Pathogen-Host Interaction Analysis of Esca-associated fungal mutant strains

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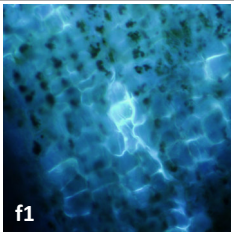
Introduction:

Esca is well known in the Mediterranean region and Western Europe as a destructive disease of grapevine caused by fungal pathogens showing severe symptoms on grapevine plants. Mainly *Phaeomoniella chlamydospora* (Pch) and *Phaeoacremonium minimum* (Pmi) growing as endophytes are involved (Fischer, 2015).

Despite the efforts made by the scientific community in the last decade, the symptom formation and the cause of those leaf symptoms is still poorly understood. In general the involvement and phytotoxic activity of the secondary metabolites scytalone and isosclerone produced by the pathogens is assumed (Andolfi et al., 2006).

During the study the ability of several gen-deletion mutants of Esca-associated fungi (namely Pch and Pmi) to infect grapevine cuttings as well as *in-vitro* plants was analyzed. The previously generated mutants show a higher sensitivity to artificial stresses, such as heat or osmotic stress, than the wild type strains. Furthermore, they have a strongly reduced melanin biosynthesis because of the lack of essential enzymes (polyketide synthases, PKS) of the scytalone biosynthesis pathway. These infection assays may help to understand the host-pathogen-interaction on a molecular level.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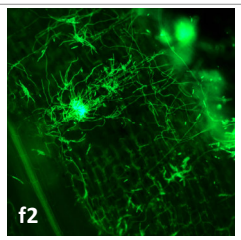
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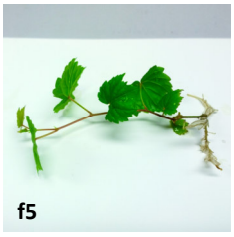
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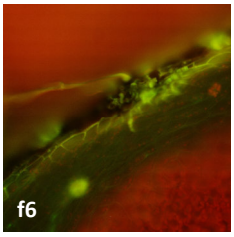
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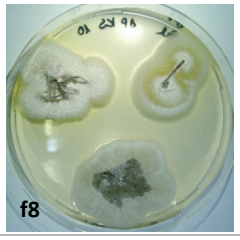
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Material and Methods:

In-vitro plants

In vitro plants (Santos et al., 2005)

Spraying of 200µL of H_2O (0.9% NaCl) $1x10^4$, $1x10^5$ or $1x10^6$ spores/mL

Monitoring for 7 to 14 dpi **[f3]**

Surface sterilisation of the plants
endophyte isolation **[f5, f8]**
microscopic evaluation **[f6]**
using e.g. fluorescence labeled strains **[f1]**

Greenhouse trials

Three-eye cions of *Vitis vinifera* cv. 'Cabernet sauvignon' (Selim et al., 2012)

Spraying of 10mL of H_2O (0.9% NaCl) $1x10^6$ spores/mL **[f7]**

Monitoring for 28 to 90 dpi **[f4]**

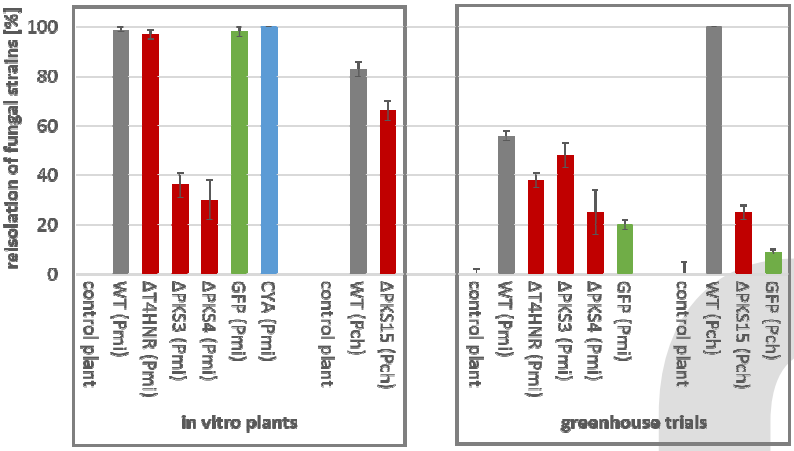
Surface sterilisation of the plants
endophyte isolation **[f8]**
microscopic evaluation **[f2]**
PCR evaluation of mutant stains using the HPT cassette as a marker

Results:

The results **[f9]** indicate that all plants tested were free of Pch and Pmi before the artificial infection assay. Furthermore, it was observed, that the WT strains and the fluorescence labeled strains had the highest infection rate in both test systems, whereas the ΔPKS strains of both fungi had the lowest reisolation rate throughout the tests. And finally, the infection rate and reisolation efforts were much lower for the greenhouse plants compared to the *in vitro* approach.

Conclusions:

The impact on the infection success of the ΔPKS-mutant strains was somehow surprising since the deletion of the Tetrahydroxynaphthalene reductase (ΔT4HNR) had little to no impact on the mutants. In the future an assay that allows a monitoring of occuring symptoms due to the infection of WT and mutant strains would be a preferable research aim.



F9: results of the conducted greenhouse and *in vitro* plant trials

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