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

# ZAŠTITA BILJA PLANT PROTECTION

VOL. 44 (2), No 204, 1993.

Zaštita bilja

Vol. 44 (2)

Br. 204 (str. 93 - 168)

Beograd, 1993.

## CONTENTS

# Original scientific papers

٠

M. Arsenijević, M. Sidor, B. Purar, S. Djurišić i D. Milošević The application of enzyme-linked immunosorbent assay /ELISA/ in proving of Erwinia carotovora subsp. atroseptica, a potato pathogen	101
S. Stojanović, D. Stojanović, B. Manojlović and M. Gavran Fungi of the genus Puccinia on weed plants in Serbia	111
O. Jovanović	
Investigation on antagonistic effect of saprophytic bacteria and fungi to Agrobacterium tumefaciens	123
O. Jovanović	
Investigation on the possibility of antibiotic use for Agrobacterium tumefaciens control	132
I. Sivčev	
Influence of temperatures on the sporulation of Aphidopathogenous fungi Pandora neoaphidis (Remaudiere et Hannebert) Humber	137-138
S. Stojanović	
Polistigna rubrum diffusion, intensity and disease dynamic at the territory of the Republic of Serbia	146
M. Vukša, B. Manojlović, A. Zabel, M. Cvetković, M. Kostić and V. Sabovljević	
Investigating on the toxity of difetialon for <i>Rattus norvegicus</i> Berk. in laboratory conditions	155
M. Vukša, B. Manojlović, A. Zabel, M. Cvetković, M. Kostić and V. Sabovljević	
Efficiency of the control of rodents in public objects and on alfaalfa by	100
Baraki Dait (Difcliaion)	166

- Lelliott, r.A., Stead, D.E. (1987): Methods for the Diagnosis of Bacterial Diseases of Plants. British Society for Plant Pathology. Blackwell Scientific Publications. Oxford, London, Edinburgh, Boston, Melbourne.
- Peltzer, S., sivasitham param, K. (1988): Scro-groups of *Erwinia carotovora* associated with water, soil, tuber and stems of potato plants in Westerna Australia. New Zealand J.Exp. Agric., Vol. 16:265-270.
- S c h a a d, N.W. (1979): Serological identification of plant pathogenic bacteria. An.Rev. Phytopath., 17:123-147.
- T a n i i, A., B a b a, J. (1975): Blackleg of Potato Plants Caused by a Scrologicaly Specific Strain of Erwinia carotovora var. carotovora (J o n e s) D y c. Ann.Phytopath. Soc.Japan.Vol.41(5): 513-516.
- Torrance, L., Jones, R.A.C. (1981): Recent development in serological methods suited for use in routine testing for plant viruses. Plant Pathology, 30:1-24.
- Ts uch iya, K., Matsuyama, N., Wakimoto, S. (1983): Studies on the Relationship between Virulence and Bacteriological Properties in *Erwinia carotovora* subsp.carotovora. J.Fac.Agr., Kyushu Univ. 27(3-4): 197-207.
- Tsuchiya, K., Karnjanarat, S., Hayashi, M., Umeno, M., Wakimoto, S. (1987): On the Strains of *Envinia carotovora* subsp. *carotovora* Isolated from Radish and Broccoli Plants. J.Fac.Agr.Kyushu Univ.31 (1-2): 63-69.

(Primljeno 19.05.1993.)

ŧ

٩,

## THE APPLICATION OF ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) IN PROVING OF ERWINIA CAROTOVORA SUPSP. ATROSEPTICA, A POTATO PATHOGEN

by M. Arsenijević M. Sidor, Božana Purar Faculty of Agriculture, Novi Sad

S. Durišté Institute for Health Protection, Faculty of Medicine, Novi Sad

## D. Milošević

Institute for Potato, Guca

## Summary

From the total number of 13 investigated strain of bacteria, isolated from the diseased plants and potato tubers, 9 strains react positively in enzyme-linked immunosorbent assay (ELISA) test (on microplates), used to prove *Erwinia carotovora* ssp. *atroseptica* (A g d i a inc., 1987). Four strains show the negative result, because they do not react with the antibodies of this bacterium (tab.1).

Filtrated or centrifugated sad, strained from the diseased potato tissue, also manifested the positive effect in the invaginations ("pools") of microplates. The positive result was obtained by using the autentic strain of *E.c.ssp.atroseptica* (P-14/1), used as positive control.

The negative reaction with the antibodies of *E.c.ssp.atroseptica* manifested the strains of the bacteria *E.c.ssp.carotovora* (P-2092), *E. chrysanthemi* (E.chr.8263/2) and *P.s.pv syringae* (96A/2), applied as negative controls (tab.1).

Uljaniščev, V.I. (1976): Opredelitelj ržavčinnjih gribov SSSR. Nauka, Leningrad, 384 p.

- W a t s o n, A.K., C h a m p a g n e, J.G. & H a r t m a n, H. (1978): /Biological weed control with plant pathogens/. Phytoprotection 58(2-3): 129, 1977. In Weed Abstract, vol. 27: 3081.
- Watson, A.K. & Koegh, W.J. (1980): Mortality of Canada thistle due to Puccinia puncitiformis. Proceedings of the V th International Symposium of Biological Control of Weeds, Brisbane, Australia, 1980: 325-332.
- W a t s o n, A.K. & B r u n e t t i, K. (1984): Puccinia carduorum on Carduus tenuiflorus in California. Plant Discase, 68: 1003-1005.

(Primljeno 1.03, 1993.)

## FUNGI OF THE GENUS PUCCINIA ON WEED PLANTS IN SERBIA

by

S. Stojanović, D. Stojanović, B. Manojlović and Mira Gavran Institute for Plant Protection and Environment, Belgrade.

## Summary

On 16 weed species 14 pathogens of the genus *Puccinia* were registered. The higher pathogenity to its host plants was manifested by *P.menthae*, *P.suaveolens*, *P.sonchi*, *P.retifera* and *P.xanthii*. All these pharasites cause leaf drying, followed by a decreasing vitality of the diseased plant, by which is prevented their expansion.

With its destructive effect especially stood out *P.suaveolens* on *Cirsium arvense*, which point to the necessity of its more detailed investigation in our conditions. The use of this fungus for biological control of Canada thistle can be restrected by the appearance of the superparasite *Fusarium sporotrichiella var. tricinctum*, which destroys pycnia, uredinia and telia of parasites.

- Panagopoulos, C.G., Psallidas, P.G., Stylianidis, D.C. (1983): Current work on the Effectiveness on Biocontrol of Crown Gall. International workshop on Crown Gall, Wadenswil, Switzerland, 1982.
- R is t a n o v i ć, M. (1964): Antagonistički odnos između nekih fitopatogenih gljiva in vitro. Zaštita bilja, br. 79, 315-319, Beograd.

Ryder, M.H., Jones, D.A. (1990): Biological control of Crown Gall. Review fo Plant Pathology 69, 4623.

- S a 11 c, A.J. (1974): Osnovni principi bakteriologije (prevod sa engleskog). Medicinska knjiga, Beograd-Zagreb.
- Schroth, M.N., Thompson, J.P., Hildebrand, D.C. (1971): Isolation of Agrobacterium tumefaciens - Agrobacterium radiobacter group from soil. Phytopathology 55, 645-647.
- Schroth, M.N., Moller, W.J. (1976): Crown gall controlled in the field with a nonpathogenic bacterium. Plant Disease Reporter 4, 275-277.
- S o l o v c j, E.F. (1972): Videljenic i izučenic gribov antagonistov po otnošeniju k vozbuditelju bakterijalnoga raka vinograda. Vsesojuznoj konf. po bakterialjnim boleznjam. Kiev.
- Š u t i ć, D., P a n i ć, M. (1969): Metode proučavanja fitopatogenih bakterija. Zavod za zaštitu bilja Poljoprivrednog fakulteta i sekretarijat za poljoprivredu, šumarstvo i vodoprivredu SR Srbije, Beograd.

Tešić, Ž., Todorović, M. (1966): Mikrobiološke metode ispitivanja zemljišta i vode. Beograd.

W a r c u p, H.J. (1960): Methods for Isolation and Estimation of Activity of Fungi in Soil. An International Symposium: The Ecology of Soil Fungi, Liverpool, 1960:

(Primljeno 16.03.1993.)

## INVESTIGATION ON ANTAGONISTIC EFFECT OF SAPROPHYTIC BACTERIA AND FUNGI TO AGROBACTERIUM TUMEFACIENS

by .

Olivera Jovanović Faculty of Agriculture, Belgrade-Zemun

## Summary

In this paper it was investigated antagonistic effect of bacteria and fungi to Agrobacterium tumefaciens (Smith and Townsend) conn, a grapevine parasite.

The isolation of 10 strains of bacteria Agrobacterium tumefaciens from the discased grapevine tumor from the locality of Velika Drenova was carried out in the course of 1988/89. From the same ground the saprophytic bacteria and fungi isolation was carried out. After that, the antagonistic effect of bacteria - by the method of small wells and fungi by disk block agar method, to Agrobacterium tumefaciens, with the control of the ecological factors (temperature, medium and age of investigated fungi cultures).

Among the saprophytic bacteria the antagonistic effect was manifested by the species Bacillus mycoides and Escherichia coli, and among the fungi: Rhizopus nigricans, Mucor pusillus, Penicillium nubrum, Alternaria humicola, Alternaria alternata, Fusarium dimerum, Fusarium oxyporum.

The obtained results proved the former knowledge from this field, but some new data, for our conditions, are also included, which can be also used for practical purposes.

- Panagopoulos, C.G., Psallidas, P.G., Stylianidis, D.C. (1983): Current work on the Effectiveness on Biocontrol of Crown Gall. International workshop on Crown Gall, Wadenswil, Switzerland, 1982.
- P a n i ć, M. (1982): Bakterioze bilja (izvodi sa predavanja za studente Odseka za zaštitu bilja). Poljoprivredni fakultet Beograd - Zemun.
- Plessis, H.J., Hattingh, M.J., Van Vuuren, H.J.J. (1985): Biological Control of Crown Gallin South Africa by Agrobacterium radiobacter Strain K 84. Plant Disease Reporter 69, 302-305.
- S a 11 e, A.J. (1974): Osnovni principi bakteriologije (prevod sa engleskog), Medicinska knjiga, Beograd -Zagreb.
- Šigacva, M.H., Tulcmisova, K.A. (1977): Antibiotiki v rastenicvodstve. Alma Ata.
- T o m i ć M i c a (1987): Ispitivanje mogućnosti primene nekih antibiotika u suzbijanju gljive Sclerotinia selerotiorum (Diplomski rad). Poljoprivredni fakultet, Beograd-Zemun.
- Weller, D.M. (1988): Biological control of soilnborn plant pathogens in the rhizosfere with bacteria. Annual Review of Phytopathology 26, 379-407.

(Primljeno 16.03.1993.)

## INVESTIGATION ON THE POSSIBILITY OF ANTIBIOTIC USE FOR AGROBACTERIUM TUMEFACIENS CONTROL

by

#### Olivera Jovanović Faculty of Agriculture, Belgrade-Zemun

#### Summary

Former knowledge proved the efficasy of the different antibiotics to Agrobacterium tumefaciens (Smith and Townsend) Conn.

In our paper ten strains of Agrobacterium tumefaciens, were used. It was investigated the effect of seven different antibiotics to these strains: pentrexyl, ceporex, streptomycin sulfat, vibramicin, chymociclar, nistatin, bactrim.

Two methods were used: antibiogram method and medium cut method. In both the methods the most efficient was streptomycin sulfat (tab. 3).

Pentrexyl, chymociclar and bactrim had a moderate effect to the strains Agrobacterium tumefaciens.

All the seven antibiotics manifest antagnistic effect, mostly in low concertations (from 10-1 to 10-4) (tab. 1-7).

Among the investigated strains of Agrobacterium humefaciens there is no higher exeptions in the susceptibility to antibiotics. Slightly higher susceptibility is manifested by the strains VL-1 and VL-2.

#### LITERATURA

- Latteur, G., Destain, J., Godefroid, J. (1983): Research on the Feasibility of Producing in Vitro an Entomophthorale Biopreparation Based on Conidia or Mycelium to Control Cereal Aphids. C.E.C. Programme on Integrated and Biological Control. 407-416. Agriculture.
- Milner, R.J. (1981): Patterns of primary spore discharge of Entomophthora spp.from the blue green aphid Acyrthosiphon kondoi. Journal of Invertebrate Pathology. 38(3), 419-425.
- Milner, R.J., Holdom, D.G., Glare, T.R. (1984): Duirnal patterns of mortality in aphids infected by entomophthoran fungi. Entomologia Experimentalis et Applicata. 36(1), 37-42.
- Rockwood, L.P. (1950): Entomogenous fungi of the family Entomophylioraceae in the Pacific North West. Journal of Economic Entomology. 43(5), 704-707.
- S i v č e v, I. (1991): Entomopatogene gljive fam. Entomophthoraceae kao regulator populacije vašiju (Homoptera. Aphididae) štetočina kupusa. Univerzitet u Novom Sadu.
- Wilding, N. (1969): Effect of humidity on the sporulation of Entomophthora aphidis and E.thaxteriana. Trans.Brit.mycol.society, 53(1), 126-130.
- Wilding, N. (1970a): Report for 1970. Annual Report Rothamsted Exp. Station. 1, 206-207.
- W i l d i n g, N. (1970b): Entomophthora conidia in the air-borne conidia. Journ.General Microbiol., 62, 149-157.
- Wilding, N. (1973): The survival of *Entomophthora* spp. in mumified aphids at different temperatures and humidities. Journal of Invertebrate Pathology. 21(3), 309-311.

(Primljeno 20.02.1993.)

## INFLUENCE OF TEMPERATURES ON THE SPORULATION OF APHIDOPATHOGENOUS FUNGI PANDORA NEOAPHIDIS (REMAUDIERE ET HANNEBERT) HUMBER

by

I. Sivčev

Institute for Plant Protection and Environment, Belgrade

#### Summary

Entomopathogenous fungi are an important regulators of the population of plant aphids. They are causer of *epizootia mycosis* on aphids on the different cultivated plants during the spring and autumn. The fungi are transmitted per horizontal type and due to this, they depend considerably on the environmental conditions. The presence of favorable environmental conditions during the sporulation is a critical period. Due to this, the aim of this paper has been to prove the temperature influence on the sporulation.

The experiments were set up at the temperatures of  $11^{\circ}$ C,  $20^{\circ}$ C,  $25^{\circ}$ C,  $30^{\circ}$ C and  $36^{\circ}$ C in dark conditions and at  $20^{\circ}$ C at light. All the treatments were with 100% of relative humidity.

The results of the experiment proved that fungus P.neoaphidis sporulates at the temperatures from 11°C to 25°C and at 30°C and 36°C it cannot sporulate. P.neoaphidis released the largest number of conidiaat the changeable temperatures such were in the field with on average 61.340 conidia per aphid and at 25°C, where was on average 52.110 conidia. The fungus formed the lowest number of conidia at a constant temperature of 11°C, where was on average 24.497 conidia per aphid. If all the conditions of favorable relative humidity and temperature are satisfied, *P.neoaphidis* sporulates at light and in dark as well.

In cabbage agrobiotype the favorable temperatures are present during a long period of aphid presence in cabbage, but they last differently. The fungus sproulates most intensively during the period from 3 to 12 horus from the beginnig of the sporulation, which in the nature is from 2 to 8 h in the morning, when conidiophore discharges up to 64% of the total amount of conidia. This proves that it needs only one favorable night to discharge the most of conidia. In the constantly favorable conditions the sporulation lasts over 36 hours. In this paper it was proved that the favorable conditions for the sporulation of limited duration, which in our conditions often arise during the summer, can cause the lost of infective inoculum quantity of this fungus.

## POLISTIGMA RUBRUM DIFFUSION, INTENSITY AND DISEASE DYNAMIC AT THE TERRITORY OF THE REPUBLIC OF SERBIA

by

S. Stojanović Institute for Plant Protection and Environment, Belgrade

#### Summary

**Polystigna rubrum** (Pers.) D.C. is very diffused plum parasite in Serbia. In all the localities in the course of 1986-1988 and 1990-1991 it was registered the appearance of this fungus (table 2). During 1986 the plum orchards with the disease intensity of 20-50% prevaled. In 1987, in the most plum orchards, the intensity ranged from 20 to 40%, and in 1988, 1990, and 1991 in the most localities it was 10%.

The disease intensity lower than 10% can be evaluated as week attack, 11-25% as middle, 26-50% as strong and over 50% as very strong attack.

On the diseased leaves of the plum, it was registered up to 75 stroma per leaf in 1986, up to 45 in 1987, up to 46 in 1988, up to 9 in 1990 and up to 5 stroma per leaf in 1991. In the first two years it was mostly 1-5 and 1-3 stroma per leaf respectively, and in 1988, 1990 and 1991 the leaves with only one stroma prevailed.

Investigating the disease growth during the vegetation (table 3), it was proved the insignificant increase of disease intensity, although the number of stroma per leaf did not increase significantly, which is the result of the enlargement of the existing stroma of *P.rubrum*, and not of new infections.

## INVESTIGATING ON THE TOXITY OF DIFETIALON FOR RATTUS NORVEGICUS BERK. IN LABORATORY CONDITIONS

by

Marina Vukša, B. Manojlović, A. Zabel, Marina Cvetković and M. Kostić Institute for Plant Protection and Environment-Belgrade

> Vesna Sabovljević Institute for Research in Agriculture "Serbia", Institute for Pesticide and Environment, Belgrade-Zemun

## Summary

The researches of the acceptability and the toxity of difetialon (1,25 g/l, producer "Reusel Uclaf", France) in Baraki baits, prepared in pellets and parafine blocks, carried out on the white laboratory rat, *Rattus norvegicus*, of the species Wistar, with once occuring and repeated treatment.

The obtained results in laboratory conditions showed that with once occuring and repeated treatment, Baraki baits in parfine blocks are more acceptable for *R.norvegicus* than the baits prepared in pellets.

With once occuring use of Baraki pellets, the animals consumed on average 13,1 g. The first symptoms of poisoning of all animals appeared after 4 days, and the mortality began between the 6th and the 9th day.

The toxity of difetialon and the acceptibility of the baits prepared in parafine blocks for *R.norvegicus* with once occuring and repeated use is more expressed than of the bait in pellets. The Ratus fed once, consumed on average 32,5 g of baits, and the loss of the body mass was on average 6,2 g each. The first symptoms of poisoning appeared also after 4 days, and they did between the 5th and the 6th day. With repeated feeding, seven-days exposure of Baraki baits prepared in parafine blocks, animals consumed on average 74,5 g each, the loss of the body mass per individual was on average 25 g. The first symptoms of poisoning appeared after 4 days, and the mortality began between the 6th and the 7th day.

## EFFICIENCY OF THE CONTROL OF RODENTS IN PUBLIC OBJECTS AND ON ALFALFA BY BARAKI BAIT (DIFETIALON)

by

Marina Vukša, B. Manoflović, A. Zahel, Marina Cvetković and M. Kostić Institute for Plant Protection and Environment, Belgrade

> Vesna Sabovljević Institute for research in Agriculture "Serbia", Institute for Pesticide and Environment, Belgrade-Zemun

#### Summary

-

1

It was researched the possibility of the control of rodents in public objects (Rattus norvegicus, R.rattus and Mus musculus) and on alfa-alfa (Microtus arvalis and Apodemus sylvaticus) with the Baraki baits (a.m. difetialon).

The testing of Baraki bait with active component of difetialon (1,25 g a. m/l, producer "Rouset Uctaf"), showed that the efficiency against *R.norvegicus* and *R.rattus* in the mixer of fodder, is satisfactory. Slightly higher acceptability and efficiency was obtained with the haits prepaired in parafine blocks, in relation to the bait in Baraki pellets.

The results showed that the efficiency of difetialon prepaired in paraline blocks for rat control in the mixer of fodder, was 83,7%. The largest quantity of the caten bait was the first day (319 g), the second (229 g), the third (154 g) and the forth day (197 g). After that, the quantity of the consumed bait decreased and the tenth day it was only 21 g. The efficiency of difetialon prepaired in pellets for rat control was 82,9%. Larger quantity of the baits was consumed the first day (241 g), the second (198 g) and the third (171 g). After that, the quantity of the consumed bait decreased and the tenth day it was only 36 g.

The check of the acceptability and the efficiency of difetialon prepaired in Baraki pellets and paraline blocks for *M.musculus* in a restaurant, shows that in the interval of eight days 107 baits in pellets and considerably more baits in parafine blocks (164 g) were caten. The efficiency in suppressing of M. musculus of 62,5% for pellets and 79,2% for parafine blocks was obtained.

On alfalfa, where the experiments were taken place, it was outbreak numerousness of the rodents. On the experimental plots of  $100 \text{ m}^2$ , from 102 to 152 active holes were registered, and from 204 to 304 individuals, respectively. The number of occurence of the species was: *Micronus atvalis* Pall. 69% (87 individuals) and *Apodemus sylvaticus* L. 31% (39 individuals).

The efficiency of difetialon prepared in pelicts for rodents control in alfalfa was satisfactory. The efficiency obtained by applying in the period of 7 days was 84,6%. But difetialon in parafine blocks, showed considerably higher efficiency (94,7%). Considerable decreasing of the number of rodents was the first day (86,8%). Suitability of the parafine blocks, besides higher efficiency, is also in the fact that they can be used in the conditions of high humidity (immediately after atmospheric rainfall).