the complex molecular interactions occurring between plant cell and fungal pathogens. As a model we are using *Uncinula necator*, the ascomycetes casual agent of powdery mildew in grape. Powdery mildew of grape, like other powdery mildew fungi, grows on the surface of leaves. In grape it also infects the fruit and other young tissue including flowers, shoots and petioles. During infection, the fungus grows its hyphae on the surface of the leaf and the hyphal tip (haustorium) penetrates into epidermal cells from which the fungus drags nutrients. However, infection of epidermal cells is not massive, instead haustoria are produced only in a limited number of susceptible upper epidermal cells. Expression analysis of infected cells in comparison to non infected cells could provide useful information on the molecular messages both from the fungus and the plant cell. Whole leaf expression profiling in this case might not be appropriate, since it is complicated by the multiple cell types present in an infected leaf: non epidermal, non infected cells, infected and non infected epidermal cells, uninfected cells adjacent to a cell in which a haustorium is present. For this reason we are interested in implementing cell specific RNA profiling, taking advantage of the LCM technology. Laser capture microdissection (LCM) is a rapid way of isolating substantially pure cellular preparations directly from heterogeneous tissues, based on conventional histological identification. By this technique, individual cells can be harvested from tissue sections while they are viewed under the microscope.

The fixation method, paraffin embedding, was utilized to isolate epidermal cells from adult grape leaves. RNA was extracted and amplified.