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## A CENTURY OF PLANT VIROLOGY

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### S u m m a r y

The first notes on a plant disease proven to be caused by a virus date from a poem written in 752 in Japan where Eupatorium yellows was described. The development of Plant Virology as a scientific discipline started with Mayer (1886), Ivanowski (1892), and above all Beijerinck's researches (1898). Beijerinck proved that the causal agent of tobacco mosaic is transmitted by the sap of diseased plants and by grafting, that it can be maintained in dry leaves and soil up to a year, and that it can infect a plant through roots, multiplying in a plant and translocating through vessels. He defined the infectious agent as a *contagium vivum fluidum*, after he had discovered that the virus easily passed through a porcelain filter, suggesting it was smaller than bacteria. He also observed that the agent could diffuse through agar that retained bacteria, and furthermore, that the virus could be cultured only in growing plants. This is the reason why Beijerinck is considered to be the father of PLANT VIROLOGY.

Further development of PLANT VIROLOGY is characterized by several phases. The first one is when many virus plant diseases were discovered and described; the second one is epidemiological; and the third one is physico-chemical which acquired the molecular approach of the study of viruses all. These phases have contributed to the progress of PLANT VIROLOGY, particularly the last full-developed one, which has led to faster detection and identification of viruses.

There have been described over 900 plant viruses, which represents 1/4 of all known viruses. New viruses have been discovered or separated from related ones which used to have the same description. Diseases caused by phytoplasmas, spiroplasmas and viroids are the most standing in the group of plant diseases caused by viruses.

In the epidemiological phase the ways of maintenance and transmission of plant viruses have been studied. About one fifth of the known plant viruses are transmitted by the seed and pollen of infected host plants. Numerous vectors, both animals (insects, mites, nematodes, snails, birds etc.) and plants have been studied.

The interaction between many viruses and their vectors has been investigated among which, the so-called "helper" components, allowing viruses to be transmitted by vectors, were proven to exist.

The physicochemical phase began with studying the shape and size of viruses by means of light scattering, electron microscopy, x-ray crystallography etc. It was proved that viruses were particles (virions) and not liquid like Beijerinck thought. It was also determined that apart from the size and shape, viruses had also a certain architecture.

Stanley (1935) started the chemical research of viruses. He purified tobacco mosaic virus and confirmed the presence of protein with the ability to crystallize. Soon after it was found viruses were nucleoproteins, which means they have both the nucleic acid (NA) and the protein. Viruses have one of four types of NA: ssRNA, dsRNA, ssDNA or dsDNA. The RNA alone has been shown to be sufficient for infection. Reconstitution of virus particles has been achieved *in vitro*, as well as the connection of one viral NA and the capsid of the other virus.

Owing to development and application of molecular biology and genetic engineering for the last ten years, general progress in virology was made above all, relating to the knowledge of viral genome structure and its replication, which was of great importance for different parts of virology.

The knowledge of the sequences of viral genome and virus coded proteins contributes very much to better explanation of mechanisms by which viral diseases have been incited. They are also important for taxonomy to be established in accordance with their origin and evolutionary interactions.

Not only was it possible to make the analysis of the genome but it also applied its cloning by a certain vector or constructions of modified versions of genes with the help of site-directed mutagenesis. Sequencing, isolation of genes, molecular cloning and implantation viral genome parts in transgenic plants, provide as new methods for the studies of viral gene function as a new approach in viral disease control. When using molecular techniques for last past years, including N-terminus serology and nucleic acid hybridization, the methods for identification of viruses and their strains and for the clarification of their interactions were improved. This is an important prerequisite for undertaking efficient precautions.

Biotechnology today has enabled the isolation of target sequences *in vitro* and amplification of nucleic acid fragments for just a few hours in an amount large enough to manipulate (PCR), thus overcoming the problems of molecular cloning by using restriction endonucleases. The ability to produce infectious RNA transcripts from cDNA copies of viral RNA genomes raises the possibility of applying genetic engineering techniques for the study of their biology at molecular level. The first report of transgenic virus resistance has been based on expression of the coat protein gene of tobacco mosaic virus. More recently there have been reports on resistance based on expression of non structural viral genes and non viral genes with anti viral activity. Also, the production of reactive virus specific recombinant antibodies has been stimulated by the expression of appropriate gene in bacterial cell or transgenic plants.

Many techniques and systems (transgenic organisms, in vitro mutagenesis and recombinant viruses, in situ transcription, PCR, defective interfering RNA<sub>s</sub> mutants of virus genom etc.) will probably in the near future influence better research on various aspects of replication, and first of all regulatory controls and mechanisms of replication, study of compatibility of virus-host plant, as well as the possibility of modification or of viral disease.

So far entire knowledge of plant viruses, have been aimed at developing the strategies of their control containing fast and reliable diagnostic methods (antigen detection, detection of NA), conventional resistant and tolerant cultivar selection and of transgenic plant production.

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## CONSTITUTIVE PLANT RESISTANCE TO VIRAL INFECTIONS

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Virus resistance is often mediated by plant genes that block specific steps in the viral life circle. Different types of virus resistance of plants have been described. Two basic types of constitutive virus resistance of plants can be discriminated. They are characterized as qualitative or localizing resistance and quantitative or non-localizing resistance, respectively.

The genetic basis of resistance can be monogenic, oligogenic and polygenic, respectively. Qualitative virus resistance is mostly controlled monogenically or oligogenically. For each gene conditioning resistance in the host plant, there is a gene conditioning avirulence in the pathogen. Inhibition of virus accumulation and/or virus short- and long-distance translocation are among the most conspicuous mechanisms of plant virus resistance. Many RNA viruses encode proteins that increase the gating capacity of plasmodesmata, enabling virus passage from cell to cell. Several virus genes play an essential role in long-distance movement. Viral entry into the phloem appears to involve an interaction with additional host components.

The hypersensitive response (HR) of plants to virus infections is an active defense reaction that leads to the necrosis of the initially infected tissues and usually prevents further virus spread within the infected plant. The HR develops only for certain plant-virus combinations, and it is determined by both the plant and the virus genomes according to a gene-for-gene interaction. Hypersensitivity and extreme resistance are controlled by single genes. Extreme resistance may be epistatic to hypersensitive resistance.

Both kinds of resistance can be overcome by distinct pathotypes. Breakdown of disease resistance can also be caused by extremely high or low temperatures. Genetic association or pleiotropism for genes conferring resistance was found in several virus-host combinations.

Enhanced durability can be expected from polygenically controlled quantitative virus resistance. This type of resistance never conditions for an absolute resistance but for a more or less weakening of the pathogenesis. It is a genetically complex form of disease resistance and is characterized by a quantitative variability. Several traits of quantitative virus resistance are described. The incidence of symptoms, virus accumulation and growth and/or yield proved to be the most important traits for assessment of the level of resistance. Several factors of both host plant and virus may influence the expression resistance traits.

Field resistance means that a plant does not become infected under natural conditions. It can be based on different types of resistance.

Recovery, characterized by the absence of severe symptoms despite a virus infection, is a character typical for the chronic phase of a disease. Recovery seems to be one of the most important forms of natural resistance to virus infection. On the other side, tolerance is characterized by less severe disease symptoms and less damage without reduced virus titer.

Several methods and procedures are known for check and evaluation of virus resistance in plants. They should take in consideration most important properties of the virus, particularly its pathogenicity, the virulence and transmissibility. Marker assisted selection provides a convenient and rapid assay of selection during breeding process, allowing the molecular detection of resistance genes.

*Key words:* virus resistance, types and traits of resistance, review.

## INTRODUCTION

Plant viruses need to overcome many obstacles to induce disease. They must enter wounded cells, uncoat, replicate, express their genes, move from cell to cell within the host, and move long distance through the plant vascular system. The complexity of the infection process, results in resistance when any of one of those steps is prevented (Dawson and Hilf, 1992, Matthews, 1991). Virus resistance is often mediated by plant genes that block specific steps in the viral life circle.

Breeding of productive cultivars of cultivated plant species led to remarkable results during last decades. Resistance to pests and diseases contributed to ever increasing success. Lind et al. (1986) called breeding of resistance "genetic plant protection". Growing resistant cultivars prove to be the superior ecological measure of pest and disease control.

This review summarizes the latest results in research on types and traits, discusses their interactions and describes methods of check and evaluation of virus resistance of plants.

## TYPES OF VIRUS RESISTANCE

Different types of virus resistance of cultivated and wild plant species have been described. One of the most important models was suggested by Cooper and Jones (1983). They proceeded on the assumption that plants can be infected or noninfected. Virus infection means that viral nucleic acid enter the cell and its replication. A plant which can be infected is a host but a plant which cannot be infected is a non-host. A non-host is immune from the distinct virus, but a host can be either susceptible or resistant. Susceptible and resistant hosts, respectively, can be sensitive or tolerant.

## FUSARIUM HEAD BLIGHT OF THE CEREAL CROPS IN RUSSIA

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Fusarium head blight (FHB) is one of the most important fungal diseases affecting cereal crops in Russia. In the study of *Fusarium* species on cereals in Russia 19 species were identified. The variety of *Fusarium* species present in the territory of Russia appears to be completely diverse. *Fusarium graminearum* is a more dangerous pathogen causing head blight on wheat. The structure of biology of *F. graminearum* populations and resistance of cultivars were investigated.

*Key words:* *Fusarium* species, population of parasite, tolerant and resistant cultivars.

### INTRODUCTION

It is known that *Fusarium* species cause considerable damage of cereal. Toxins produced by these fungi have proved to be health hazard to humans and animals. In the last 10-15 years Fusarium head blight (FHB) of cereals has been rather widely spread in Russia. Just in the Krasnodar district (North Caucasus) head blight has spelled three large epidemics. The loss of wheat crop has reached 25-50% and the contamination of cereal grains by mycotoxins increased over 25 times (Levitin et al., 1994a). In 25-80% of wheat samples, the concentration of deoxynivalenol (DON) exceeded the permissible level. During 1989-1992 about 23% of cereal samples (wheat, barley, rye) in Russia were contaminated by DON. Amongst them, 9% of samples contained DON in concentrations exceeding the permissible level. In 0.4% of samples of bread and groat products, the concentrations of mycotoxins cannot meet the hygienic standards (Tuteljan, 1995).

Russia is country large enough to cover 17,075.4 thousands square kilometres. The territory of Russia stretches from the west to the east for about 7,000 km and from the north to the south for over 3,500 km. The country is characterized

**ANTAGONISTIC EFFECT OF SAPROPHYTIC BACTERIA ON  
*MONILINIA* SPP. *IN VITRO***

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**S u m m a r y**

Apart from already existing agrotechnical and chemical control measures of *Monilinia fructigena* Pers., *M. laxa* (Ehrenb.) Sacc. and *M. linhartiana* Prill. et Dell. Growing attention will be paid to biological control measures of these important pathogens of pome- and stone-fruits, in the future. In this investigation 50 strains of saprophytic bacteria deriving from 21 plant species were collected from different localities, nine of which were capable of inhibiting colonial growth *in vitro* of species of genus *Monilinia*.

The most powerful in antagonistic activity were the strains Hr-1 and Fo-9, identified as members of the genus *Bacillus* as well as the strains Fo-6 and Ru-4 determined as members of genus *Pseudomonas*.

Various methods of investigation did not influence the degree of antagonistic effect of the selected strains of the saprophytic bacteria on *M. fructigena*, *M. laxa* and *M. linhartiana in vitro*. There were also no major differences in antagonistic activity of the investigated strains of saprophytic bacteria in various isolates *Monilinia fructigena* and *M. laxa*, deriving from different host. The degree of the antagonistic effect was mostly dependent on the saprophytic bacterium suspension concentration. The strongest antagonistic effect on *Monilinia fructigena* was obtained when using suspension at the concentration of  $10^8$  cfu/ml, while the weakest effect was at the concentration of  $10^1$  cfu/ml.

Investing the morphological, cultural, biochemical and physiological properties of 10 selected strains of saprophytic bacteria, it was found that 3 strains (Hr-1, Fo-9 and Gl-4) belonged to the genus *Bacillus*, 5 strains (Ru-4, Fo-6, Fo-7, Ht-1 and L-69) to the genus *Pseudomonas*, while 2 strains (Gl-3 and PP-17) was a part of an unidentified genus of gramnegative bacteria.

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**PATHOGENIC, BIOCHEMICAL AND PHYSIOLOGICAL  
CHARACTERISTICS OF *XANTHOMONAS CAMPESTRIS* PV.  
*VESICATORIA* STRAINS OF THE PATHOGENS  
OF PEPPER PLANTS IN SERBIA**

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**S u m m a r y**

Numerous bacterial strains were isolated from the diseased pepper plants, showing symptoms of necrotic leaf spot and collected from different pepper growing areas in the Republic of Serbia.

Based on the results of biochemical, physiological and serological (ELISA) tests, investigated strains were identified as *Xanthomonas campestris* pv. *vesicatoria* (Table 1). Since our strains do not hydrolyze starch, they belong to the genetic group "A" of nonamyolytic strains of *X. c.* pv. *vesicatoria*.

The pathogenicity tests showed that the investigated strains are able to infect pepper plants only. Inoculated tomato plants (cv. Walter) reacted hypersensitively. Reaction of pepper differential varieties, (Early Colliender) and their isogenic lines (ECW-10R, ECW-20R, ECW-30R), indicated that our strains belong to pepper races 1 (P1) and 3 (P3) of *X. c.* pv. *vesicatoria* (Table 2).

*Key words:* pepper plants, leaf spot, bacterium, *Xanthomonas campestris* pv. *vesicatoria*, biochemical and physiological properties, (serological properties), pathogenic characteristics; races.

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**EFFECT OF DIFFERENT PREY SPECIES ON LARVAL DEVELOPMENTAL TIME AND FEEDING CAPACITY OF COCCINELLA SEPTEMPUNCTATA L. AND HIPPODAMIA VARIEGATA GOEZE. (COLEOPTERA: COCCINELLIDAE)**

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**S u m m a r y**

Three aphid species *Acyrtosiphon pisum* (Harris), *Aphis craccivora* Koch. and *Brachycaudus helichrysi* Kalt. were used as prey for larvae of *Coccinella septempunctata* L. and *Hippodamia variegata* Goeze. According to the rate of larval development and due to the overall survival, pea aphid, *A. pisum* was the most suitable prey for larvae of both coccinellid species. *Aphis craccivora* was more suitable prey for *H. variegata*, while for *C. septempunctata*, it was acceptable but inadequate food. Larval development time was 21,3 and 16,0 days, and the total rate of survival 56,6% and 90,0% respectively. *B. helichrysi* provides a similar feeding value, and as a prey may be ranged at the same level of suitability for larvae of both coccinellid species. Adults of both coccinellids fed as a larvae on *A. pisum* were larger than those fed on *A. craccivora* and *B. helichrysi*.

*Key Words:* *Coccinella septempunctata*, *Hippodamia variegata*, developmental time, Feeding capacity, prey suitability, *Acyrtosiphon pisum*, *Aphis craccivora*, *Brachycaudus helichrysi*

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