



Doctoral Thesis

**Towards an integrative management of Eutypa dieback and esca disease of grapevine**

**Author(s):**

Christen, Danilo G.

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**TOWARDS AN INTEGRATIVE MANAGEMENT OF EUTYPA DIEBACK AND  
ESCA DISEASE OF GRAPEVINE**

A dissertation submitted to the  
**SWISS FEDERAL INSTITUTE OF TECHNOLOGY, ZÜRICH**  
for the degree of  
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Presented by  
**DANILO G. CHRISTEN**  
Dipl. Ing. Agr. ETH

born September 23<sup>rd</sup>, 1969  
citizen of Affoltern im Emmental BE

Accepted on the recommendation of

Prof. Dr. Geneviève Défago, referent  
Prof. Dr. Bruce McDonald, co-referent  
Prof. Dr. Raphaël Tabacchi, co-referent

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## SUMMARY

Eutypa dieback and esca disease are two trunk diseases of grapevine affecting the sustainability and the productivity of vineyards worldwide. They are characterized by a slow decline leading to the death of the plants. Eutypa dieback is caused by *Eutypa lata*. *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum*, and *Fomitiporia mediterranea* are the causal agents of esca disease. Due to a long latency time of several years, foliar symptoms become visible tardy, when the diseases had already largely developed in the trunk. Trunk disease pathogens produce toxic compounds, like eutypine, 4-hydroxybenzaldehyde, or 3-phenyllactic acid, which participate in the symptom development. No *Vitis* variety is resistant to trunk diseases, but Merlot is tolerant to Eutypa dieback. This tolerance is probably due to the presence of an aldehyde reductase metabolizing eutypine (a toxin of *E. lata*) to the non-toxic eutypinol. Since the banning of sodium arsenite worldwide, prophylactic measures are recommended to control effectively these diseases.

Trunk diseases of grapevine can be latent for several years. Therefore, we aimed in this project to investigate deeply this essential period in the development of the symptoms by exploring possible nondestructive methods to detect early the diseases. We opted for acoustic emission, computer tomography and drill resistance to detect wood deteriorations, like necrosis, decay, or white rot in grapevine trunk. In order to calibrate the measurements, we first assessed these methods in the laboratory. Due to the small diameter of grape trunks, acoustic emission did not provided enough differences between healthy and affected wood. On the contrary, computer tomography supplied good representations of wood deterioration. Nevertheless, drill resistance was the most promising method to give informations on the structure of the wood. Differences between healthy and affected wood were detectable. Furthermore, this method differentiated between the hard structure of a necrosis and the soft structure of a white rot. More studies are needed to assess drill resistance as a suitable method to diagnose early wood decay in vineyards.

Trunk disease pathogens expend in the xylem vessels, and perturb the water transport in the plant. Physiological changes in the plant and particularly disturbance of the photosynthetic efficiency were assessed using *in situ* fluorescence monitoring. All measurements were carried out in June 2003 on healthy leaves with Handy-PEA chlorophyll fluorescence analyzer. Esca symptoms were controlled on the same plants in August 2003. Asymptomatic and symptomatic

plants were analyzed separately using the JIP-test. Disorder of the photosynthetic apparatus could be revealed two months before the apparition of foliar symptoms in autumn and a functional behavior pattern of the photosystem II could be defined for esca foliar symptoms. In parallel, drought stress was also evaluated with this method in a greenhouse experiment and the functional behavior pattern found was different from the esca symptom pattern. The comparison of both type of stress suggests that esca foliar symptoms cannot simply be interpreted as a water stress and that other physiological changes probably occur in plants affected by esca pathogens. These results are very promising and suggest that an early diagnosis method based on fluorescence monitoring could be useful to detect early different types of stress and especially trunk diseases of grapevine in the latent period.

No direct control of trunk diseases of grapevine is available. Therefore, we also aimed to investigate new biological control mechanisms based on microbial degradation of biotic toxins. Known biocontrol agents (*Fusarium lateritium*, *Trichoderma sp.*) were tested for their ability to degrade some of the trunk diseases toxins. Detoxification processes were investigated by incubating these biocontrol agents in a liquid medium containing the individual pure toxins. An HPLC-based method using UV- and MS-detection was developed to analyze toxin metabolization, and a quantification of the degradation products was performed. For eutypine and for 4-hydroxybenzaldehyde, the reduction of the aldehyde function to the correspondent alcohol was found, as by the tolerant cultivar Merlot. A supplementary detoxification pathway, not present in Merlot, where the aldehyde was oxidized to an acid, was revealed. These intermediates disappeared totally at the end of the time courses performed with some biocontrol candidates. When biological assays on cells of *V. vinifera* cv. Chasselas were carried out, the degradation products exhibited a lower toxicity than the toxins. Thus, the selection of better biocontrol candidates could be facilitated.

An aldehyde dehydrogenase (ALDH) was supposed to be responsible for the oxidation of the toxins. Using PCR with degenerate oligonucleotide primers, *ALDH* was found in potential biocontrol agents. The specific amplicons were sequenced. The deduced amino acid sequence revealed a high level of identity (55% – 79%) with ALDH from fungal origin. Several amino acid motifs containing important catabolic active site or NAD-cofactor binding site were highly conserved, as well as important residues that remain invariant. A fragment of the *ALDH* gene has been cloned in a plasmid that was used for the transformation of *Trichoderma atroviride* P1. Using protoplast-mediated transformation techniques, disrupted mutants could unfortunately not

be produced. In the future, we plan to use other more efficient transformation techniques and to check if the ALDH is responsible for toxin degradations.

This thesis deals with a better understanding of the interactions between the different pathogens involved in trunk diseases and the grapevines at first, and with the impact of fungal biocontrol candidates on these interactions. New diagnosis methods have been developed to early detect wood deterioration and esca foliar symptoms. Furthermore, new possible biocontrol mechanism based on microbial detoxification was found and selection of better biocontrol agents was discussed. The global understanding of the epidemiology of trunk diseases, and more particularly of the central role of the toxins implicated in the diseases, provide a basis for the development of new integrated strategies to control successfully trunk diseases of grapevine.