UDK 632.9

YU ISSN 0372–7866

INSTITUT ZA ZAŠTITU BILJA I ŽIVOTNU SREDINU - BEOGRAD
INSTITUTE FOR PLANT PROTECTION AND ENVIRONMENT - BELGRADE

ZAŠTITA BILJA
PLANT PROTECTION

VOL. 50(2), N^{O} 228, 1999.

Zaštita bilja, Vol. 50(2), N°228: 91–181, 1999, Beograd

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Original scientific papers

SENSITIVITY OF SOME MODIFICATIONS OF ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) IN DETECTING POTATO VIRUS Y

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Summary

ELISA test has been successfully applied since mid 1970s for the detection of plant viruses. Different variants of this test were described: direct (DAS), indirect, and triple. In order to improve antigen binding for antibodies and effect of signals of positive reaction, the modifications of this test were applied: "cocktail", "amplified", and MUP ELISA. In this paper the suitability od DAS, "cocktail", and MUP test in the detection of PVY from the dilution scale of purificated virus suspension of known virus concentration was detected.

Antiserum was produced on PVY (isolate D-2-1), conjugated with alcaline phosphatase and applied in DAS, "cocktail" and MUP ELISA technique. The optimal dilution of antibodies (1/600) and conjugate (1/400) in PVY detection from infected sample (Table 1), continued to be applied in the tests.

ELISA techniques were carried out according to the procedure of Clark and Adams (1977). Enzyme, conjugate, and antigen were incubated at the same time in "cocktail" test (van den Heuvel and Peters, 1989), and in MUP test (Torrance and Jones, 1981), instead of nitrofenyl phosphate, 4-metilumberiferil was added in substrate buffer.

Minimum concentrations of different strains of PVY were proved: Y^{o}-F and Y^{N}-F and isolates D-2-1, which can be detected by the mentioned techniques (Table 2). The effect of the concentration of the virus in the sample on absorption values at 405 nm (A_{405}) according to DAS and "cocktail" ELISA was also investigated. The dependence of A_{405} of the tested concentration was compared by statistical procedure of correlational analysis (Table 3).

When applying the produced serum in DAS and MUP ELISA tests, different strains of Y virus can be detected: Y^{o}-F, Y^{N}-F. Minimal concentration of virus detected from "cocktail" suspension and in MUP test was 22-25 ng/ml, and by DAS test it was ten times bigger (Table 4). The obtained corelation coefficients for DAS test of r=0,937 and for "cocktail" of r=0,951 point to the conclusion of high dependence between A_{405} and the tested virus concentration, as well as that virus concentration in the sample is directly proportionate to the value of A_{405}.

Key words: DAS, Cocktail; MUP ELISA test, potato virus Y, purificated virus suspension, potato.

(Received: February 2, 1999)
(Accepted: June 20, 2000)

Plant Protection, Vol. 50(2), N° 228: 95–104, 1999, Belgrade

DISTRIBUTION, IMPORTANCE AND PLANT HOSTS OF THE BACTERIUM *ERWINIA AMYLOVORA* ON SOUTHERN SERBIAN TERRYTORY

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Summary

Occurrence, spread, economic importance, and host plants of the bacterium *E. amylovora* are studied on the territory of Southern Serbia (Jablanica and Pčinja district) which is known by its intensive fruit trees cultivating (Map 1.).

In this area *E. amylovora* is recorded almost in all localities where pomaceous trees are grown (Map 2-5 and Table 1). During 1997 it has been found in fruit tree plantations,which were socially owned in the surface of 492,45 ha as follows, in plantations under pears at 179,45 ha, apples at 217,00 ha and quince at 96,00 ha. Besides, in socially owned large plantations, the pathogen was also found in smaller private plantations, home fruit trees, apple maiden trees in nurseries, parent fruit trees, individual pomaceous fruit trees in home gardens, as well as on plants of spontaneous flora - wild pear and hawthorn, at boundaries and pastures near plantations (Fig. 1.).

So far, 300,18 hectares of apple cultivars were cleared and the damages caused by the clearing are about 3.020.240 DEM (Table 2).

Host plants of the bacterium *E. amylovora* have been studied using the specimen with typical fire blight symptoms (pear, apple, quince, medlar, wild pear and hawthorn), and as well as the plants containing fire blight like symptoms (*Pyracantha coccinea, Chaenomeles japonica, Cotoneaster horizontalis, Sorbus domestica, Forsythia intermedia, Prunus laurocerasus, Buxus sempervirens, Prunus armeniaca, Prunus cerasus, Prunus persica, Prunus domestica* and *Prunus cerasifera).* However, the pathogen has been isolated and identified only from specimens with typical symptoms of the disease but not from specimens with fire blight-like symptoms so that plant specimens cannot yet be considered the host plants of *E. amylovora* in the region of South Serbia (Fig. 1,2 and 3).

Pathogen identification has been performed by the abridged procedure of Gram stain using 3% KOH, artificial inoculations of unripe pear and plum (cv. Stanley) fruits in order to determine tissue necrosis and occurrence of bacterial exudate drops, tabacco hipersensitivity, and absence of green fluorescent pigment on King's medium B, as well as by the means of the slide agglutination test.

Therefore, on the basis of the results obtained the bacterium *E. amylovora* was registered as pathogen of pear, apple, quince, medlar, wild pear and hawthorn, on this territory up till now (Table 3 and Map 6-11).

Key words: *Erwinia amylovora*, fire blight, occurrence, spread, importance, host plants.

(Received: September 20, 1999)
(Accepted: June 20, 2000)

Plant Protection, Vol. 50(2), N° 228: 115–149, 1999, Belgrade

DISTRIBUTION AND INTENSITY OF THE OCCURENCE OF SOME PEPPER PATHOGEN VIRUSES IN SERBIA

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Summary

In this paper we presented the data about the distribution and the occurence of some pepper pathogen viruses in Serbia.

Enzyme-linked immunosorbent assay (DAS-ELISA) was used for virus identification. Samples of diseased pepper, collected from different pepper growing localities, were tested for the presence of the following viruses: cucumber mosaic virus (CMV), tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), alfalfa mosaic virus (AMV), potato virus Y (PVY), tomato spotted wilt virus (TSWV) and potato virus X (PVX) (Tab. 1.)

According to the results obtained, 91.48% of the pepper samples were infected with some of the investigated viruses. Mixed infection with two or more viruses was found in 52.73% of the samples collected. Infection with two viruses was found in 35.27%, with three - 7.75%, four - 7.36%, five - 1.55% and six - 0.8% of the pepper samples (Fig 1.). Regarding intensity of the occurrence of investigated viruses, our results indicated that out of total number of the samples, 72.81% contained CMV, 25.96% - TMV, 25.19% - PVY, 22.48% - ToMV, 13.17% - PVX, 11.62% - AMV and 7.75% pepper samples were infected with TSWV (Fig. 2).

Regarding the virus appearance and high percentage of infected samples in all pepper growing localities, it can be concluded that investigated viruses are widely present in pepper production in the Republic of Serbia.

Cucumber mosaic virus caused the most severe economic losses in pepper production. In some localities, 100% of the tested plants contained this virus.

Key words: pepper, viruses (CMV, TMV, ToMV, AMV, TSWV, PVY, PVX), DAS-ELISA, distribution, intensity of the occurence

(Received: August 19, 1999.)
(Accepted: June 20, 2000)

Plant Protection, Vol. 50(2), N° 228: 151–159, 1999, Belgrade

Therefore, on the basis of the results obtained the bacterium *E. amylovora* was registered as pathogen of pear, apple, quince, medlar, wild pear and hawthorn, on this territory up till now (Table 3 and Map 6-11).

Key words: *Erwinia amylovora*, fire blight, occurrence, spread, importance, host plants.

(Received: September 20, 1999)
(Accepted: June 20, 2000)

Plant Protection, Vol. 50(2), N° 228: 115–149, 1999, Belgrade

STUDY OF POPLAR TREE GALL MIDGES

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Summary

In numerous localities of our country, we have recognized 10 out of the total sum of 12 known species of *Cecidomyiidae* living on poplar tree among which are two species of the genus *Contarinia* (*C. petioli* and *C. populi*), two species of the genus *Dasineura* (*D. populi* and *D. populnea*), five species of the genus *Harmandia* (*H. pustulans, H. cavernosa, H. populi, H. globuli* and *H. tremulae*) and the species *L. populnea* of the genus *Lasioptera* for the last several years.

All cited species, except *Contarinia petioli* which develops on the leaf stalk and *Dasyneura populnea* living on a bud, develop leaf galls.

The galls sometimes resemble each other in their appearance so that we should study the inner structure of the gall too in order to achieve the precise determination instead of forming the gall as well as spatulae of the larvae.

The species *Dasyneura populnea* Kieff. has been recorded so far only for The Middle Europe and we have not found any data about it since 1929. *D. populnea* is new for the fauna of Yugoslavia and the Balkans.

Nearly all mentioned species have recently appeared in the increased number although we have not stated greater damages on the poplar tree caused by their attack.

Key words: poplar tree, *Cecidomyiidae,* the Balkans, South Slavic Region

(Received: July 8,1999)
(Accepted: June 20, 2000)

Plant Protection, Vol. 50(2), No228: 161–169; 1999,Belgrade