ISHS
International Society for Horticultural Science

ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

13th International Fire Blight Workshop
2.-5. July 2013, Zürich, Switzerland
ETH Zürich Main Building, HG E7

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The epiphytic survival of *Erwinia amylovora* on the apple fruit surface

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Using molecular and conventional detection methods the presence of *Erwinia amylovora* was confirmed on the surface of 4% of tested apple fruits cv. Antonovka picked from moderately infected trees and on 23% apples collected from severely infected cv. James Grieve trees. In another experiment, the survival of *E. amylovora* was tested on the calyx of apple fruits cv. Gala collected from the orchard where fire blight symptoms were not observed. Two hundred µl of *E. amylovora* suspensions at the concentration of 10^3 or 10^7 cfu/ml were introduced on the calyx and afterwards apples were stored for 5 months at 3˚C and humidity of 85%. Every month, 10 fruits were tested for the presence of *E. amylovora.* On the fruits which were contaminated with suspension at lower concentration, the pathogen was not detected already after two months, but in case of apples treated with pathogen suspension at higher concentration, *E. amylovora* presence was confirmed even after 5 months of storage. Analysis of the influence of the humidity on the survival of pathogen on calyx showed that the humidity of about 100% was less favorable for bacteria survival than that of about 30%. The longevity of pathogen could be restricted by associated epiphytic microorganisms. We found out that some isolates of bacteria and fungi obtained from tested fruits inhibited the pathogen growth *in vitro*.

HrpW interacts with NADH dehydrogenase of apple

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During the infection, *Erwinia amylovora* secretes in the host cell at least twelve different effectors, which are involved in the establishment of the disease. Harpins are part of the proteins delivered in the plant cell via the type three secretion system. These proteins are known to be heat stable, glycine rich, without cysteine residues, and trigger a HR in non-host plant. In particular, the mechanism of action of harpin N has been well studied. HrpW, one of the other harpin members, is present in many phytopathogenic bacteria such as *E. amylovora* and *Pseudomonas syringae* pv tomato, and possesses a class III pectate lyase domain, but nobody so far has reported a pectate lyase activity. Also *hrpW* mutants induce symptoms as strong, or even stronger, than wild-type strains, suggesting that this protein, contrarily to HrpN, is not required for a full virulence of the bacteria. However, recently HrpW has been described as a HR negative modulator in non-host plants such as *Nicotiana tabacum* and *Arabidopsis thaliana*. On *Arabidopsis* cell cultures, depending on its concentration, it could trigger, like HrpN, a defense response, or act antagonistically to HrpN, inhibiting cell death and ROS production.
At present nobody has deeply investigated the effect of HrpW in host plants. We undertook this project to identify apple proteins interacting with this effector by screening a yeast two hybrid apple library. We identified several potential protein interactors that have been further tested by Bimolecular Fluorescent Complementation (BiFC). This test in tobacco confirmed that HrpW is able to interact with the N domain of the apple NADH dehydrogenase. Indeed when HrpW and the apple NADH dehydrogenase are co-infiltrated in tobacco an HR response can be observed after 2 days. The Interaction between HrpW and the apple NADH dehydrogenase located in the mitochondria can the ROS production and induce the HR reaction observed in non-host plant. The effect of this interaction in apple is under investigation.