Nowadays there is a tendency to implement the Lemmerzahl-test more and more in non-official testing (screening new breeding lines) in the Netherlands. As Germany and Poland also use the Lemmerzahl-test, implementing this test in the Netherlands will most probably fasten up harmonisation and mutual acceptance of test results.

In the Netherlands four pathotypes occur: pathotypes 1(D1), 2(G1), 6(O1) and 18(T1) (Baayen *et al.*, 2006). These pathotypes are distinguished by differential reactions on a set of potato cultivars (bio-assay). Currently, six differential cultivars are used in our laboratory. Disadvantages of the bio-assay are: 1) long period of incubation, 2) availability of differential cultivars sometimes problematic, 3) purity of tubers. A new issue nowadays is the development of a molecular test to distinguish pathotypes. This project is called "Ontwikkeling van moleculaire toetsen om fysio's van obligate quarantaineschimmels te onderscheiden", and led by Bonants and Van der Lee (PRI, Wageningen).

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Session Pathogen biology

Epidemiological evidence that vegetatively-propagated solanaceous plant species act as sources of Potato spindle tuber viroid inoculum for tomato

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Over the last few years many latent infections by *Potato spindle tuber viroid* (PSTVd) have been detected in various crops, mainly vegetatively-propagated ornamentals. In a survey in the Netherlands in 2006, infections were found in 42% and 72% of the professionally grown lots of *Brugmansia* spp. and *Solanum jasminoides*, respectively. The infected lots contained 73,985 and 448,474 plants, respectively. Sequence analysis showed that most genotypes of PSTVd isolates from *Brugmansia* spp. clearly differed from *S. jasminoides* isolates. In addition, phylogenetic studies showed that PSTVd genotypes from *Brugmansia* spp. and *S. jasminoides* cluster apart from each other and from PSTVd isolates from the vegetatively-propagated crops of *Solanum tuberosum* (potato) and *Physalis peruviana* (Cape gooseberry). The studied PSTVd genotypes from the generatively-propagated crop tomato did not form a separate group but clustered in the groups of *P. peruviana*, *S. jasminoides* and *S. tuberosum*. Furthermore, repeated mechanical inoculations of several of these genotypes to tomato appeared successful without resulting in substantial genome mutations. Therefore, latently-infected plants of the vegetatively-propagated solanaceous crops may have been sources of infection for tomato.

Outstanding: the dispensable chromosomes of Mycosphaerella graminicola

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Analysis of two genetic linkage maps of the wheat pathogen *Mycosphaerella graminicola* identified dispensable chromosomes that were present in both parents but absent in 15-20 % of the progeny. These Copy Number Polymorphisms (CNPs) were confirmed with a Comparative Genomic Hybridization whole-genome array based on the finished genome of *M. graminicola* (http://genome.jgi-psf.org). Chromosomes 14- 21 were frequently absent among isolates, without visible effect on viability or virulence, whereas chromosomes 1-13 were invariably present. Genetic analyses showed that CNPs arise during meiosis, usually from nondisjunction at anaphase II. Overall, *M. graminicola* has the highest number of dispensable chromosomes reported. Varying from 0.41 to 0.77 Mbp, they comprise 38% of the chromosome number and 11.6% of the genome.

The dispensable chromosomes are smaller and have significantly lower gene densities. Most of their genes are duplicated on the essential chromosomes and show a different codon usage. Dispensable chromosomes also contained a higher density of transposons, pseudogenes, and unclassified genes, which could encode novel proteins. Moreover, the dispensable chromosomes show extremely low synteny with other Dothideomycete genomes. We hypothesize that the dispensable chromosomes of *M. graminicola* are adaptive in some yet unknown way.

A selective sweep in Fusarium asiaticum populations in southern China.

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The world's most important food and feed crops wheat, maize, and barley are hosts for a complex of *Fusarium* species causing Fusarium Head Blight (FHB) in wheat and barley and stalk rot in maize. The disease pressure in several regions in China is among the highest in the world. Consumption of food and feed contaminated with the mycotoxins produced by *Fusarium* species poses a great threat as they can cause serious illnesses and immune-suppression in humans and animals. *Fusarium asiaticum* is the predominant causal agent of Fusarium head blight (FHB) in southern China. The genetic diversity was assessed by analyzing 448 single-spore *F. asiaticum* isolates from 18 sampling sites that were 10 km to 2000 km apart. This analysis showed a significant ($P < 0.001$) degree of population subdivision among populations from upper, middle and

lower valleys of the Yangtze River, with little gene flow. We observed a strong association between this genetic population subdivision and the mycotoxin produced. Our results show that the dramatic decline in trichothecene chemotypes may be explained by a recent and significant sweep of 3ADON producers in FHB pathogen composition in the middle valley. Using Bayesian statistics we found a biased gene flow from 3ADON to NIV populations.

In addition, we observed significant genetic differentiation and linkage disequilibrium between NIV and 3ADON producing isolates at the same sampling sites. We discuss the impact of this shift on the increase of FHB in Southern China.

Session Scientific tools and pathogen detection

Trends in plant science: detecting air-borne chemicals sent off by plants to monitor their state of health

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Traditional inspections of greenhouse crops are done by greenhouse personnel and rely on the presence of visual symptoms on the crop. This method has its limitations. Namely, visual symptoms are often difficult to observe, or when seen, it may be too late to remedy the problem. For example, early signs of pathogen infections and herbivore infestations often appear on the abaxial side of leaves or on stem parts that are hidden by the foliage. When these symptoms remain unnoticed, such infections or infestations may disperse rapidly and result in irretrievable crop damage.
These limitations have led to the emergence of a wide range of methods to improve the inspection of greenhouse crops. Ideally, such methods would enable continuous monitoring of individual plants in order to reveal health problems at an early stage. This would enable a grower to take early action, and prevent further crop damage. One approach to monitor the health status of plants is based on the volatile organic compounds (VOCs) emitted from them. This approach was successfully tested at both laboratory-scale (Jansen *et al.*, 2009a) as well as greenhouse-scale (Jansen *et al*,. 2009b, Jansen *et al.*, 2009c).
In general, measurement of plant emission consists of three steps: (1) collection of plant-emitted VOCs, (2) separation of plant-emitted VOCs in the mixture, and (3) identification, and/or quantification of separate VOCs.
In the first step, a fraction of the compounds emitted from the plants is collected. This sampling step is usually combined with pre-concentration of the VOCs in the air to achieve the detection limits of commonly applied analytical instruments. Two methods are generally applied to pre-concentrate VOCs present in air. The first method is based on the dynamic pre-concentration of VOCs. This method is referred to as dynamic because the air is actively pumped through a cartridge packed with a material that traps the compounds of interest. The second method is based on the static pre-concentration of VOCs. In this case, a material is exposed to the air, in which the trapping of VOCs mainly depends on mass diffusion processes. In both cases, the selection of the material is crucial in order to trap the VOCs of interest.
Before identification and/or quantification of the plant-emitted volatiles, the mixture of compounds is often separated, mostly by gas chromatography. This method is a type of chromatography in which the mobile phase is a carrier gas, usually an inert gas such as helium, and the stationary phase is a layer of a polymer on an inert solid support, inside a glass or metal column. The properties of this column should be selected with care since they have a large effect on the ability to separate plant-emitted volatiles. There are various types of detectors available on the market to identify and quantify plant-emitted VOCs. The most popular detectors in use are the flame ionization detector and the mass spectrometer. Electronic noses are also widely used to detect plant-emitted VOCs in air. More recently, biosensors have emerged as promising tool to identify and quantify low levels of VOCs in ambient air.

At this moment, we consider gas chromatography coupled to mass spectrometry (GC-MS) as the best method for monitoring the health status of crops on the basis of plant-emitted VOCs at high-input greenhouse facilities. This preference is based on its favourable combination of high selectivity and resolution, good accuracy and precision, wide dynamic concentration range, high sensitivity, and the current commercialization of robust GC-MS systems. Only due to the high costs, we are years away from having this kind of instruments in horticultural practice. But, the ongoing expansion and intensification of greenhouse production and the concern among consumers about the potential intake of pesticide residues on fruits and vegetables will support the prospected application of plant health monitoring in a commercial setting.

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What can we learn from the Botrytis cinerea genome sequence?

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Botrytis cinerea (also known as grey mould) is a serious pre- and post-harvest pathogen in a wide range of fruit, vegetable and ornamental crops. The application of molecular genetic tools in the past two decades has provided an increased insight into the mechanisms by which *B. cinerea* infects plants. This insight may be useful for developing novel, rational control strategies to reduce pathogen damage, either by using novel chemicals or by enhancing plant resistance.

The previous molecular-genetic studies on *B. cinerea* have resulted in the cloning and functional analysis of many dozens of genes, but cloning of individual genes can be time-consuming when the sequences are unknown. In the 1990's the genome of one *B. cinerea* strain has been determined by Syngenta. More recently, a different strain has been sequenced by an international consortium, coordinated by French partners. The genome sequences of both *B. cinerea* strains, as well as of the closely related pathogen *Sclerotinia sclerotiorum*, are in the process of annotation. An important part of the analysis is dedicated to the comparison between the two fungal species and the two *B. cinerea* isolates.

I will present an overview of the current status of the *B. cinerea* genome analysis and highlight what we have learned thus far from the sequence with respect to evolution, (sexual and asexual) reproduction and the infection strategies of *B. cinerea*.