## 124 - Development of a Multiplex TaqMan Real-Time PCR Assay for Sensitive and Rapid Detection of Phytoplasmas Infecting Rubus Species

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Rubus stunt, a disease associated with phytoplasma infections in wild and cultivated *Rubus* species, is a major challenge in the production of raspberries (*Rubus ideaeus* L.), blackberries (*Rubus fruticosus* L.), and loganberries (*Rubus x loganobaccus*) throughout Europe, north-eastern USA, and Turkey. Symptoms range from stunting, shoot proliferation, small leaves, short internodes, enlarged sepals, phyllody, and flower proliferation to fruit malformations. Phytoplasmas are cell wall-less bacteria inhabiting the phloem and are transferred by phloem feeding insects. As the time between infection of a plant and the development of disease symptoms can take up to 1 year and *Rubus* plants are produced by vegetative propagation, an early detection of phytoplasmas using highly sensitive and rapid molecular methods is of great importance to minimize their spread. Thus far, phytoplasmas belonging to the 16Sr groups of elm yellows (16SrV), X disease (16SrIII), aster yellows (16SrI), and stolbur (16SrXII) have been identified in *Rubus* species. Therefore, in this study, two multiplex real-time PCR assays using TaqMan probes in combination with up to four different kinds of fluorophores in the same reaction were developed. The assay combines TaqMan probes previously published in literature with newly designed probes, allowing a rapid and simultaneous detection of phytoplasmas in general as well as a more specific detection of the above mentioned groups of phytoplasmas infecting *Rubus* species. In addition, a primer and probe set for the detection of plant host DNA was used as an internal control, enabling both the conformation of a successful DNA extraction and the exclusion of false negative results due to potential inhibition of the PCR. This assay will now be used to monitor presence and distribution of phytoplasmas in *Rubus* plants of different geographic origins, cultivars and cultivation systems as well as in putative insect vectors like leafhoppers.

## 125 - Praxiserfahrungen mit dem BIOTEST zum Nachweis von *Clavibacter michiganensis* ssp. *sepedonicus* (CMS)

*Practical experience to detect Clavibacter michiganensis ssp. sepedonicus (CMS) by using bioassay*

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Akkreditierung, Validierung und Verifizierung von Untersuchungsmethoden sind in den Pflanzenschutzlaboren von zunehmender Bedeutung. Insbesondere die Quarantäneschadorganismen wie *Clavibacter michiganensis ssp. sepedonicus* (CMS) stehen dabei im Focus. In Rheinland-Pfalz wurden umfangreiche Untersuchungen durchgeführt, um den BIOTEST auf *Clavibacter michiganensis ssp. sepedonicus* (CMS) zu mit abgesicherten Prüfergebnissen zu untermauern.
Das Prüfverfahren wird nach OEPP/EPPO Diagnostic protocol of Clavibacter michiganensis subsp. Sepedonicus OEPP/EPPO, Bulletin 36, PM 7/59 (1), S. 99–109 von 2006 durchgeführt. Gemäß den Vorgaben der EPPO durch die Festlegung der Standards für Diagnoselabore Diagnostics - Basic requirements for quality management in plant pest diagnosis laboratories (OEPP/EPPO 2007) und Diagnostics - Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity (OEPP/EPPO 2010) gliedern sich die Untersuchungsschwerpunkte nach den fünf analytischen Kenngrößen.