Pre-symptomatic detection of *Plasmopara viticola* infection in grapevine leaf tissue using plant optical signals in relation to the production of *trans*-resveratrol and 2,4,6-trihydroxyphenanthrene-2-*O*-glucoside. Field study.

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Motivation and Introduction

Global climate change can cause enhanced effect of biotic stress effect on crops (*Vitis vinifera* L.,1753 subsp. vinifera). Grapevine plants has many potential pests, which may cause decrease of yield or even complete yield destruction if no chemicals are applied. Thus, early detection of infection is in the center of interest of winegrowers. One of the major disease is downy mildew (*Plasmopara viticola)* that attacks mostly leaves. Visual symptoms appear ca 5-9 days after infection. The infection causes changes in carbon metabolism (photosynthesis), pigment composition as well as production of phytoalexins (e.g., stilbenes). All these changes are reflected in changes in plant optical signals. Chlorophyll fluorescence emission (Chl-F) can be used to detect the changes in photosynthesis thanks to their close relationship (e.g., Papageorgiou and Govinjee, 2011). It has been already succesfully used for pre-symptomatic detection of *Plasmopara viticola* infection in lab conditions (Cséfalvay *et al.,* 2009). UV-induced fluorescence can be used for non-invasive detection of stilbenes (e.g., Bellow *et al*., 2012); and spectrum of reflected light (Refl) and the vegetation reflectance indices for the changes in pigment composition (e.g., Ustin *et al.*, 2004). **The use of plant optical signals can lead to early information of crop physiology and possible detection of disease directly in field. This can than resulted in effective strategy of wine producer and the resulting proper management.**

Aims:

- to verify usability of Chl-F for detection of *Plasmopara viticola* infection in field
- to determine possibility of *in-vivo* detection and estimation of resveratrol concentration in leaves
- to find optimal reflectance indices for quantification of chlorophylls and carotenoids concentration changes in infected leaves

Material and Methods

Plant material: Leaves of six susceptible (Cabernet Sauvignon, Chardonnay, Muscatel, Saint Laurent, Müller Thurgau, Alibernet) and four resistant grapevine varieties (Cerason, Malverina, Hibernal, Laurot) have been sampled in experimental vineyard (Department of Viticulture and Oenology, Lednice, Czech Republic during 2009 and 2010. Healthy and infected leaves were collected both from sun exposed and shade part ca 5 - 7 days after the infection. In total, 200 leaves have been collected, 10 healthy and 10 infected leaves from each grapevine variety.

Chl-F measurement: 15 min of dark adaptation; F_0 determination in dark, F_M measurement under saturating pulse (1500 µmol(photons) m^{-2} s^{-1}, 1.8 s), FluorCam FC 800-0 (P.S.Instrument, Czech Republic).

Calculated Chl-F parameter: $F_V/F_M = (F_M-F_0)/F_M$.

UV induced fluorescence: fluorescence emission spectrum under excitation 320 nm, JobinYvon (Horiba Scientific, Japan).

Reflectance measurement: reflectance spectrum under white light (halogen, 400 – 800 nm), hemispherical from adaxial leaf side, Spectrometer SM 9000 (P.S.Instrument, Czech Republic). *Calculated reflectance indices*: chlorophylls estimation - R_{750}/R_{700}, carotenoids estimation: $CRI_{700}=1/R_{510}-1/R_{700}$)

Chemical analysis: Chlorophylls, carotenoids – spectroscopically according to Lichtenthaler (1987), Stilbenes - HPLC analysis (HP 1050 (Ti-series) on a 3 µm, 150 mm x 2mm, Luna C18 (2) column (Phenomenex, Torrance, USA) with water-acetonitrille-o-phosphoric acid mobile phase.

Conclusions

- F_V/F_M declined at the same level in sensitive and resistant varieties but the FV/FM distribution over the leaves differed.
- Chl-F parameter F_V/F_M can be used for *Plasmopara viticola* infection in field both in sensitive and resistant varieties
- Concentrations of the stilbenes were significantly higher in infected leaf tissue
- High stilbenes content in infected leaves can be detected *in vivo* by means of UV-induced fluorescence
- Changes of pigments concentrations can be followed by reflectance indices: R_{750}/R_{700} (for chlorophylls, Gitelson *et al*., 1996), CRI index (for carotenoids, Gitelson *et al*., 2002)
- **Combination of Chl-F, UV induced fluorescence and reflectance offers unique opportunity to develop non-invasive method for an early detection of infection and large-scale screening of quantitative resistance to *P. viticola***

References

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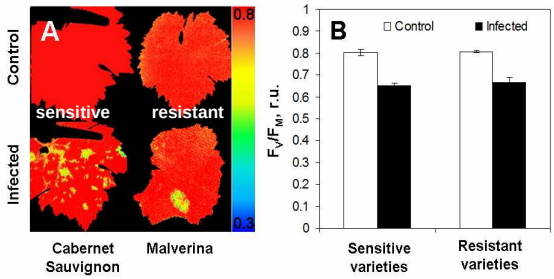
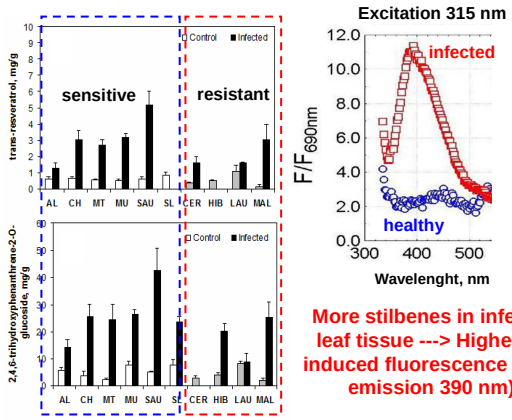
Chlorophyll fluorescence - F_V/F_M



Maximum quantum yield of PSII photochemistry (F_V/F_M) is reduced by ~ 20 % in infected leaf spots.

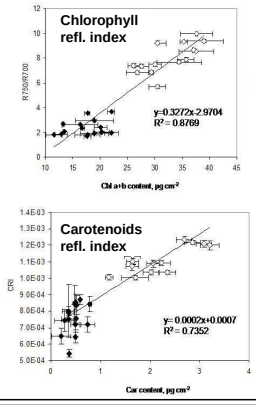
Stilbenes and UV induced fluorescence



More stilbenes in infected leaf tissue ---> Higher UV induced fluorescence (max. emission 390 nm).

Photosynthetic pigments and reflectance

Cabernet Sauvignon	CONTROL	INFECTED
Chla, ug/g	691.84	546.84
Cha+b	1055.01	819.42
Car	148.21	136.91
Pinot Gris	**CONTROL**	**INFECTED**
Chla, ug/g	763.77	460.81
Cha+b	1303.68	870.37
Car	77.65	46.05
Pinot Blanc	**CONTROL**	**INFECTED**
Chla, ug/g	592.05	524.90
Cha+b	1085.48	936.05
Car	33.28	74.40



Lower pigment content in infected leaf tissue can be detected by means of reflectance indices.

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