РЕЗЮМЕТА НА НАУЧНИТЕ ПУБЛИКАЦИИ

на доц. дн Иванка Любенова Каменова

във връзка с участие в конкурс за заемане на академичната длъжност "професор" по професионално направление 6.2. "Растителна защита", научна специалност "Растителна защита" (вирусология)

Научни публикации в списания с импакт фактор

Kamenova I. and Adkins S., 2004. Comparison of detection methods for a novel Tobamovirus isolated from Florida hibiscus. *Plant Disease* 88: 34-40.

Abstract. A novel tobamovirus recently was isolated from hibiscus in Florida. Serological and molecular methods, including enzyme-linked immunosorbent assay (ELISA), dot-blot immunoassay (DBIA), tissue-blot immunoassay (TBIA), and immunocapture reverse-transcription polymerase chain reaction (IC-RT-PCR) were compared to evaluate their usefulness for diagnosis of this virus. Each method was tested with partially purified virus preparations and tissue samples from infected hibiscus and *Chenopodium quinoa* plants. Indirect ELISA was more sensitive than double-antibody sandwich (DAS)-ELISA with all samples tested. The Florida hibiscus virus was detectable in hibiscus leaves and bark up to 1:12,800 and 1:6,400 dilutions, respectively, by indirect ELISA and up to 1:3,200 and 1:400 dilutions by DAS-ELISA. End-point dilutions of partially purified virus preparations from indirect and DAS-ELISA were 4 and 31 ng/ml, respectively.
Florida hibiscus virus was detected by DBIA in sap from hibiscus bark and leaves at dilutions up to 1:400 and 1:800, respectively, showing that DBIA was less sensitive than either ELISA method. The virus also was detected reliably by TBIA from leaves and bark of hibiscus plants. The most sensitive method was IC-RT-PCR, which could detect as little as 500 pg/ml of virus in partially purified preparations and was 16- and 32-fold more sensitive than DAS-ELISA with hibiscus bark and leaf extracts, respectively. Over 600 hibiscus samples were tested by various combinations of these methods to validate their usefulness.

Kamenova I. and Adkins S., 2004. Transmission, in planta distribution, and management of *Hibiscus latent Fort Pierce virus*, a novel tobamovirus isolated from Florida hibiscus. *Plant Disease* 88: 674-679.

Abstract. Three aspects of the infection process of a new tobamovirus species, *Hibiscus latent Fort Pierce virus*, recently isolated from hibiscus in Florida, were examined: (i) transmission efficiency of rub-, slash-, and cut-inoculation for two hibiscus cultivars, Pink Versicolor and Brilliant Red; (ii) distribution within infected hibiscus plants; and (iii) treatments to prevent infection during plant propagation and pruning. Rub-, slash-, and cut-inoculation methods were all effective and yielded infection rates of 66, 74, and 70%, respectively, in Pink Versicolor and 50, 56, and 38%, respectively, in Brilliant Red. Analysis of virus distribution in infected plants over time revealed that the virus moved from the place of inoculation to the roots and then toward the bottom (oldest) leaves of the plants. Virus was found in all leaves on branches of Brilliant Red plants at 210 days post inoculation, whereas it remained restricted to the bottom and middle leaves of Pink Versicolor plants at 290 days post inoculation. Although several treatments of tools reduced infection of hibiscus during experiments mimicking plant propagation and pruning, 10% (wt/vol) sodium hypochlorite and 20% (wt/vol) nonfat dry milk completely prevented infection.

Kamenova I., Rosskopf E. N., Lewandowski D. J. and Adkins S., 2004. Characterization of a tobamovirus from tropical soda apple *Phytopathology* 94:S48. Publication no. P- 2004-0325-AMA.

Abstract. Foliar symptoms suggestive of virus infection were recently observed on the noxious weed, tropical soda apple (*Solanum viarum*), in Florida. An agent was mechanically transmitted to *Nicotiana benthamiana* and virions were isolated from systemically infected leaves. Rod-shaped particles ~300 nm in length were observed in the purified preparations by electron microscopy. The host range determined by mechanical inoculation with purified virions included predominantly plants from the *Solanaceae*. Purified virions reacted both with IgG specific for *Tobacco mosaic virus* and also with IgG that recognizes TMV and related tobamoviruses (including *Tomato mosaic virus*) in indirect enzyme-linked immunosorbent assay. Sequence comparisons of reverse transcription-polymerase chain reaction products amplified from total
RNA of infected *N. benthamiana* using primers specific for the coat protein (CP) of *Pepper mild mottle virus* (PMMoV) indicated that the CP sequence of the isolated virus was most closely related to PMMoV, with CP nucleotide and amino acid identities of 80 and 83%, respectively.

Kamenova, I., Tsvetkov I. and Atanassov A., 2007. Virus testing of certified grapevine planting material in Bulgaria. *Biotechnology and Biotechnological Equipment* 21, (1): 66-68.

Abstract. In accordance with the European Legislation and the recently developed national system for production of certified ed vine growing material, 1295 samples from grapevine varieties and rootstocks were checked for their virus status in the period of 2004- 2006. The results obtained showed that total of 30.50% of the ELISA tested *Vitis vinifera* vines and rootstocks were infected by one (86.08%) or more viruses (13.92). The most widespread virus was *Grapevine flack virus* (GFkV) (23.3%), followed by *Grapevine fanleaf virus* (GFLV) (5.05%) and *Grapevine leafroll associated viruses* 1 and 3 (GLRaV 1 and 3) (3.34% and 2.78%), respectively. *Grapevine Bulgarian latent virus* (GBLV) (0.37%) was scarcely represented, while *Arabis mosaic virus* (ArMV), *Grapevine leafroll associated vir*uses 2 and 7 (GLRaV 2 and 7), *Grapevine virus A* (GVA) and *Grapevine virus B* (GVB) were completely absent. The mixed infection showed eight virus combinations. The associations of GFkV + GLRaV 1 (8.3%) and GFLV + GLRaV 3 (8.3%) were the most widespread ones. All tested for ArMV, GFLV and GBLV wild vines (*Vitis vinifer*a ssp. *sylvestris*) were virus free.

Adkins S., Kamenova I., Rosskopf E. N. and Lewandowski D. J., 2007. Identification and characterization of a novel tobamovirus from tropical soda apple in Florida. *Plant Disease* 91: 287-293.

Abstract. Foliar symptoms suggestive of virus infection were recently observed on the noxious weed tropical soda apple (*Solanum viarum*) in Florida. An agent was mechanically transmitted to *Nicotiana benthamiana*, and virions were isolated from systemically infected leaves. Rodshaped particles ~300 nm in length were observed in the partially purified preparations by electron microscopy. The host range determined by mechanical inoculation with purified virions included all tested plants in the *Solanaceae* (16 species including the important vegetable crops, pepper and tomato) and *Chenopodiaceae* (2 species) but excluded all tested plants in the *Amaranthaceae*, *Apocynaceae*, *Brassicaceae*, *Caryophyllaceae*, *Cucurbitaceae*, *Fabaceae*, *Lamiaceae*, *Malvaceae*, and *Tropaeolaceae*, including several common virus indicator hosts. Comparisons of the coat and movement protein nucleotide and deduced amino acid sequences of this putative tobamovirus with recognized members of this genus indicate that it is a novel tobamovirus that shares the highest level of sequence identity with *Pepper mild mottle virus* followed by other members of the *Solanaceae*-infecting subgroup of tobamoviruses. The virus, for which the name *Tropical soda apple mosaic virus* (TSAMV) is proposed, was found to be widespread in tropical soda apple in peninsular Florida during an initial survey. TSAMV contamination of seed from infected tropical soda apple plants was found, suggesting that seed transmission may be important
for TSAMV dissemination and epidemiology.

Baker, C. A., Kamenova I., Raid R. and Adkins S., 2007. *Bidens mottle virus* identified in Tropical Soda Apple in Florida. *Plant Disease* 91 (7): 905.

Abstract. Tropical soda apple (TSA) (*Solanum viarum* Dunal), a plant native to South America, was first identified in Florida in 1988. It rapidly became a noxious weed in pastures throughout the state and it is known to be a reservoir for *Cucumber mosaic virus, Potato leafroll virus, Potato virus Y* (PVY), *Tobacco etch virus* (TEV), *Tomato mosaic virus,* and *Tomato mottle virus,* viruses that infect important vegetable crops in Florida (3). During a routine survey of Florida weeds during May of 2004, a TSA plant with chlorotic, young leaves found near Okeechobee, FL was determined to be infected with a potyvirus by using a commercially available enzyme linked immunosorbent assay kit (Agdia, Elkhart, IN). The results of a host range study indicated this potyvirus was neither PVY nor TEV. The virus caused local lesions in *Chenopodium amaranticolor* and systemic symptoms in *C quinoa, Coreopsis* sp. (C. A. Baker, *unpublished*), *Helianthus annus, Nicotiana benthamiana, Petunia × hybrida, Verbena hybrida,* and *Zinnia elegans*. It did not infect *Gomphrena globosa, N. glutinosa, Pisum sativum,* or *Phaseolus vulgaris* (1). Cylindrical inclusions consistent with those observed in plants infected with *Bidens mottle virus* (BiMoV) were observed in *Z. elegans*. Immunodiffusion tests with antiserum to BiMoV (Department of Plant Pathology, University of Florida) gave a reaction of identity with leaf extracts of the symptomatic zinnia, a known sample of BiMoV originally isolated from *Bidens pilosa* and a recent isolate of BiMoV from lettuce in Belle Glade, FL (C. A. Baker and R. Raid, *unpublished*). A partial polyprotein gene fragment (GenBank Accession No. EF467235) was amplified from total RNA of an inoculated *C. quinoa* plant by reverse transcription (RT)-PCR with previously described degenerate potyvirus primers. Analysis of the RT-PCR product sequence confirmed the host range results and indicated that the potyvirus infecting TSA was neither PVY nor TEV. However, the nucleotide and deduced amino acid sequences of a 247-bp portion of the RT-PCR product were 94 and 98% identical, respectively, with the coat protein sequence (GenBank Accession No. AF538686) of Sunflower chlorotic spot virus (SCSV). SCSV is a tentative potyvirus species described from Taiwan that is not yet recognized as an accepted species by the International Committee on Taxonomy of Viruses. On the basis of our concurrent host range, inclusion body, and serological data, it is likely that SCSV is in actuality the previously described and currently accepted potyvirus species BiMoV, for which no previous sequence data existed. As part of a comprehensive viral disease management plan, it is recommended that TSA plants growing in and around lettuce-production areas be controlled along with other weed hosts of this virus.

Kamenova I., Batchvarova R., Flasinski S., Dimitrova L., Christova P., Slavov S., Atanassov A., Kalushkov P. and Kaniewski W., 2008. Transgenic resistance of Bulgarian potato cultivars to the Colorado potato beetle based on Bt technology. *Agronomy for Sustainable Development,* 28 (4): 481-488.

Abstract. Colorado potato beetle, *Leptinotarsa decemlineata* Say, is the most destructive insect pest of potatoes. When the population of beetles is high, plants can be completely defoliated and commercial potato production is nearly impossible without control of the beetle. The beetles have shown a tremendous ability to develop resistance against insecticides. Previously, a biotechnology approach to control Colarado potato beetle based on the use of the synthetic Bt gene was developed. In this article, a transformation procedure for three commercial Bulgarian potato cultivars was developed and potentially commercial transgenic lines have been selected based on field resistance to Colorado potato beetles and yield. Plants were transformed with the *Bacillus thuringiensis* (Bt) *cry*3A gene using *Agrobacterium*-mediated transformation. 110 plants from the three cultivars were regenerated and tested by double antibody sandwich enzyme-linked immunosorbent assay (DASELISA). The Cry3A protein accumulation varied across the transgenic lines, rating from very low to 71.5 μg/g fresh weight. 21 transgenic lines expressing the Cry3A protein at levels above 10 μg/g fresh weight were tested in two successive years in filed conditions

at two different locations of the country. All transgenic lines compared with the controls, non-transgenic potatoes from the respective cultivar, were consistently protected from foliar damages from all developmental stages of the beetle. The comparison of all properties of the tested transgenic lines, including variety phenotypes and tuber yield, allowed the selection of the most promising 2–3 lines per cultivar. Selected lines produced tuber amounts 80–100% higher compared with the control, non-transgenic plants. Those lines were grown for mass propagation during the third year of field experiments. The presence of the transgene in these lines was confirmed with the use of primers specific to the transgene by polymerase chain reaction (PCR). Additionally, the results from the insect bioassay showed that these lines were highly resistant to insect feeding, leading to 100% of mortality of larval populations. In summary, we generated potentially commercial potato lines highly resistant to Colorado potato beetle using Bt technology that may have a profound impact on development of sustainable agriculture in Bulgaria. This is one of the several agriculture biotechnology products entirely developed and tested in Bulgaria.

Kamenova I., Dallot S., Bozkova V. and Milusheva S., 2011. First report of the *Plum pox virus* recombinant strain on peach in Bulgaria. *Plant Disease,* 95 (10): 1320.

Abstract. *Plum pox virus* (PPV) causes sharka, the most damaging viral disease of stone fruit species. Seven distinct PPV strains are known; PPV-M, PPV-D, and PPV-Rec are the most common (3). PPV-Rec is a unique recombinant (3) between PPV-M and PPV-D and has been reported from plum, apricot, Japanese plum, myrobalan, and blackthorn in eastern and central Europe, but has never been found in peach as a single natural infection (2). A survey was conducted during spring 2009 in eight peach orchards located in the southwest, southeast, and south central regions of Bulgaria to assess the incidence of PPV infection. A total of 98 leaf samples from individual trees showing PPV-like symptoms were collected and analyzed by triple-antibody sandwich (TAS)-ELISA with the universal monoclonal antibody (MAb) 5B (Agritest, Valenzano, Italy). Sixty one samples reacted positive for PPV (optical density 0.161 to 1.267) and these samples were further analyzed with PPV-M (AL) and PPV-D (4DG5) specific MAbs (1). All 61 samples reacted positively with PPV-M specific MAbs. To distinguish PPV-M and PPV-Rec strains, which are serologically identical, immunocapture (IC)-reverse transcription (RT)-PCR was carried out with PPV-M (CIP-M: 5′-GTC GCA GCA TTT GTA GCC CTT GTT-3′, CIP-MR: 5′-CCA ACA CGT TAA CGC CAT GCT TCA-3′) and PPV-D (CIP-D: 5′-ATG ATG CTG TTT GAC TCG GAG CGA-3′, CIP-DR: 5′-TCG CAA CTG CTT GCA CAC ATT CTC-3′) specific primers targeting the 6K1-CI genomic region. A PCR fragment of ~880-bp amplified with PPV-M specific primers obtained from 59 samples confirmed that these were PPV-M isolates. However, the remaining two samples (both coming from infected tress located in two different orchards in the southwest region) yielded a 468-bp PCR fragment with PPV-D specific primers, suggesting that these two samples belonged to PPV-Rec strain. These samples together with controls of PPV-M, PPV-D, and PPV-Rec strains were further analyzed by RTPCR using mD5/mM3 primers spanning the recombination breakpoint (4). Both peach samples and the PPV-Rec strain control produced a single 605-bp PCR product. The two peach amplicons were purified and sequenced directly with the same primers. The nucleotide (nt) sequences obtained were 100% identical to each other. BLAST analysis of the two samples with PPV-Rec (No. AF421118.1) showed maximum nt identity of 98%. Percent maximum nt identity with PPV-M (No. AY324837.1) and PPV-D (No. AB576062.1) were 93 and 87%, respectively. The deduced amino acid sequences of the two isolates were 98% identical to PPV-Rec (No. No. AF421118.1), 93% identical to PPV-M (No. M92280.1), and 84% identical to PPV-D (No. AB576062.1). Analyzed samples were further transmitted from the diseased trees to peach seedlings (GF 305) by chip-budding in a greenhouse during the fall of 2009. Six months later, faint vein clearing on the leaves of inoculated seedlings was observed. The presence of PPV was confirmed by TAS-ELISA and PPV-Rec presence was shown by IC-RT-PCR (mD5/mM3 primers). One of the generated 605-bp products was sequenced and showed 100% nt identity with the isolate used for inoculation. To our knowledge, this is the first identification of PPV-Rec strain in naturally infected peach trees, a finding that calls for further large-scale investigations of PPV-Rec incidence in peach in Bulgaria.

Vidal E., Zagrai L., Milusheva S., Bozhkova V., Tasheva-Terzieva E., Kamenova I., Zagrai I. and Cambra M., 2013. Horticultural mineral oil treatments in nurseries during aphid flights reduce *Plum pox virus* incidence under different ecological conditions. *Annals of Applied Biology,* 162: 299-308.

Abstract. The application of horticultural mineral oil (HMO) treatments has been reported as a possible control strategy to reduce *Plum pox virus* (PPV) incidence in *Prunus* nurseries. The effect of Sunspray Ultrafine HMO at 1% on the natural viral spread was evaluated in experimental nursery plots of Nemaguard and Mariana GF8-1 *Prunus* rootstock blocks established under high natural inoculum pressure of the most prevalent PPV-types. Tests were conducted in experimental nursery plots in Plovdiv, Bulgaria (PPV-M and PPV-Rec), in Bistrita, Romania (PPV-D and PPV-Rec) and in Llıria, Spain (PPV-D). Horticultural mineral oil treatments were applied weekly during the vegetative period from spring to fall (treatments were interrupted in the summer). Nursery plants were analyzed yearly by double-antibody sandwich enzyme linked immunosorbent assay with 5B-IVIA/AMR monoclonal antibodies. The population dynamics of the aphids visiting plants in each experimental nursery plot was monitored by the sticky-shoot method and also by Moericke yellow water traps. At all three locations, the aphid population first peaked in the springtime. Furthermore, a variable second peak of aphid population was observed in Plovdiv and Bistrita in autumn. The treatments reduced PPV incidence in the three experimental locations and plots and in both assayed *Prunus* rootstocks grown under high PPV-inoculums pressure. A reduction from 10% to 20% of PPV-incidence between treated and control plants ($P<0.05$) in Plovdiv and Bistrita, respectively, was observed at the end of the tests. However, HMO treatments did not prevent PPV infection altogether, probably because of the high PPV prevalence in the area near the experimental nursery blocks. The control of PPV in nursery blocks based on HMO is presented as an environmentally friendly strategy based on the physical action of the treatments.

Vidal E., Zagrai L., Milusheva S., Bozhkova V. Tasheva-Terzieva E., Kamenova I., Zagrai I. and Cambra M., 2013. Effect of mineral oil treatments in reducing *Plum pox virus* prevalence and spread. *Canadian Journal of Plant Pathology* 35 (1): 138.

Abstract. The application of horticultural mineral oil (HMO) treatments, as a physical barrier, has been reported to reduce *Plum pox virus* (PPV) incidence in *Prunus* nurseries. In the frame of SharCo EU project (FP7/2007-Agreement 204429) the effect of Sunspray Ultrafine at 1% (HMO) on the natural viral spread, as a unique phytosanitary treatment, was evaluated in three different European ecological areas. Experimental nursery plots of Nemaguard and Mariana GF8-1 *Prunus* rootstock blocks were established in the immediate vicinity of high natural PPV inoculums pressure in Plovdiv (Bulgaria)/PPV-M and - Rec, Bistrita (Romania)/PPV-D and -Rec and in Llíria (Spain)/PPV-D. Aphid species visiting plants were monitored and the maximum peak of aphid population at the three locations occurred at springtime. HMO treatments were weekly sprayed during the vegetative period from springtime to fall (in summertime the treatments were interrupted). Nursery plants were yearly analyzed by Magic DAS-ELISA based on 5B-IVIA monoclonal antibodies (Plant Print Diagnostics) for two consecutive years. The treatments significantly reduced ($p < 0.05$) PPV incidence in the three experimental locations and in both assayed rootstocks, although did not avoid PPV infection. A more accurate control of PPV could be afforded in areas with low or occasional PPV outbreaks, in both nurseries and young plantations, through this environmentally friend strategy.

Scorza R., Callahan A., Dardick C., Ravelonandro M., Polak J., Malinowski T., Zagrai I., Cambra M. and Kamenova I., 2013. Genetic engineering of *Plum pox virus* resistance: 'HoneySweet' plum-from concept to product. *Plant Cell, Tissue and Organ Culture*, 115: 1-12.

Abstract. Sharka disease, caused by *Plum pox* virus (PPV) was first recorded in Bulgaria during the early twentieth century and since that first report, the disease has progressively spread throughout Europe and more recently to Asia, Africa, North and South America. Few PPV resistance genes have been found to naturally occur in *Prunus* and this has led to the investigation of biotech approaches to the development of resistance through genetic engineering (GE). A

notable example of the utility of this approach is 'HoneySweet' plum. PPV protection in this case is based on RNA interference (RNAi) and resistance has been shown to be highly effective, stable durable, and heritable as a dominant trait. Extensive testing and risk assessment of 'HoneySweet' in laboratory, greenhouse and in the field for over 20 years has demonstrated not only the effectiveness but also the safety of the technology. 'HoneySweet' has been cleared for cultivation in the USA. By the appropriate regulatory agencies. The development and regulatory approval of 'HoneySweet' demonstrate the ability of RNAi technology to contribute to the sustainability of stone fruit production in regions impacted by PPV. Although it has taken almost 100 years since the identification of sharka, we are now able to effectively protect stone fruit species against this disease through the application of GE.

Kamenova I., Mavrodieva V., Levy L., Milusheva S., Dragoiski K., Borisova A. and Stefanova B., 2013. *Plum Pox Virus* survey of sweet and sour cherry in Bulgaria. *Bulgarian Journal of Agricultural Science*, 19 (4): 732-736.

Abstract. *Plum pox virus* (PPV), the causal agent of Sharka disease affecting the major stone fruit species is endemic in Bulgaria. To investigate PPV incidence level and distribution on sweet cherry (*Prunus avium*) and sour cherry (*P. cerasus*) 28 commercial and abandoned orchards were surveyed in 2009 and 2010, as well as some residential and wild cherries. A total of 1141 samples from individual tree were collected and tested using a commercial DAS-ELISA detection system. Some samples were subjected to additional testing by RT-PCR using PPV-specific primers. A commercial sweet cherry sample showing PPV-like symptoms, e.g. chlorotic rings and spots on the leaves and fruits was bud-grafted on GF305 hybrid peach woody indicator seedlings and tested by RT-PCR. PPV was not detected in any samples using serological and/or molecular based tests. Nevertheless, periodic systemic surveys should be conducted to evaluate PPV disease status of sweet and sour cherries in Bulgaria.

Kamenova I., 2014. A recombinant strain of *Plum pox virus in peach in* Bulgaria. *Journal of Plant Pathology*, 96 (2): 411-414.

Abstract. Of 88 leaf samples collected from three peach orchards in south-west Bulgaria and tested for *Plum pox virus* (PPV) by TAS-ELISA, 32 (36.4%) were positive for PPV-M. When these samples were tested by IC-RT-PCR targeting the (Cter)NIb-(Nter)CP genomic region using PPV-M and PPV-Rec strain-specific primers the presence of the M strain was confirmed in 16 of them (50%), whereas the Rec strain was detected in 15 samples (47%). Mixed infection of both strains was found in a single sample. The nucleotide sequences of the (Cter)NIb-(Nter)CP genomic fragment of 13 PPV isolates showed a 98-100% identity with PPV-Rec isolates and 97-98% identity with the reference isolates. The maximum nucleotide identity of PPV-Rec isolates with PPV-M and PPV-D isolates was 92-93% and 84-85%, respectively. These results show that PPV-Rec is widely distributed in peach orchards in southwestern Bulgaria.

Публикации в международни списания без импакт фактор

Allen J. E., Kamenova I., Adkins S. and Hanson F. S., 2005. First report of *Hibiscus latent Fort Pierce virus* in New Mexico. *Plant Health Progress* 10. Online: *Plant Health Progress*, doi:10.1094/PHP-2005-0105-01-HN.

Abstract. *Hibiscus* spp. are common landscape and potted ornamental plants throughout the southern United States. Two new tobamovirus species have recently been isolated from *Hibiscus rosa-sinensis* plants with diffuse chlorotic spots and rings and an overall chlorotic mottle. One of these viruses was first identified in Florida, and it was named *Hibiscus latent Fort Pierce virus* (HLFPV) to reflect the location and host from which it was isolated. The other virus was first identified in Singapore and was named *Hibiscus latent Singapore virus* (HLSV). During the summer of 2003, foliar symptoms including chlorotic spots and chlorotic mottling were observed on *H. rosasinensis* and *H. syriacus* plants in and around Las Cruces, NM. Fifty *Hibiscus* spp. plants

including indoor potted plants, landscape plants, and local nursery stock were sampled from eight different locations, including three local nurseries. Twenty-eight of the 50 samples had virus-like symptoms, whereas the remaining samples were from randomly selected asymptomatic plants. Twenty-three of the 28 symptomatic plants had mild symptoms, including chlorotic spots, which were consistent with previous reports of HLFPV, whereas five of the plants had more severe symptoms such as leaf distortion, stunted growth, and a lack of flowering. Initial testing for HLFPV was by tissue blot immunoassay (TBIA) using IgG prepared to HLFPV virions as previously described. No cross reaction of this IgG with HLSV infected hibiscus has previously been observed in double antibody sandwich enzyme-linked immunosorbent assays. TBIA identified 16 HLFPV-infected samples, all of which came from plants with virus-like symptoms. HLFPV was not detected by TBIA in any of the 22 asymptomatic plants. Electron microscopic analysis of leaf dips from symptomatic leaves revealed rigid, rod shaped particles with dimensions of ~15 nm in width and ~250 to 300 nm in length, consistent with tobamovirus virions and supporting the LFPV diagnosis by TBIA. Similar particles were not observed in leaf dips from asymptomatic leaves.
The presence of HLFPV was confirmed by amplification of the capsid protein gene by immunocapture reverse-transcription polymerase chain reaction (ICRT- PCR) using previously described methods and primers specific for HLFPV. The expected size product (535 bp) was amplified from a TBIA-positive, symptomatic *H. syriacus* sample. Sequence analysis of the ICRT-PCR DNA fragment revealed 100% nucleotide identity with the corresponding portion of the HLFPV capsid protein gene. This finding supports the identification of HLFPV in New Mexico, and distinguishes it from HLSV, which only shares 68% nucleotide identity with the HLFPV capsid protein gene. No DNA fragments were amplified by IC-RT-PCR from uninfected *Nicotiana tabacum*, TBIA negative *H. rosa-sinensis* and *H. syriacus* leaves, or HLSV-infected *H. rosa-sinensis* leaves.

Kamenova I., Adkins S. and Achor D., 2006. Identification of *Tomato mosaic virus* infection in Jasmine. *Acta Horticulture*, 277: 277-283.

Abstract. Virus-like symptoms were recently observed on leaves of landscape and nursery downy and star jasmine *(Jasminum multijlorum)* and wax jasmine *(J. gracile)* in southeast Florida. Foliar symptoms included mottling, chlorotic ring spots and chlorotic line patterns. An agent was mechanically transmitted with difficulty from symptomatic leaves of downy jasmine to *Nicotiana debneyi* and *N. tabacum* 'Xanthi' ·and subsequently from these hosts to *Chenopodium quinoa* and other herbaceous test plants. Virions were isolated from *N. tabacum* 'Xanthi.' Rod-shaped particles (297 x 18 nm) similar to tobamoviruses were observed in partially purified virus preparations, and in leaf dips from symptomatic star jasmine and indicator plants. Extraction of viral-associated double-stranded (ds) RNA revealed a profile consistent with that of a tobamovirus. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis showed that the virus contained one polypeptide with an approximate molecular weight (Mr) of 18 kDa. The virus reacted specifically with IgG for *Tobacco mosaic virus* (TMV) and related tobamoviruses [including *Tomato mosaic virus* (ToMV)] in double antibody sandwich, enzyme-linked immunosorbent assay. No reaction was observed with TMV specific IgG. Reverse transcription - polymerase chain reaction with total RNA isolated from symptomatic jasmine leaves and infected *N. tabacum* 'Xanthi' using ToMV coat protein specific primers amplified the expected product from downy and star (but not wax) jasmine and *N. tabacum* 'Xanthi.' The nucleotide and amino acid sequence of the products were 100% identical to the corresponding fragment of a Brazilian isolate of ToMV from *Impatiens.* To our knowledge, this the first report of ToMV in jasmine in the USA.

Adkins S., Kamenova I., Chiemsombat P., Baker C. and Lewandowski D., 2006. T*obamoviruses* from hibiscus in Florida and beyond. *Acta Horticulture*, 277: 65-70

Abstract. Malvaceous plants have not been known as hosts for any of the recognized tobamovirus species until quite recently. Three sub-groups of tobamoviruses have been described that infect solanaceous plants, brassicas, and cucurbits or legumes. We recently isolated a new tobamovirus species, *Hibiscus latent Fort Pierce virus (HLFPV) from landscape plantings of the malvaceous*

plant hibiscus (Hibiscus rosa-sinensis) in Florida, and a related hibiscus-infecting tobamovirus, *Hibiscus latent Singapore virus (HLSV*), has been reported from *Singapore*. The experimental host range of *HLFPV* is mostly limited to the *Malvaceae,* which includes fiber and food crops such as cotton, kenaf and okra, in addition to economically important ornamental crops like hibiscus. Serological and molecular methods were compared to evaluate their usefulness for diagnosis of HLFPV. An initial survey by enzyme-linked immunosorbent assay for IILFPV in landscape hibiscus plants in Florida has shown a high level (56%) of incidence. Subsequent analysis of related malvaceous species has identified HLFPV infection of Turk's cap *(Ma/va viscus arhoreus),* rose of Sharon *(H. syriacus),* scarlet rosemallow *(H. coccineus)* and common rosemallow *(H. moscizeutos).* A similar virus has been detected in *H. rosa-sinensis* in Thailand by tissue-blot immunoassay. Cloning of the coat protein gene of Thai virus isolates and subsequent analysis showed the nucleotide and amino acid sequences to be nearly identical to HLFPV. Dixie rosemallow *(H. mutahi/is)* and *H. rosa-sinensis* plants have been found in Florida infected with tobamoviruses that are serologically distinct from HLFPV but related to HLSV. Cloning and sequencing of the HI.FPV genome indicates that it is distinct from but related to HLS%. However, the coat protein genes and deduced proteins of HLFPV and HLSV are only 37-53% identical to all other tobamovirus species, suggesting the existence of a malvaceous-infecting subgroup of tobamoviruses.

Borisova A.; Borovinova M. and Kamenova I., 2014. Major diseases of apple trees in Kyustendil region of Bulgaria. *Türk Tarım ve Doğa Bilimleri,* 2014, 6: 695-700.

Abstract. Surveys were done in different apple orchards at the Institute of Agriculture –Kyustendil and private orchards and nurseries in Kyustendil region of Bulgaria between 2004 and 2013. Generally accepted methods in plant pathology were used. Fungal diseases such as scab (Venturia inaequalis) and powdery mildew (Podosphaera leucotricha) are the most important diseases of apple in Kyustendil region. Crop losses mainly depend on the frequency of infection periods of V. inaequalis and cultivar susceptibility. Damages by black rot (Botryosphaeria obtusa) of trunks and branches are problem at biological growing system and apple orchards with cut-wounded, cold, hail or insect–injured apple trees. Brown rot (Monilinia fructigena) and blue mold (Penicillium spp) radically infect apple fruits damaged by Cydia pomonella. Bacterial disease fire blight (Erwinia amylovora) occurs in some years, depending on certain abiotic and biotic factors in region and caused damages. The most common virus infected apples in Kyustendil region is Apple chlorotic leaf spot virus (ACLSV), followed by Apple stem grooving virus (ASGV). Apple mosaic virus (ApMV) has not been detected up to now in the species Malus domestica in the region. The phytoplasma 'Candidatus Phytoplasma mali' the causal agent of Apple proliferation disease (AP) was identified, too.

Kamenova I., Borissova A., Dragoyski K., Stefanova, B. Milusheva S., Dallot S. and Glasa M., 2015. *Plum pox virus* strains in Bulgaria. *Acta Horticulture,* 1063: 47-54.

Abstract. Sharka or plum pox has a long presence in Bulgaria. In this study, 790 plum, peach and apricot samples were analyzed by TAS-ELISA. 465 samples (58.9%) reacted positive for PPV. The highest level of PPV infection was detected in the plum orchards (86.06%), followed by the peach (46.5%) orchards/single grown trees and in the apricot (32.05%) orchards/single grown trees. Molecular typing performed by strain-specific IC-RT-PCR analyses showed that the most prevalent strain was PPVM (46.7%), followed by PPV-Rec (43.9%) and PPV-D (7.9%). The rate of mixed infection was 1.5%. Distinct epidemiological situations depending on the stone-fruit species were evidenced. On plum, PPV-Rec was the most prevalent strain (69.1%), followed by PPV-M (20.1%) and PPV-D (8.3%). The rate of mixed infections was 2.5%. On the contrary to the plums, peaches and apricots were essentially infected by PPV-M (89.2 and 80.6 %, respectively). On apricot, PPV-Rec was detected more frequently than PPV-D (11.9 and 7.5% respectively), whereas on peach the opposite pattern was found (7.5 and 3.3% for PPV-D and PPV-Rec, respectively). To assess the genetic diversity, 27 isolates were partially characterized by direct sequencing of the PCR products spanning the (Cter)NIb-(Nter)CP and the (Cter)P3-6K1-(Nter)CI coding regions. Phylogenetic analysis of these isolates confirmed their RT-PCR based strain typing. Bulgarian PPV-Rec isolates clustered with PPV-Rec isolates retrieved from the NCBI database in both analyzed regions.

Phylogenetic analyses of PPV-M isolates based on the NIb-CP genomic region showed that they fell into the Ma and Mb clusters, while on the base of the P3-6K1-CI genomic region, part of the isolates formed a third sub-cluster more related to Mb, than to Ma isolates. Regardless of the analyzed region, the analyzed PPV-D isolates grouped together with D strain isolates from Europe, Canada and USA.

Milusheva S., Bozhkova V. and Kamenova I., 2015. Results from survey on resistance of plum cultivar 'Jojo' to *Plum pox virus. Acta Horticulture*, 1063: 93-97.

Abstract. The aim of this study was to evaluate the reaction of plum cultivar 'Jojo' to plum pox virus (PPV) infection by combination of biological indexing with PPV-M. –D and –Rec strain isolates and field observations in Bulgarian commercial orchards, planted with 'Jojo', under the agro-biological conditions of three locations of the Plovdiv region. Development of visual symptoms on the field-grown trees' leaves and fruits was monitored during three successive vegetative cycles (2010-2012). PPV presence in plant tissues was detected by enzyme-linked immunosorbent assay (ELISA) and immuno capture–reverse transcription-polymerase chain reaction (IC-RT-PCR). The biological indexing experiment showed that initially small chlorotic spots developed on the leaves of the inoculated plants that turned brownish (hypersensitive reaction) later on, followed by falling of the necrotic tissues. In the field survey, similar symptoms of hypersensitivity were observed on the leaves of some 'Jojo' trees. Leaf samples from plants that manifested hypersensitive reaction (in inoculated plants and field growing trees) reacted negative with antiserums against PPV in ELISA and no amplification was obtained in IC-RT-PCR with the primer pair P1/P2 used. Several of the trees grown in the field showed some virus-like leaf symptoms, during the third vegetative period. Samples collected from these trees reacted negative for PPV in ELISA and none of the samples tested by IC-RT-PCR produced PPV-specific PCR products. However, the symptomatic trees reacted positive with antiserum against *Prunus necrotic ring spot virus* (PNRSV). No symptoms on fruits of the inspected 'Lojo' trees were recorded. The results obtained suggest that 'Jojo' cultivar could be resistant to PPV infection, both in the experimental biological indexing and under the agro-biological conditions of Plovdiv region of Bulgaria.

Публикации в български сборници

Kamenova I., 2012. Genetic variability of *Plum pox virus* - D strain in Bulgaria. *Third Congress of Virology,* 25-27 October, 2012, Sofia, Bulgaria, 90-97. Book of procedings: 90-97.

Abstract. *Plum pox virus* (PPV), the causal agent of sharka disease is an important pathogen on *Prunus* species as plum, peach and apricot in Bulgaria. The presently known PPV isolates are assigned to seven biologically, serologically and molecularly different types of strains, from which PPV-M, PPV-D and PPV-Rec strains are detected in the country.

The aim of this study was to asses the genetic variability of 14 isolates collected from different *Prunus* species and fruit-growing regions, serologically and molecularly defined as PPV-D strain. Immunocaptured reverse-transcription-polymerase chain reaction (IC-RT-PCR) analyses were performed with P3D/P4 and PCI/PP3 pair of primers targeting PPV most variable regions as (Cter)-NIb-(Nter)CP and (Cter)P3-6K1-(Nter)CI, respectively. The obtained PCR products were sequenced directly and phylogeneticaly analyzed with several PPV-D isolates retrieved from the GenBank. Construction of phylogenetic trees (Kimura 2-model) and the estimation of average nucleotide divergence values were performed by MEGA4 programme.

Phylogenetic analyses of Bulgarian PPV-D strains in both regions showed that they formed a group separated from the used as reference PPV-D isolates with origin from USA, Europe, Canada and Japan. The only reference isolate that clustered with the Bulgarian PPV-D isolate was an isolate from Slovakia. In CP region the analyzed PPV-D strains split in 4 clusters connected with their geographical origin and the observed intra group diversity (0.037±0.005) was more than three times higher than the reported by other authors.

Phylogenetic analyses of (Cter)P3-6K1-(Nter)CI genomic region showed that the three analysed peach isolates clustered together and appear to be closely related, while the two analysed isolates from apricot clustered together with the isolates from plum. The mean nucleotide

divergence among Bulgarian PPV-D isolates was relatively low (0.017±0.003) and only slightly higher from the reported one.

In this study the knowledge about genetic variability of PPV-D strain spread in Bulgaria was broadened.

Публикации в международни сборници

Rizov I., Vlahova M., Kamenova I. and Atanassov A., 2003. Near infrared spectroscopic study of tobacco plant engineered with *Potato virus Y* CP DNA. Proceedings of 11th International Conference on Near Infrared Spectrocopy. Edited by A.M.C., Davied and A. Garrido-Varo.

Abstract. The NIR transflectance spectroscopy was employed for classification of transformed and non-transformed tobacco plants with coat protein (CP) of *Potato virus* Y (PVY). The results obtained from the comparative study of NIR transflectance spectra of fresh, ground with silica gel and lyophilized tobacco leaves could be assumed as evidence that the water structured in a biological matrix made the difference on DNA level detectable for NIR.

Публикации в български научни издания

Янкулова М., Цветков И., Каменова И., Кондакова В., Атанасов А. и Вълчев В., 2007. Вирусни болести по лозата у нас. *Лозарство*, №1, 19 - 24.

Abstract. Progress in monitoring of grapevine virus diseases in Bulgaria during the period 1999-2003 was presented. Totally, 1072 samples from two years previously breeding selected 86 grapevine varieties, 13 rootstocks and 20 hybrid forms from different vineyards in Bulgaria were analyzed for 8 economically important viruses: GFLV (Grapevine Fanleaf Virus), GBLV (Grapevine Bulgarian latent virus), ArMV (Arabis Mosaic Virus) AILV (Artichoke Italian latent virus), GRRaV 1 and 3 (Grapevine leafroll associated viruses 1 and 3, GFkV (Grapevine Flack Virus), and GVA (Grapevine Virus A). The results showed that total of 73.18% of the ELISA tested *Vitis vinifera* vines and rootstocks were infected by one or more viruses. The most wide-spread virus was *Grapevine flack virus* (GFkV) (18.00%), followed by *Grapevine leafroll associated viruses* 1 and 3 (14.41%) and *Grapevine fanleaf virus (GFLV)* (11.69%). There are not detected ArMV and AILV. Virus free varieties and rootstocks were included in AgroBioInstitute pre-basic genebank after 5 in vitro propagation passages.

Borisova A., Kamenova I. and Sotirov D., 2013. Sanitary status of rootstocks for sweet and sour cherry. *Journal of Mountain Agriculture on the Balkans,* 16 (1): 151-163.

Abstract. The aim of this research was to study the sanitary status of the widely used in the practice rootstocks for sweet and sour cherry. In total 109 trees from 22 different genotypes of mahaleb, wild cherry and sour cherry from the collection and mother gardens of Institute of

Agriculture, Kyustendil were tested by DAS-ELISA for infection with *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Plum pox virus* (PPV), *Cherry leaf roll virus* (CLRV) и *Raspberry ringspot virus* (RpRSV) in the spring of 2012.

The overall percentage of viral infection was 6.4%. PNRSV was identified in 2.75% of tested samples. The same rate of infection was established for PDV. Mixed infection of PNRSV and PDV was found in only one tree. All infected trees were timely eradicated. No infection of CLRV, PPV and RpRSV in tested rootstocks was detected.

Borisova, A., Kamenova I. and Borovinova M., 2013. Viral and fungal diseases of stine fruit species in Kyustendil region. *Journal of Mountain Agriculture on* the Balkans, 16 (1): 164-182.

Abstract. This article is presented the results obtained over a long period of study on the agents of viral and fungal diseases of stone fruit species growing in Kyustendil region.

The investigation was carried out during the period 2000 – 2012 at the sweet cherry, sour cherry and plum orchards of the Institute of Agriculture- Kyustendil and private orchards and nurseries in the municipalities of Kyustendil, Nevestino, Dupnitza and Bobov Dol.

It was used generally accepted methods in plant pathology. The highest percentage of virus infection among the investigated fruit tree species was in plum (62.3%), followed by sour cherry (59.8%) and sweet cherry (22.6%).

The most common virus in plum is PPV, in sour cherry PNRSV, and in sweet cherry PDV. Strain characterization revealed that the most distributed PPV strain on plum in Kyustendil region is PPV-Rec (84.1%), followed by PPV-M (12.7%) and PPV-D (3.2%) strain. No infection of CLRV, ApMV, ArMV and RpRSV in tested trees was detected.

The numerous infection periods, the high rate of attack on tree of controls (unsprayed) and the need of annual treatments are determined cherry leaf spot (*Blumeriella jaapii*) as the most important (key) fungal disease in sweet cherry in Kyustendil region.

The fungi *Monilinia laxa* and *Monilinia fructigena* are caused significant damage to the stone fruit species (sweet and sour cherry and plum). They are economical important during years with a

Kamenova I., 2016. Non-transmission of *Plum pox virus* through seeds of myrobalan and apricot. *Bulgarian. Journal of Agricultural Science,* 22: 267–271.

Abstract. Seeds from wild-grown myrobalan (*Prunus cerasifera* Ehrh.) infected with PPV-D strain and from apricot (*P. armeniaca*) infected with PPV-Rec strain were investigated for their potential role in virus transmission. The presence of PPV was checked in fully ripe seeds, geminated seeds and seedlings. While the results obtained by DASI-ELISA tests of whole seeds and separated seed coats from myrobalan were negative for PPV, IC-RT-PCR showed virus presence in forty six and forty seven samples out of 50 tested, respectively. Both DASI-ELISA and IC-RT-PCR detected PPV in the whole seeds and seed coats from apricot displaying pale spots and/or rings on the stones and did not detect the virus in the seeds coming from stones without symptoms. The virus was identified in the cotyledons containing the embryo only by IC-RT-PCR and only in apricot seeds showing symptoms on the stone. Seedlings investigated for a period of two years never showed symptoms and were found PPV-negative, both by DASI-ELISA and IC-RT-PCR. On the base of the results obtained it can be concluded that the seeds from *P. cerasifera* Ehth. infected with PPV-D and from apricot infected with PPV-Rec strain do not transmit the virus.

Borissova A. and Kamenova I., 2016. Occurrence of phytoplasmas of the apple proliferation group in fruit trees in Kyustendil region of Bulgaria. *Bulgarian Journal of Agricultural Science,* 22(3): 465-469.

Abstract. Apple, pear and plum trees showing symptoms typical for apple proliferation, pear decline and European stone fruit yellow from Kyustendil region of Bulgaria were analyzed for phytoplasma infection by polymerase chain reaction technology. PCR was performed by the use of specific for Apple proliferation group set (Loewe Phytodiagnostica, Germany) and the protocol recommended by the manufacturers. The results obtained showed the presence of phytoplasmas in 85.0% and 62.0% tested apple and pear trees, respectively and in all tested plum trees. Restriction fragment length polymorphism analysis revealed that apple and pear were infected with apple proliferation and pear decline phytoplasmas, respectively, while European stone fruit yellows was detected in plum trees. Because many trees showed symptoms like those of sampled and analyzed trees, this preliminary assay suggest a high incidence of phytoplasmas from AP group in the surveyed region of the country.

Глави от книги

Atanassov A., Dzhambazova T., Kamenova I., Tsvetkov I., Georgiev V., Dincheva I., Badjakov I., Mihaylova D., Kakalova M., Pavlov A. and Mollov P. Chapter: Modern Biotechnologie and Phytonutritional Improvement of Grape and wine. Engineered resistance to viruses. In: Phytonitritunal improvement of crops. Wiley (Submitted and accepted).

Abstract. Grapevine is one of the most cultivated fruit crops worldwide, which reflects its great economy significance. Due to its extensive exploitation for wine production and because of the beneficial health effects of grape metabolites, grapevine got into the spotlight of science and has become a model woody species in plant biotechnology. As a result, few years ago the grapevine genome was fully sequenced, presenting a molecular basis for further studies. *In vitro* propagation techniques and genetic markers have boosted the development of grape breeding in a dynamic industry with a focus on creating cultivars with improved metabolite profile, enhanced tolerance to pathogens and better adaptation to wide ecological factors. The final touch of grape biotechnology is made by metabolomic analyses, which estimate the efficiency of genome improvement and the influence of environmental factors and enological practices on grape and wine quality.

Atanassov A., Dzhambazova T., Kamenova I., Tsvetkov I., Georgiev V., Dincheva I., Badjakov I., Mihaylova D., Kakalova M., Pavlov A. and Mollov P. Chapter: Modern Biotechnologie and Phytonutritional Improvement of Grape and wine. Diagnosis of grapevine viruses. In: Phytonitritunal improvement of crops. Wiley (Submitted and accepted).

Abstract. Control strategies of viruses infecting grapevine used nowadays are preventive measures which are based on sanitary selection and chemical control of the known vectors (nematodes, mealybugs). EU Directive 2002/11/EC rules require that the initial plant material for vegetative propagation be free of *Grapevine fanleaf virus* (GFLV), *Arabis mosaic virus* (ArMV), *Grapevine fleck virus* (GFkV), *Grapevine leafroll-associated virus 1* (GLRaV-1) and *Grapevine leafroll-associated virus 3* (GLRaV-3). Thus, sanitary certification programmes depend on a reliable and sensitive detection of these viruses.

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