

reported in other European countries. Symptoms generally appeared after humid climate periods; they make the affected batches nonmarketable. In France the disease is responsible for about 10% losses every year (2). So far contaminated seeds or infested soil are considered as the major infection sources (2, 3).

To further investigate the epidemiology of the disease a research project was started, which first aims at improving the serological and DNA-based detection of the pathogen in plants and seeds. Since the bacteria may further grow during packaging and trade, highly sensitive and specific detection methods are required.

Using different *Av* isolates several polyclonal antisera (pAS) and a broad panel of monoclonal antibodies (mAbs) were developed. While all pAS strongly crossreacted with other bacterial species some of the mAbs displayed a high specificity to *Av*. They were successfully applied in a TAS-ELISA format to detect the pathogen in extracts from infected plants. After enrichment of bacterial cells through incubation in a semiselective culture medium (4) the method could be used for *Av* detection in seed lots too. Several mAbs were also applicable for immuno-histochemical detection of *Av*. Bacterial cells could be visualised in ultrathin sections for the first time in intercellular spaces of infected corn salad leaf tissue by immunogold labelling and transmission electron microscopy.

On the genome level 50 isolates of *Av* were analysed by amplified rDNA restriction analysis (ARDRA) (5) for sequence differences in their 16S rDNA and for variations in genome organization by BOX PCR (6). First results indicate that the *Av* isolates can be classified into two main groups by ARDRA pattern. This finding was confirmed by BOX PCR. The members of ARDRA groups show very similar BOX pattern respectively and are near clustered by analysis with the GelCompare software. In a next step several BOX PCR fragments were cloned and sequenced to obtain species specific sequences for DNA based detection of *Av*.

(1) MOLTSMANN, E. et al., 2000: Blattflecken an Feldsalat durch das Bakterium *Acidovorax valerianellae*. Gemüse 36(12), 10-12.

(2) GRONDEAU, C., R. SAMSON, 2009: Detection of *Acidovorax valerianellae* in corn-salad seeds, seed transmission of the pathogen and disease development in the field. Plant Pathology 58, 846-852.

(3) GRONDEAU, C., V. CERCEAU, C. BUREAU, R. SAMSON, 2003: Evidence that *Acidovorax valerianellae*, bacterial black spot of corn salad (*Valerianella locusta*) agent, is soil transmitted. *Pseudomonas syringae* and related pathogens. Biology and Genetics, 89-91.

(4) GRONDEAU, C., C. MANCEAU, R. SAMSON, 2007: A semiselective medium for the isolation of *Acidovorax valerianellae* from soil and plant debris. Plant Pathology 56, 302-310.

(5) VANECHOUTTE, M. et al., 1992: Rapid Identification of Bacteria of the Comamonadaceae with Amplified Ribosomal Dna-Restriction Analysis (Ardra). Fems Microbiology Letters 93, 227-234.

(6) MARTIN, B. et al., 1992: A Highly Conserved Repeated Dna Element Located in the Chromosome of *Streptococcus pneumoniae*. Nucleic Acids Research 20, 3479-3483.

(DPG AK Phytobakteriologie)

## 5) Untersuchungen zu Anfälligkeiten verschiedener Apfelsorten gegenüber Feuerbrand im Glashaus

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Die zunehmende Ausbreitung des Feuerbranderreger (*Erwinia amylovora*) in Österreich und damit verbundene Rodungen stel-

len die Obstbaupraxis verstärkt vor die Frage der Sortenwahl bei Nachpflanzen oder Neupflanzungen. Um die unterschiedlichen Empfindlichkeiten verschiedener Apfelsorten unter gleichen, kontrollierten Bedingungen zu erarbeiten, wurden an der AGES, Institut für Pflanzengesundheit, in den Jahren 2007 und 2008 Versuche durchgeführt.

In Glashaus-Quarantänekabinen wurden an dreizehn verschiedenen in Europa bedeutenden oder für die Zukunft relevanten Kulturapfelsorten bzw. Unterlagen künstliche Inokulationen mit einem österreichischen Referenzstamm von *E. amylovora* durchgeführt um die Feuerbrandanfälligkeit dieser Sorten zu vergleichen. Die Sorten 'Jonagored supra'®, 'Gala Galaxy selecta'®, 'Fuji KIKU 8'®, 'Golden Delicious Kl. B'®, 'Elstar', 'Braeburn Mariri Red'®, 'Rewena'®, 'Cameo'® Caudle, 'Crimson Crisp'® COOP39 (alle auf M 9, T337), 'Topaz'® auf Malus M7, MM111 und M9 (T337) sowie die Unterlagen M9 (T337), Malus M7, Malus MM 111, Supporter 4 (Pi80) und Malus A2 wurden auf ihre Blüten- und Triebanfälligkeit untersucht.

Bei den untersuchten Apfelsorten war keine Sorte dabei, die sich bei visueller Bonitur an Trieben und Blüten als Feuerbrand-tolerant erwies. Nur eine Sorte zeigte trotz künstlicher Inokulation keine Symptome an den Blüten ('Crimson Crisp'® COOP39). Die Häufigkeit von Feuerbrand-Blütensymptomen variierte zwischen 2% ('Rewena'®) und 48% ('Golden Delicious Kl. B Laimburg'®). Weiters erwiesen sich 'Braeburn Mariri Red'®, 'Elstar', 'Fuji KIKU 8'®, und 'Cameo'® Caudle als wenig empfindlich, 'Golden Delicious Kl. B'®, 'Gala Galaxy selecta'® und 'Jonagored supra'® als anfällig.

Um die Ausbreitungsgeschwindigkeit des Erregers in der Pflanze festzustellen, wurden Bäumchen in 10 cm lange Stückchen geschnitten und je nach Beschaffenheit so aufgearbeitet, dass *E. amylovora* Bakterien extrahiert werden konnten.

Mit einer adaptierten qPCR Methode konnte *E. amylovora* auch an symptomlosen Pflanzenteilen in unterschiedlichen Dichten nachgewiesen werden. Die Ausbreitungsdynamik des Erregers scheint sortenspezifischen Unterschieden unterworfen zu sein. Auch in den Pflanzen derselben Apfelsorte zeigt die Vermehrung des Erregers deutliche zeitliche und räumliche Schwankungen. Zur Aufklärung dieser Mechanismen sind weitere vergleichbare Untersuchungen notwendig.

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## 6) Reliability and sensitivity of diagnostic methods for detection of *Clavibacter michiganensis* subsp. *michiganensis* in seeds and plant material

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*Clavibacter michiganensis* subsp. *michiganensis* (CMM), the causal agent of bacterial canker of tomato, was at first described 1909 in Michigan (USA) and has since spread to nearly all main tomato growing areas world-wide by infested seeds and latently infected tomato plantlets. For an effective control of the disease a sensitive and reliable semi-selective growth medium for CMM is very decisive. Therefore, we tested 5 published semi-selective media on 30 CMM strains originating from many different countries. We found that the mean recovery rates on the media mSCM, D2ANX, SCM, CMM-1 and the medium suggested in 2005 (ANONYMOUS, 2005) by the European Plant Protection Organisation (EPPO) reached 6.86%, 70.34%, 95.87%, 93.81% and 0%, respectively within 7 days. After 10 days the mean recovery rates were: 35.64%, 70.34%, 96.51%, 93.81% and