ÚSTAV ZEMĚDĚLSKÝCH A POTRAVINÁŘSKÝCH INFORMACÍ

OCHRANA ROSTLIN

PLANT PROTECTION

2

ČNÍK 33 (LXX)
AHA 1997
ISSN 0862-8645

ČESKÁ AKADEMIE ZEMĚDĚLSKÝCH VĚD

OCHRANA ROSTLIN

PLANT PROTECTION

Journal for Phytopathology, Pest, Weed Research and Plant Protection published by the Czech Academy of Agricultural Sciences and with the promotion of the Ministry of Agriculture of the Czech Republic

Abstracts from the journal are comprised in Agrindex of FAO (AGRIS database), in Bibliographie der Pflanzenschutzliteratur published by Zentralstelle für Agrardokumentation und -information (Phytomed database), in Biological Abstracts of Biosis (BIOSIS Previews database), and in Review of Agricultural Entomology and Review of Plant Pathology of CAB International Information Services (CAB ABSTRACTS database) and AGROINDEX.

Aim and scope: The journal publishes original scientific papers, short communications, and reviews, that means papers based on the study of technical literature and reviewing knowledge in the given field. Published papers are in Czech, Slovak or English.

Subscription information: Subscription orders can be entered only by calendar year and should be sent to the contact address.
Subscription price for 1997 is 204 Kč, 51 USD (Europe) and 53 USD (overseas)

Periodicity: The journal is published four times a year.

Contact address: Slezská 7, 120 56 Praha 2, Czech Republic
tel.: 42 2 25 10 98; fax: 42 2 24 25 39 38; e-mail: braun@uzpi.agrec.cz

Ochr. Rostl., *33*, 1997 (2) : 81–88

ON THE EPIDEMIOLOGY OF PLUM POX VIRUS IN THE CZECH REPUBLIC

Jaroslav POLÁK

Research Institute of Crop Production – Division of Phytomedicine, Prague, Czech Republic

Abstract: The distribution of natural sources of plum pox virus (PPV) that infects stone fruits in the Czech Republic was investigated. Wild growing plums are almost completely infected with PPV in central Bohemian and Moravian regions. Myrobalan (*Prunus cerasifera* ssp. *myrobalana*) is probably the second main natural source of PPV. The virus was also detected in some plants of blackthorn (*Prunus spinosa*) growing in PPV infected areas. Leaves of these plants showed distinct symptoms, which were very similar to those on plums. PPV was not detected in blackthorn shrubs growing in forest and in areas where PPV is not or only occasionally present. *Prunus spinosa* is considered to be only a secondary source of PPV infection.

plum pox virus; epidemiology; natural sources; wild plum; myrobalan; blackthorn

Sharka disease of plums (caused by plum pox virus, PPV) was first detected in the Bohemian part of the Czech Republic in 1952. The disease has spread to the major plum-producing areas of the country, mainly in central, western and eastern Bohemia, and in southwestern and northern Moravia. PPV causes economic losses on plums, apricots and peaches, especially on susceptible cultivars of plum and apricot, and also attacks *Prunus cerasifera* ssp. *myrobalana* L. (Ackermann, 1994).

The disease has progressively spread from eastern Europe to the European continent, including the Czech region (Roy, Smith, 1994). The primary sources of PPV infection were most probably infected grafts and rootstocks of fruit trees used in nurseries, mainly in the fifties and sixties. PPV was spread by infected trees from nurseries, and its spread continued in orchards and gardens, and in natural conditions by aphids. We had earlier proved the occurrence of natural infection of myrobalan trees with PPV (Polák, 1989). Blackthorn (*Prunus spinosa* L.), often mentioned to be a symptomless host of PPV, was also supposed to be an important source of PPV.

To study the epidemiology of sharka it is necessary to know the importance of individual sources of infection, with special attention on natural sources of it.

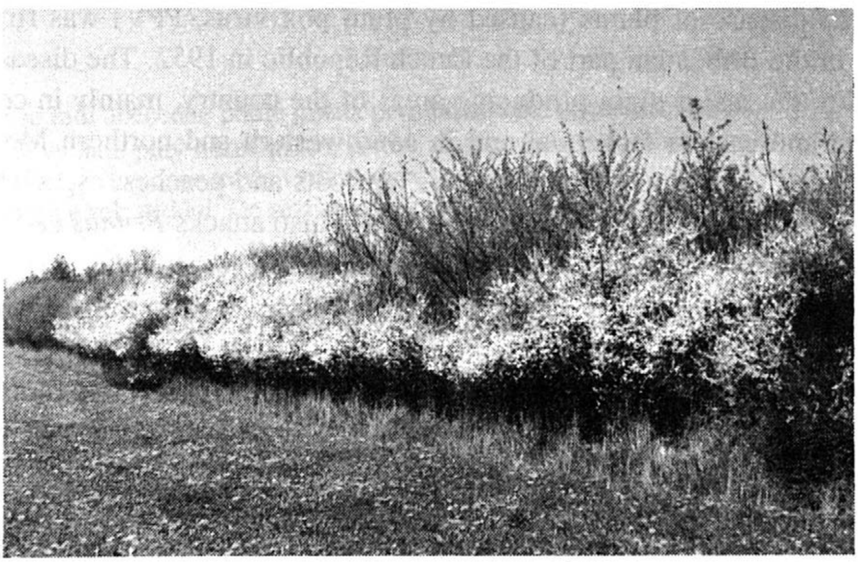
Wild growing plums, myrobalans and blackthorns are supposed to be important natural sources of PPV infection in the Czech Republic. The role of blackthorn as a natural source of PPV was questioned by Ranković and Dulić-Marković (1992).

The aim of our study was to prove the presence and distribution of PPV in blackthorn in the Czech Republic and to demonstrate its importance as a natural source of PPV infection. We also paid attention to the distribution of PPV in wild plums and myrobalans.

MATERIAL AND METHODS

Sampling of plants

Samples of flowers of blackthorn were collected mostly from different localities of central Bohemia in May. Thirty flowers were usually taken from one plant. Leaves were collected from the same plants during the summer from June to August (Fig. 1).



1. Hundreds of *Prunus spinosa* shrubs in marginal associations of the forest (locality Dohalice near Hradec Králové)

Wild growing plums and myrobalans or plums along roads in areas of central Bohemia and northwestern Moravian were visually evaluated for the presence of PPV symptoms during the summer. Wild growing plums are trees or shrubs of plums that have established themselves by seed or sucker. Occasionally, samples of flowers or leaves were taken for serological tests.

Serological evaluation

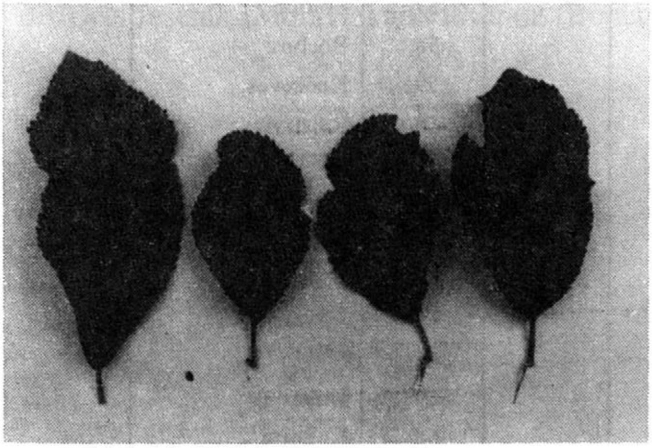
PPV antiserum was prepared in our laboratory by the procedure published by Albrechtová et al. (1986); it was used in the DAS-ELISA method (Clark, Adams, 1977). Samples for ELISA were prepared by grinding thirty flowers or 0.2 g of blackthorn leaf tissue in phosphate buffered saline pH 7.4 (ratio 1 : 20) with 2% polyvinylpyrrolidone and 0.2% of egg albumin. Plates were evaluated using a MR 5000 reader (Dynatech) at 405 nm.

Visual evaluation

Presence of PPV and intensity of symptoms in leaves of blackthorn, myrobalan and plum were evaluated in June, July or August. Photo documentation of leaf symptoms was also provided.

RESULTS

The ELISA tests on samples from blackthorn shrubs from different localities of the Czech Republic proved the presence of PPV in 12 blackthorns out of 68 tested (Table I); thus, 17.6% of the plants were infected.

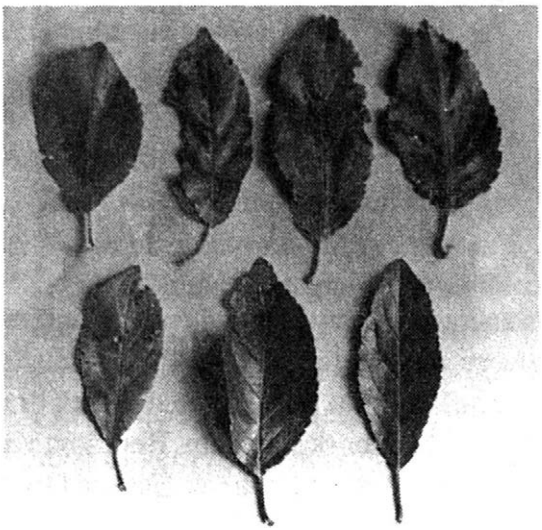


2. *Prunus spinosa*, leaves with severe rings and oak line pattern as symptoms of PPV

I. Distribution of PPV determined by ELISA on *Prunus spinosa* L. in the Czech Republic

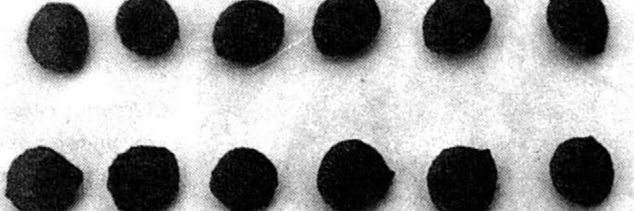
Nr. of sample	Sampling site	Results of ELISA PPV	Nr. of sample	Sampling site	Results of ELISA PPV
1.	Praha-Troja	–	35.	Praha-Smíchov	–
2.	Praha-Bohnice	–	36.	Mochov	–
3.	Praha-Dolní Chabry 1	+	37.	Králíky	–
4.	Praha-Dolní Chabry 2	–	38.	Holice	–
5.	Kralupy 1	–	39.	Hradec Králové	–
6.	Kralupy 2	–	40.	Hořice	–
7.	Zákolany	–	41.	Městec Králové	–
8.	Dřešťovice	–	42.	Poděbrady	–
9.	Hlubočepy 1	–	43.	Poběžovice	–
10.	Hlubočepy 2	–	44.	Myštěves	–
11.	Slivenec 1	+	45.	Noný Bydžov	–
12.	Slivenec 2	–	46.	Kanice	–
13.	Lochkov	–	47.	Chvojenec	+
14.	Radotín	–	48.	Dohalice 1	+
15.	Černošice	–	49.	Dohalice 2	+
16.	Černolice 1	–	50.	Miletín	–
17.	Černolice 2	–	51.	Sulom	–
18.	Třebenice 1	–	52.	Žitenice	–
19.	Třebenice 2	–	53.	Podbořany 1	–
20.	Košťálov 1	+	53.	Podbořany 2	–
21.	Košťálov 2	+	55.	Drahonice	–
22.	Kuzly	–	56.	Lubenec	–
23.	Velemyšleves	–	57.	Žlutice	–
24.	Kličín	+	58.	Bochov	–
25	Blšany	–	59.	Kněževes	–
26.	Bedlov	–	60.	Řeporyje	–
27.	Šanov	–	61.	Mníšek	–
28.	Rakovník 1	+	62.	Zbelítov	–
29.	Rakovník 2	–	63.	Lety	–
30.	Rakovník 3	–	64.	Mikulov	–
31.	Věšín 1	+	65.	Valtice	–
32.	Věšín 2	+	66.	Strážnice	+
33.	Pálava	–	67.	Dubá	+
34.	Nelahozeves	–	68.	Kokořín	–

Blackthorn plants infected with PPV showed distinct symptoms consisting of diffuse spots, rings and oak line patterns in leaves (Fig. 2). Symptoms of necrotic rings and oak line patterns appeared in leaves of two PPV infected shrubs (Fig. 3). No symptom, or mild pitting was found on the fruits, and red spots appeared on the stones of fruits of PPV infected blackthorns (Fig. 4).

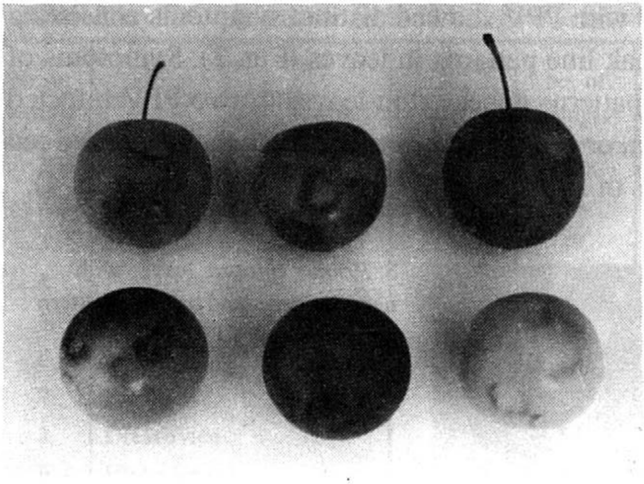


3. *Prunus spinosa*, leaves with necrotic rings and oak line pattern as symptoms of PPV

Wild growing plums and plums along roads in areas of Central Bohemia and northwestern Moravia (Šumperk and Jeseník districts) used to be severely infected with PPV. Myrobalan was often used as rootstock for plums planted along roads. We observed hundreds of rootstock myrobalans in the districts Jeseník (Moravia) and Příbram (Bohemia) with distinct PPV symp-

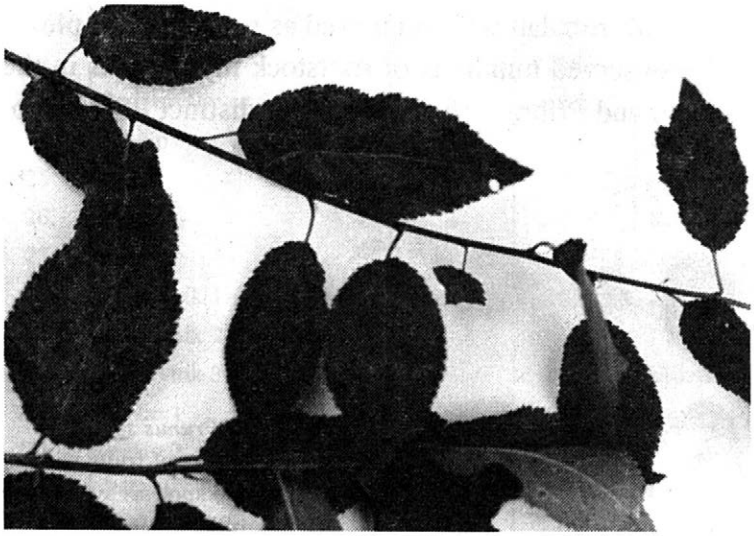


4. *Prunus spinosa*, stones of fruits with red spots as symptoms of PPV



5. *Prunus cerasifera* ssp. *myrobalana*, fruits with rings and spots as symptoms of PPV

toms on leaves. Grafted plums also showed heavy leaf and fruit symptoms of PPV. Wild growing plums were very often infected with PPV in different places of central, western and eastern Bohemia (e.g. in the districts Prague West and East, Kladno, Mělník, Kolín, Nymburk, Příbram, Benešov, Kutná Hora, Plzeň, Litoměřice, Teplice, Louny, Hradec Králové, Ústí nad Orlicí). We also observed myrobalans in central Bohemia with distinct PPV symptoms on fruits (Fig. 5) and leaves. Symptoms of vein mosaic and diffuse mosaic spots appeared on leaves (Fig. 6).



6. *Prunus cerasifera* ssp. *myrobalana*, leaves with vein mosaic and diffuse mosaic spots as symptoms of PPV

DISCUSSION

Our results proved that only part of the blackthorns in Central Bohemia are infected with PPV. Brunt et al. (1996), when reviewing references to PPV, described blackthorn as a latent host of the virus. We were, however, able to prove distinct symptoms on leaves of PPV infected blackthorns. Fifteen to 20% of blackthorn shrubs were infected in the area where the occurrence of PPV in plums is severe. Our findings differ from those of Ranković and Dulić-Marković (1992) who found only 0.9% of blackthorns to be infected with PPV in Yugoslavia. Such a lower occurrence can be explained by higher temperatures during the vegetation period, causing possible inactivation of the virus in aphid vectors. Nonpersistent PPV is probably transmitted by aphids to blackthorn more sporadically. The situation in both Yugoslavia and the Czech Republic suggests blackthorn to be only a secondary source of PPV infection. Blackthorns surrounded by contaminated plums can be infected step by step. The role of *P. spinosa* in the epidemiology of PPV in the Czech Republic seems to be limited at present, but it can increase in future. We intend to verify our theory in the next years. PPV occurs only occasionally in several marginal areas of highland in the Czech Republic. Therefore, our investigations will be concentrated on those marginal areas where we expect no incidence of PPV on blackthorns.

Wild growing plums are the main natural source for PPV infection of cultivated plums. Myrobalans are probably the second most important source of PPV infection, with an important role in the epidemiology of virus.

Acknowledgements

The autor thanks Mrs. J. Pívalová and Miss M. Bartoňová for excellent technical assistance.

References

ACKERMAN, P. In: ROY, A. S., SMITH, I. M. (1994): Plum pox situation in Europe. Bull. OEPP/EPPO Bull., *24*: 516–517.

ALBRECHTOVÁ, L., HOLUBCOVÁ, L., JOKEŠ, M. (1986): Purifikace viru šarky švestky a příprava antiséra vhodného pro stanovení viru metodou ELISA. Ochr. Rostl., *22:* 161–167.
BRUNT, A. A., CRABTREE, K., DALLWITZ, M. J., GIBBS, A. J., WATSON, M. (1996): Viruses of Plants. Wallingford, U. K., CAB Intern.: 1484 pp.
CLARK, M. F., ADAMS, A. N. (1977): Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. gen. Virol., *34*: 51–57.
POLÁK, J. (1989): Diagnosis of plum pox virus in infected symptomless trees of apricot, peach and *Prunus cerasifera* ssp. *myrobalana* by ELISA and ISEM. Acta Horticult., *235*: 299–303.
RANKOVIĆ, M., DULIĆ-MARKOVIĆ, I. (1992): Evaluation of *Prunus spinosa* L. as host of sharka and other viruses. Acta Horticult., *309*: 151–155.
ROY, A. S., SMITH, I. M. (1994): Plum pox situation in Europe. Bull. OEPP/EPPO Bull., *24*: 515–523.

Received October, 21 1996

Epidemiologie viru šarky švestky v České republice

V České republice je uskutečňován výzkum rozšíření přírodních zdrojů infekce peckovin virem šarky švestky (PPV). Planě rostoucí švestky jsou téměř úplně infikovány PPV v moravských oblastech a ve středních Čechách. Myrobalán (*Prunus cerasifera* ssp. *myrobalana*) je pravděpodobně druhým hlavním přírodním zdrojem PPV. Virus šarky švestky byl také stanoven v některých rostlinách trnky (*Prunus spinosa*) rostoucích v oblastech infikovaných PPV. Na rostlinách trnky infikovaných PPV byly zřetelné příznaky na listech, které byly velmi podobné příznakům na švestkách. PPV nebyl stanoven v keřích trnky rostoucích v lese a v oblastech, kde PPV není, nebo je pouze příležitostně přítomen. *Prunus spinosa* je považován za pouze sekundární zdroj PPV infekce.

virus šarky švestky; epidemiologie; přírodní zdroje; planá švestka; myrobalán; trnka

Contact address:

Ing. Jaroslav Polák, DrSc., Výzkumný ústav rostlinné výroby. odbor rostlinolékařství, 161 06 Praha-Ruzyně, Česká republika
tel.: 00 420 2 360 851, fax: 00 420 2 365 228

Ochr. Rostl., *33*, 1997 (2) : 89–102

THE DIAGNOSIS OF *Fusarium culmorum* BY POLYCLONAL ANTIBODIES – PREPARATION AND CHARACTER OF ANTIGENS AND ANTIBODIES

Jiřina KRÁTKÁ, Blanka KYNĚROVÁ, Andrea ZEMANOVÁ, Světlana SÝKOROVÁ

Research Institute of Crop Production – Division of Phytomedicine, Prague, Czech Republic

Abstract: Eighteen monospore *Fusarium culmorum* (*FCu*) isolates with different pathogenicity were cultivated on five liquid media. Czapek Dox and Minimal medium were suitable media. The morphological and physiological characteristics were determined (chlamydospore and conidia production, conidia dimension, character of mycelia, colour of media, growth dynamics of mycelium and weight of dry mycelial mats). The composition of media influenced morphological and physiological characters of isolates. Pathogenicity was not correlated with character of isolates. Antigens were prepared by extraction of proteins from mycelial mats in a special buffer, then purified by centrifugation and ultracentrifugation of supernatant. The composition of media influenced quantity and quality of proteins of antigens. The pathogenicity of *FCu* isolates was not correlated with protein content of antigens. Specificity and sensitivity of polyclonal antibodies after immunization of rabbits with *FCu* antigens were studied. Different reactions of antigens of *FCu* isolates were not found. Anti-*FCu* IgG reacted with antigens of another species of *Fusarium (oxysporum, solani, equisetum, nivale, sambucinum, poae, avenaceum)*, the differences were quantitative. Cross-reactions of anti-*FCu* IgG with antigens of *Pseudocercosporella herpotrichoides, Gaeumannomyces graminis, Rhizoctonia solani, Trichoderma viridae* (pathogenic isolates of wheat) were not observed. Reaction of antisera and IgG with antigens were evaluated by agar double diffusion and ELISA.

Fusarium culmorum; media; isolates; morphological and physiological character; antigen; proteins; polyclonal antibodies; sensitivity; specificity; cross-reactions

Fusarium culmorum is the dominant pathogen of winter wheat heads in the Czech Republic. Also widespread and economically important species are *F. graminearum, oxysporum, heterosporum, avenaceum, poae* and *nivale* (Kováčiková et al., 1992).

Pathogenic *Fusarium* sp. can grow on winter wheat in all phases of development. Infection causes lower germination, death of seedlings, yellow stems and leaves, root rot and head blight.

Infection of wheat kernels in particular lowers the yield. When the infection is very weak, grains may contain toxins. Recent research has shown that the production of some toxins can be modified by biotic and environmental factors and is not always correlated with virulence of the isolates and/or resistance of the variety (Mesterházy, Bartók, 1992; Manka et al., 1989). It is clear that a reliable, fast and specific diagnosis of the pathogens will not be found on biochemical methods (detection of mycotoxins), but on immunochemical (antigen–antibody interaction) or molecular methods (polymorphism of DNA). Detection of the pathogens in seeds is desirable mainly from a commercial point of view.

The present publication summarises our research on optimal separation and character of antigens suitable for obtaining polyclonal antibodies for the detection of *Fusarium culmorum* in host plants.

MATERIAL AND METHODS

Biological material

All analyses were performed with monospore cultures of *Fusarium culmorum* (*FCu*). Three groups of isolates were used: no. 46, 67, 62, 40, 47, 1, 35, 33 – high pathogenicity, no. 5 – medium pathogenicity, no. 7, 21, 4, 11, 48, 6, 28, 29, 2 – low pathogenicity. The isolates were obtained and tested at RICP in 1992–1995 (Ondřejová, Kováčiková, 1992; Kováčiková et al., 1992; Hájková – personal communication). Analyses were also performed with other pathogens of *Fusarium* sp. (*oxysporum, solani, equisetum, nivale, sambucinum, poae, avenaceum)* and with other important pathogens on wheat (*Pseudocercosporella herpotrichoides, Gaeumannomyces graminis, Rhizoctonia solani, Trichoderma viridae*). The isolates were chosen according to tests of pathogenicity performed at RICP (Sychrová – personal communication).

Cultivation of fungi

FCu isolates were cultivated on different liquid media: Czapek Dox medium (CD), Minimal medium (MM) – Puhalla (1985) and SNA medium

(SNAM1) – Nierenberg (1976), SNA medium (SNAM2) – Bilai's medium modified by Joffe (1974), SFA medium (SFAM) – Tio et al. (1977) (comprehensive Ribeiro, 1978). Liquid media suitable for growth of *FCu* isolates were evaluated according to weight of mycelial mats after 25–36 days. Other fungi (*Fusarium* spp. and other pathogenic fungi isolated from wheat) were cultivated on CD (liquid).

Morphological and physiological classification of mycelium

The influences of two media (CD and MM) on morphological and physiological characters of *FCu* isolates (with different pathogenicity) were evaluated: colour of media, character of mycelia, growth rate, dry matter of mycelia and incidence of spores (macroconidia, chlamydospores).

Preparation of antigens

Mycelial mats were ground with a pestle in a mortar and then extracted in buffer pH 8.8 (containing: 0.03M Tris, 0.005M $MgCl_2$, 0.001M Na_2EDTA, 0.001M dithiothreitol, 0,001M ascorbic acid, and 20% glycerol) with PVP at 4 °C 24 h. After centrifugation (10 000 rpm, 15 min) the supernatant was ultracentifuged (35 000 rpm, 90 min) and concentrated (20% polyethyleneglycol). The concentrate (1 ml) was lyophilised and diluted in PBS pH 7.4 (Amouzou-Alladaye et al., 1988; Krátká et al., 1995). The content of proteins in antigens was determined according to Lawry et al. (1951).

Determination of quality and quantity of antigen proteins

The starting content of extracted proteins was approximately 3 mg/ml.

a. *Separation of native proteins*

Native protein electrophoresis according to Davis (1964) was used (modification according to Bielenina et al., 1988). Electrophoresis was carried out on a Desphor VA instrument using 4.6% polyacrylamide stacking gel and 7.7% separating gel in vertical slab mold. Electrode buffer was 0.025M Tris and 0.19M glycin at pH 8.3 (Jones, 1987). The solution of bromphenol blue in electrode buffer (one drop) and saccharose (20% w/v) were added to protein extracts. 30 µl of extract were placed into wells in the gel. Electrophoresis was performed for 6 h at 50 mA and max. 200V at 4 °C. The gels were fixed 30 min in 20% trichloracetic acid, rinsed 15 min in methanol : acetic acid : water (25 : 10 : 65), visualised by staining overnight with 0.0375% Coomassie Brilliant Blue R 250 in the same solution, destained 2 h with the solution and rinsed overnight in destilled water.

The REM (relative electrophoretic mobility) values of particular protein bands were defined according to formula: REM = (100 / *M*) . *x* (*M* = interval between start and coloured front in cm, *x* = interval between start and measured band in cm).

b. *Separation of SDS-dissociated proteins*

The extracts of soluble native proteins were mixed 1 : 3 with disruption buffer (0,5M Tris HCl pH 6,8 : glycerol 4% : SDS 4% : mercaptoethanol 10%) and boiled in a water bath for 2 min. Vertical discontinuous SDS polyacrylamide gel electrophoresis was used according to Laemmli (1970). Fixation, staining and destaining were as described for separation of native proteins.

The intensity of colouring bands (native and dissociated proteins) was expressed on a five-grade increasing scale 1–5 according to Sýkorová and Hadačová (1992).

Preparation of polyclonal antibodies

Intramuscular immunization of rabbits (race Činčila velká) was applied with *FCu* antigen (isolate 67). Injection schedule: day 1 (with Freund's complete adjuvant), 14, 21, 28, (all with incomplete adjuvant), 35 (booster). 1.5 mg proteins per dose in all cases. Blood was sampled three times, in weekly intervals.

Reproduction of antiserum quality (anti-*FCu*) was evaluated by comparing the reaction of anti-*FCu* with the serum from rabbits before immunization (i.e. control) and by comparing the reaction of anti-*FCu* for each rabbit separately. Reaction of *FCu* antigens with antisera were evaluated by agar double diffusion according to Ouchterlony (1968).

IgG was prepared by permeatic gel filtration on DEAE-cellulose. Reaction of *FCu* and other *Fusarium* sp. antigens with IgG were evaluated by ELISA (DAS- and PTA-). Cross-reactions of anti-*FCu* were evaluated by testing against the antigen of *Pseudocercosporella herpotrichoides, Gaeumannomyces graminis, Rhizoctonia solani, Trichoderma viridae* (fungi isolated from winter wheat) by ELISA.

ELISA was performed in polyvinyl 96-microtitre plates (GAMA), the absorbance was measured with an automatic reader (Dynatech) at 405 nm (Krátká et al., 1996).

RESULTS AND DISCUSSION

In preliminary experiments we found that CD and MM media were most suitable for the cultivation of *FCu* isolates. This was determined by the increase of mycelium weight in a defined period (Table I). Therefore, the following results are restricted to tests with the CD and MM media – for cultivation of fungi and preparation of antigens from mycelial mats.

I. Cultivation of *Fusarium culmorum* isolates on liquid media

Medium	Isolate →																	
	46	67	62	40	47	1	35	33	5	7	21	4	11	48	6	28	29	2
MM	**	**	**	***	***	***	***	***	***	***	***	***	**	***	**	***	**	***
CD	**	**	**	**	**	**	**	**	**	**	*	**	**	*	**	**	**	*
SNAM 1	minimal growth																	
SNAM 2	minimal growth																	
SFAM	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

*	0,1–1,0 g	MM	26 days	CD	35 days
**	1,1–5,0 g	SNAM 1	35 days	SNAM 2	35 days
***	5,1–10,0 g	SFAM	35 days	——→	pathogenicity decrease

The composition of liquid media influenced the morphological and physiological character of *FCu* isolates. The pathogenicity of *FCu* isolates was not correlated with their morphological and physiological classification in any case (Table II).

The composition of liquid media affected quality and quantity of native and dissociated proteins and total protein content of antigens prepared from mycelial mats of *FCu* isolates. When the *Fusarium* isolates were cultivated on the same medium, differences were found in the quality and quantity of native and dissociated proteins. But the pathogenicity of *FCu* isolates was

II. Morphological and physiological classification of *Fusarium culmorum* isolates

Medium	Mycelium								Spores					
	growth [mg/ml]		colour of medium		character of culture		dry matter [g]		macroconidia				chlamydospores	
									number per ml	number of septa	number per ml	number of septa	number per ml	number per ml
Isolate	MM	CD	MM	CD	MM	CD	MM	CD	MM		CD		MM	CD
46	4.0	3.7	c	e	1	7	3.7	3.0	*	4.1	*	3.2	*	*
67	4.6	3.0	c	f	2	8	4.1	2.5	***	5.0	**	3.4	*	
62	2.8	3.5	d	f	1	7	2.6	2.9	*	4.0	*	2.8	**	*
40	14.3	2.8	b	f	2	8	9.9	1.9	*	3.6	*	2.7	*	
47	11.4	1.9	b	d	1	7	7.9	1.3	****	4.6	*	4.1		*
1	9.0	2.8	a	e	2	7	6.2	1.9	***	4.8	*	3.8		
35	12.8	3.2	a	b	3	5	8.8	2.2	*	5.2	*	2.0	*	
33	10.6	2.5	c	f	1	6	7.3	1.7	**	3.0		2.0		
5	9.6	2.0	a	f	2	5	6.6	1.4	****	4.3	*	3.8	*	
7	9.0	1.6	a	d	1	6	6.2	1.1	**	4.6	*	4.1	*	
21	10.3	0.9	a	c	3	8	7.1	0.6	*	4.0	*	3.6		*
4	11.3	2.5	a	f	2	6	7.8	1.7	*	4.4	*	4.3	*	
11	2.7	2.5	d	c	4	8	2.9	0.5	*	4.0	*	3.0	**	
48	10.4	1.4	a	d	2	6	7.2	1.0	–	–	*	3.0		*
6	4.0	2.5	d	e	3	5	3.7	2.0	*	4.0	*	3.2	*	*
28	12.6	2.8	c	e	1	7	8.7	1.9	**	4.8	*	3.2	*	
29	3.4	2.6	b	c	3	5	3.1	2.3	**	4.1	*	3.0	*	
2	7.5	1.8	d	c	4	6	5.2	0.7	**	5.3	*	1.7	**	*

Explanation to Table II:

character of mycelium:
1 – compact and dark pigment, air mycelium
2 – compact and dark red pigment, air mycelium
3 – compact and dark pigment, brown air mycelium
4 – compact pigmentless, air mycelium colourless
5 – compact light yellow, air mycelium colourless
6 – compact light yellow, no air mycelium
7 – compact rosy, air mycelium colourless
8 – compact rosy, no air mycelium

colour of medium:
a – red–wine red
b – light red–red
c – rosy–light red
d – yellow–brown
e – yellow
f – light yellow

number of macroconidia in 1 ml:
* 1– 100
** 101– 500
*** 501–1000
**** 1001–

number of chlamydospores in 1 ml:
* 1– 10
** 11–50

MM: Minimal medium (Puhalla, 1985)
CD: Czapek–Dox medium

growth: mycelium mats after 26 days

III. Content of proteins in antigens (Lawry et al., 1951) mg/g (dry matter of mycelium)

Medium	Isolates → (pathogenicity decrease)																	
	46	67	62	40	47	1	35	33	5	7	21	4	11	48	6	28	29	2
MM	97.7	95.6	102.2	50.8	49.5	72.8	64.0	69.0	66.4	70.6	58.6	70.0	103.3	64.8	70.8	57.3	124.7	76.3
CD	59.2	33.5	46.3	47.8	24.7	35.6	35.6	12.6	41.1	27.1	34.0	16.4	34.0	14.3	48.1	46.1	37.3	38.8

→ pathogenicity decrease

Ochr. Rostl., 33, 1997 (2): 89–102

Ochr. Rostl., *33*, 1997 (2) : 89–102

IV. Electrophoresis of native proteins

Isolate	Intensity → → →																	
	46		67		62		40		47		1		35		33		5	
REM	MM	CD	MM	CD	MM	CD	MM	CD	MM	CD	MM	CD	MM	CD	MM	CD	MM	CD
3,5			1	1						1		1						
4,7																		1
5,5			1	1						1								
6,6			1		1	1				1								1
8,6			1						1			1						1
10,5					2			1			2				1		1	
15,1															1	3	1	3
17,4													2	3				
19,1	2	2	4	1	1	1	1	3	1			1	1					
22,5	1	1	1					1				1						
24,8	2		1		1			2								1	1	
27,6										2								
29,9			1														2	
33,2			1	1 1	1						1	1						
41,6		1	1	1		1	1	1	1		1	1	1	1				
44,1	1		1		2		1		1			1	1		1	1		1
51,2				1			1											
54,3					1	1	1	1		1		1	1		1			
56,5																1	1	
61,4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
65,8				1												1		
69,9			1															
76,6												1	1					
82,5												1	1		1	1		1

→ pathogenicity decrease
REM: relative electrophoretic mobility
MM: Minimal medium (Puhalla, 1985)
CD: Czapek Dox medium

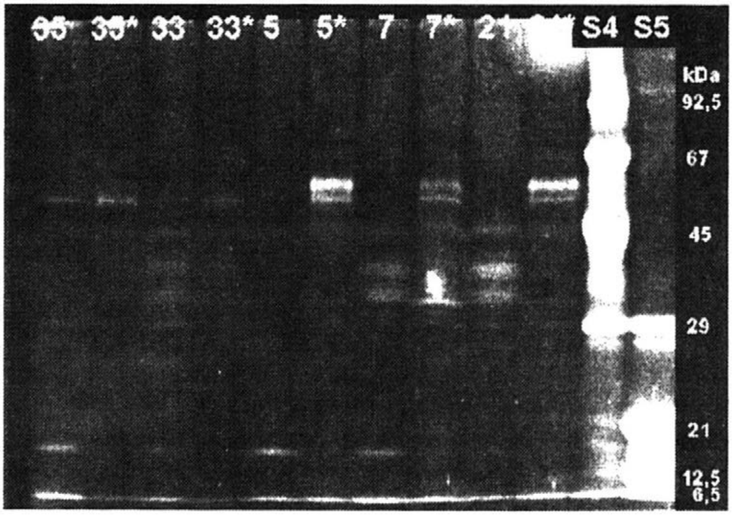
Table IV to be continued

Intensity →

7		21		4		11		48		6		28		29		2	
MM	CD	MM	CD	MM	CD	MM	CD	MM	CD	MM	CD	MM	CD	MM	CD	MM	CD
										1	1		1				
													1				
			1				1	1			1		1	1			
				1													1
	1		1			1			1			1			1		
1																	
1		1	2	3	3	2	2	2	2	1	2	2			1		
	2										1		2				2
				1	3	1	1		1								
				1	2						1				1		
1					2	1			1	1		2					
		1								1							
										1			1				
	1			1			1			1	1	1	1	1	1	1	
1		1	1		1	1		1	1			1					
									1	1	1						
			1	1		1							1				
1										1							
1	1		1			1	1	1	1	1	1	1	1		2		
					1												1
										3	1	1					

Intensity of band colouring (Sýkorová, Hadačová, 1992):

1 – very low intensity
2 – low intensity
3 – medium intensity
4 – high intensity
5 – very high intensity



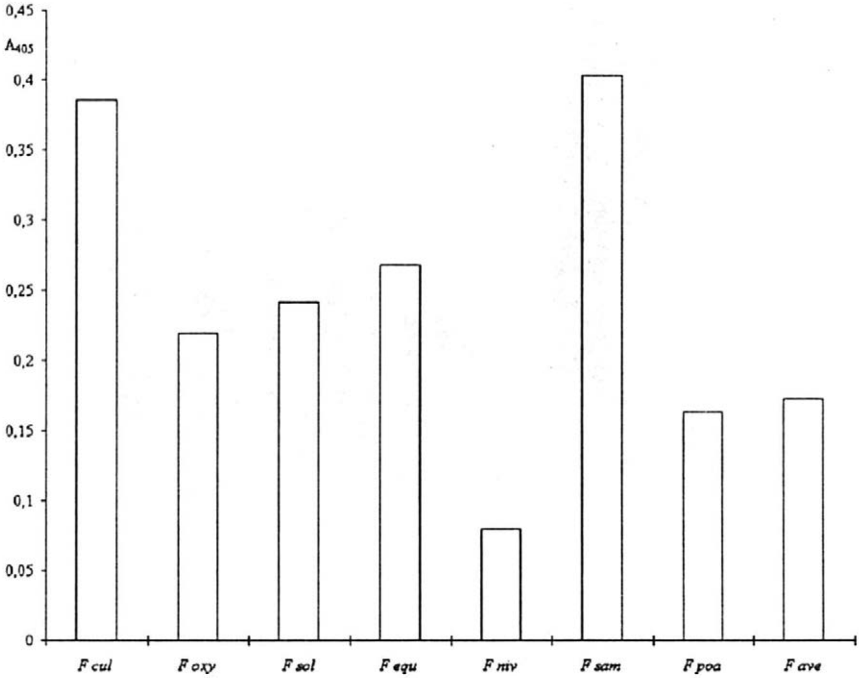
5, 7, 21, 33, 35: *Fusarium culmorum* isolates cultivated on Minimal medium
5*, 7*, 21*, 33*, 35*: *Fusarium culmorum* isolates cultivated on Czapek-Dox medium
S4, S5: standards
1. Electrophoresis of SDS dissociated proteins

not correlated with the quantity and quality of antigens protein in any case (Fig. 1, Table III and IV).

We determined the titre and specificity of anti-*FCu* with agar double diffusion tests. Titre of antiserum (immunization of rabbits with isolate no. 67) was 1/16. The anti-*FCu* reacted with all antigens of *FCu* isolates. The specificity of anti-*FCu* is low as it reacted with antigens of other *Fusarium* spp. Concentrations of proteins in antigens were 4 mg/ml and 1 mg/ml.

The two immunoenzymatic methods were used for the identification of the genus of *Fusarium.* DAS-ELISA was less specific for detection of the fungi. Reactions of IgG with purified antigens were successfully tested by PTA-ELISA (Fig. 2). The optimal concentrations of reactants were determined as follows: anti *FCu* IgG 1 μg/ml, anti rabbit IgG coupled with alkaline phosphatase 250 mU (20 μg/ml IgG), concentration of proteins in antigens 0,5–1 μg/ml. Anti-*FCu* IgG reacted with:

- all antigens prepared from isolates of *Fusarium culmorum*, differences were not observed,
- all antigens prepared from *Fusarium* spp., differences in the reaction were quantitative (Fig. 3).



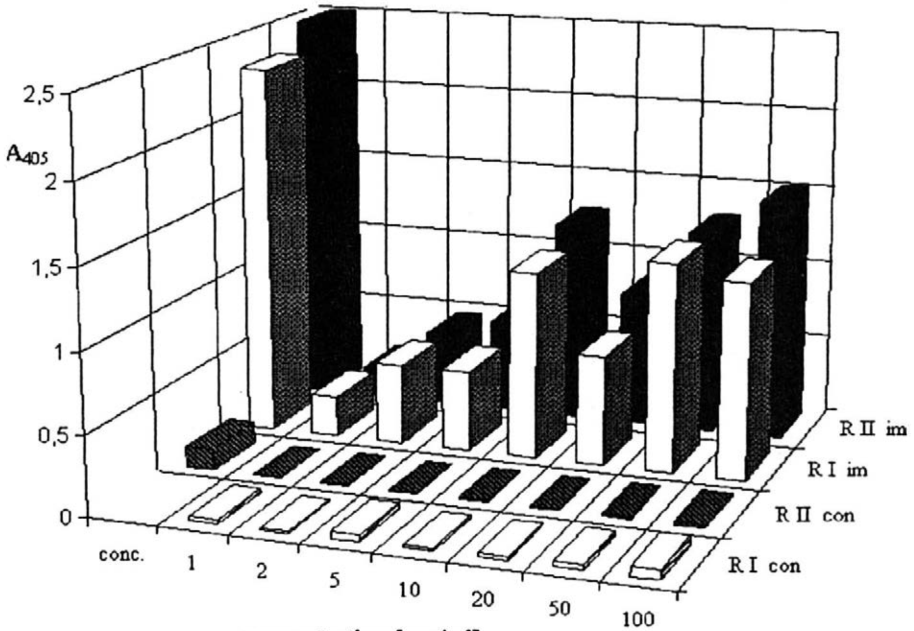
2. Reactions of anti-*FCu* IgG with other members of the genus *Fusarium*

The lowest positive absorbance was determined on the basis of both reactions – immunized and unimmunized rabbits.

There were no cross-reactions of anti-*FCu* IgG with antigens isolated from other pathogenic fungi of wheat.

The presented datas demonstrated that anti-*FCu* detected only members of the genus *Fusarium*. Quantitative differences between species could be suitable for taxonomic determinations. We had similar experiences with immunodiagnostic tests of *Phytophthora* spp. (unpublished). The polyclonal antibodies will be suitable for rapid diagnosis of *Fusarium* sp. in agronomic practice. Most species detected in the seeds of wheat are pathogenic and their harmfulness is similar to *F. culmorum*.

Only monoclonal antibodies are able to ensure the specific determination of species, subspecies or races of most pathogenic fungi. These problems are well known from the publications of many authors (comprehensive review by Dewey, 1991, 1992, 1996; Miller, Joaquim, 1993; Werres, Steffens, 1994).



R – rabbit; con – control; im – immunization

3. Reaction of anti-*FCu* IgG with *FCu* antigen (isolate n. 67)

The utilization of poly- or monoclonal antibodies depend on the demands of diagnosis specificity, the immunochemical methods (the most frequent ELISA) are in all cases rapid and unequivocal.

References

AMOUZOU-ALLADAY, E., DUNEZ, J., CLERJEAU, M. (1988): Immunoenzymatic detection of *Phytophthora fragariae* in infected strawberry plants. Phytopathology, *78*: 1022–1026.

BIELENINA, A., JEFFERS, S. J., WILCOX, W. F., JONES, A. L. (1988): Separation by proteins electrophoresis of six species of *Phytophthora* associated with deciduous fruit crops. Phytopathology, *78*: 1402–1408.

DAVIS, B. J. (1964): Discs electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci.: 121p.

DEWEY, M., EVANS, D., COLEMAN, J., PRISTLEY, R., HULL, R., HORSLEY, D., HAWES, C. (1991): Antibodies in plant science. Acta Bot. Neerl., *40*: 1–27.

DEWEY, F. M. (1992): Detection of plant-invading fungi by monoclonal antibodies. In: DUNCAN E. M., TORRANCE L. (Eds.): Techniques for the Rapid Detection of Plant Pathogens. UK, Blackwell Sci. Publ.: 47–62.

DEWEY, F. C. (1996): Production and use of monoclonal antibodies for the detection of fungi. In: BCPC Symp. Proc. No 65: Diagnostics in Crop Production: 85-91.

JONES, B. L. (1987): Identifying United States malting barley varieties by electrophoresis of hordeins and esterase enzymes. In: Biochemical identifications of varieties. Materials of III. Inter. Symp. ISTA, Leningrad, USSR: 195–199.

KOVÁČIKOVÁ, E., ONDŘEJOVÁ, J., VLASÁKOVÁ A. (1992): Vztah hostitel patogen v ontogenezi pšenice ozimé po inokulaci houbou *Fusarium culmorum*. Ochr. Rostl., *27*: 27–30.

KRÁTKÁ, J., SÝKOROVÁ, S., KYNĚROVÁ, B. (1995): The diagnosis of *Phytophthora* sp. by polyclonal antibodies the separation and character of the antigen. Ochr. Rostl., *31*: 81–92.

LAWRY, H. O., ROSENBOUGH, N. J., PARR, A. L.(1951): Protein measurement with Folin phenol reagent. J. Biol. Chem., *193*: 265–275.

LAEMMLI, V. K. (1970): Cleavage of structural proteins during assembly of the head bacteriophage T4. Nature (London), *227*: 680–685.

MANKA, M., CHELKOVSKI, J., BRAYFORD, D., VISCONTI, A., KWASNA, H., PERKOWSKI, J. (1989): *Fusarium graminearum* Schwabe (telemorph *Giberella zea* Schw. Petch) – Cultural characteristics, pathogenicity towards cereal seedlings and ability to produce mycotoxins. J. Phytopathol., *124*: 143–148.

MILLER, S. A., JOAQUIM, T. R. (1993): Diagnostics techniques for plant pathogens. In: Biotechnology in Plant Control. Wiley-Liss, Inc.: 321–339.

MESTERHÁZY, J., BARTÓK, T. (1992): Resistance and pathogenicity influencing toxin (DON) contamination of wheat varieties following *Fusarium* infection. Hodow. Rosl. Aklimat. i Nasiennic., *37*, part II: 9–15.

ONDŘEJOVÁ, J., KOVÁČIKOVÁ, E. (1992): The frequency of *Fusarium* occurence in roots of affected genotypes of winter wheat in the Czech Republic. In: Proc. XII Czechoslov. Plant Prot. Conference, Praha: 181–182.

OUCHTERLONY, Ö. (1968): Handbook of Immunodiffusion and Immunoelectrophoresis. Ann Arbor, Michigan, Ann. Arbor Scient. Publ.

PUHALLA, J. E. (1985): Classification of strains of *Fusarium oxysporum* on the basis of vegetative compability. Can. J. Bot., *63*: 179–183.

RIBEIRO, O. K. (1978): A source Book of the Genus *Phytophthora*. J. Cramer, Ganter Verlag: 417p.

SÝKOROVÁ, S., HADAČOVÁ, V. (1992): Methodical notes to the use of esterase isoenzymes in characterization of spring barley (*Hordeum vulgare)* varieties. Scientia Agric. Bohemoslov., *24*: 15–28.

WERRES, S., STEFFENS, C. (1994): Immunological techniques used with fungal plant pathogens: aspects of antigens, antibodies and assays for diagnosis. Ann. appl. Biol., *125*: 615–643.
WIESSE, M. V. (1977): Compendium of Wheat Diseases. 2nd ed. St. Paul, Amer. Phytopatol. Soc.: 16–17.

Received March 17, 1997

Diagnostika *Fusarium culmorum* pomocí polyklonálních protilátek – příprava a vlastnosti antigenů a protilátek

Osmnáct monosporových izolátů *Fusarium culmorum* (*FCu*) s rozdílnou patogenitou bylo kultivováno na pěti tekutých médiích. Nejvhodnější pro další využití byly určeny média Czapek Dox a Minimální médium. Bylo zjíštěno, že media ovlivňují jak morfologické, tak fyziologické vlastnosti jednotlivých izolátů. Sledované vlastnosti příp. jejich změny nejsou v korelaci s patogenitou sledovaných izolátů *FCu*. Antigeny byly připraveny z myceliální masy extrakcí ve speciálním pufru a purifikovány centrifugací a ultracentrifugací supernatantu. Tekutá média ovlivnila kvantitu i kvalitu protenů antigenů. Bylo zjištěno, že obsah a složení proteinů, příp. jejich změny nejsou v korelaci s patogenitou izolátů *FCu*. Z krve imunizovaných králíků antigenem *FCu* byly získány polyklonální protilátky a byla sledována jejich citlivost a specifičnost. Nebyly zjištěny rozdílné reakce mezi jednotlivými izoláty *FCu*. Anti-*FCu* IgG reagovalo s antigeny dalších druhů rodu *Fusarium (oxysporum, solani, equisetum, nivale, sambucinum, poae, avenaceum)*, rozdíly byly kvantitativní. Nebyly zjištěny křížové reakce s dalšími patogeny izolovanými z pšenice (*Rhizoctonia solani, Gaeumannomyces graminis, Pseudocercosporella herpotrichoides, Trichoderma viridae*). Reakce antisér a IgG s antigeny byly sledovány pomocí dvojité difúze v agaru a ELISA.

Fusarium culmorum; média; izoláty; morfologické a fyziologické vlastnosti; antigen; proteiny; polyklonální protilátky; citlivost; specifičnost; křížové reakce

---

Contact address:

RNDr. Jiřina Krátká, DrSc., Výzkumný ústav rostlinné výroby
odbor rostlinolékařství, 161 06 Praha 6-Ruzyně, Česká republika
tel: 00 420 2 36 08 51, fax: 00 420 2 36 52 29

Ochr. Rostl., *33*, 1997 (2) : 103–112

THE APPLE BLOSSOMS FROST DAMAGE DECREASED BY BIOLOGICAL CONTROL AGENTS OF INA$^+$ *Pseudomonas syringae**

Alois BILAVČÍK, Blanka KOKOŠKOVÁ, Jiří ZÁMEČNÍK

Research Institute of Crop Production – Division of Plant Medicine, Prague, Czech Republic

Abstract: We have concerned for selection of antagonistic bacterial strains reducing the population of ice nucleation active (INA$^+$) *Pseudomonas syringae (Ps)* bacteria on apple blossoms with an aim of their possible use in biological control of fruit trees from frost injury. About 25 % of 71 screened bacterial strains produced antibiotic substances which inhibited the growth of INA$^+$ strain *Ps* CCM 4073 *in vitro*. The selected antagonistic strains were non-pathogenic and also ice nucleation non-active (INA$^-$). The three selected INA$^-$ antagonistic strains with the highest antibiosis *in vitro* were tested in experiments on detached flowering shoots of apple cv. Spartan in environmental chamber. Antagonistic strains induced freezing of 50% of separated flowers from –5.2 to –7.9 °C, however strain INA$^+$ *Ps* CCM 4073 at –3.9 °C. The most effective INA$^-$ antagonistic strain lowered the supercooling temperature by 4 °C and consequently avoided frost injury of separated blossoms. However the blossoms connected with spur froze at –4.3 °C in average. The intrinsic ice nuclei of plants were found in shoots. The ice from spurs propagated to the blossoms and causes frost injury. Although the intrinsic ice nuclei of apple tree could weaken the effect of INA$^-$ antagonistic bacteria, there is a space for their use. Especially during radiative spring frosts, when the temperature of tender flowers is lower then spur temperature, the effect of INA$^-$ antagonistic bacteria could reach greater benefit in lowering frost injury.

ice nucleation activity; *Pseudomonas syringae*; frost injury; biological control

Flowers of fruit trees are often injured by spring frosts in the nature. Frost damage of plant cells is caused by growth of ice crystals and/or by dehydration of plant tissue during withdrawing of water from cells to form of ice crystals. Plants can survive under frost conditions by tolerance of ice crystals in their tissues or by avoidance of ice crystal forming. One of the avoidance mechanism is for example supercooling, e.g. decreasing of temperature below the melting point of water without forming of ice crystals.

* This work was supported by the Grant Agency of The Czech Republic grant no. 513/94/1520.

It is well known, that small amounts of water are able to supercool down to –40 °C, the bigger volumes to temperatures from –10 to –20 °C. Certain number of water molecules must order into lattice structure to trigger ice crystals forming. Close –40 °C the crystallisation of ice occurs spontaneously (homogennic nucleation). At few degrees below zero ice crystallises in supercooled water only in addition of ice crystals or suitable initiators (heterogennic nucleation). In contrast, different inorganic compounds pertain to heterogeneous ice nuclei active between –8 to –15 °C. Certain organic compounds in crystal form, e.g. steroids, aminoacids, proteins, or some living organisms, e.g. bacteria (*Pseudomonas syringae*, *Erwinia herbicola*, *Xanthomonas campestris* pv. *translucens*) are active as an ice nuclei at the range of temperature from zero to –5 °C.

Supercooling ability of different plant tissues is related to their structure, ontogenetic development, season, weather condition and so on. Supercooling of water in huge parenchymatic cells and xylem vessels is in an unstable state, which lasts at most for few hours. Small cells and cells with divided vacuoles are able to supercool to lower levels. Bud meristems and xylem parenchyma of woody perennials from temperate zone are able to supercool close to –40 °C (Ashworth, Wisniewski, 1988). Generative organs as flower buds, flowers and fruitlets are very sensitive to frost at the beginning of growth period. Strang et al. (1980) found the increasing of frost injury in pear from pink flower to small fruit stage, also wet flowers were more damaged than dry ones. Critical temperatures for supercooling of blossoms are dependent on plant species and duration of frost. Frost damage of intact blossom or fruitlet could be caused only by one nucleus. When ice starts to form on plant surface, ice crystals grow rapidly through stomatos, hydatodes, lenticels and/or through wounds to inner parts of the plant (Lindow, 1983b). Dostálek (1957) found differences in frost resistance of blossoms of different fruit trees only at temperatures at which the frost damage appears whereas the lowest surviving temperature of various stone blossoms and fruits did not differ much from –4 °C.

The highest ice nucleation activity of plant tissues themselves (endogenous ice nuclei) arises only in certain sorts of tissues over –5 °C. The endogenous ice nucleation of spur from flowering apple shoot appears at –4.9 °C (Bilavčík, Zámečník, 1995).

About 95% of ice nuclei active from 0 to –5 °C on the leaf surface show bacterial origin (Lindow et al., 1982). Only 0.1–10.0% of bacteria living on plant surfaces belong to strains that are ice nucleation active. Population frequencies of plant pathogenic INA^+ bacteria, especially *Pseudomonas syringae* correlate to frost damage of blossoms in spring (Lindow et al., 1982; Lindow, Connel, 1984). The natural antagonism exists between INA^+ bacteria and bacteria that are INA^-. This natural antagonistic features can be enhanced and used for decreasing of INA^+ bacteria (Lindow, 1983a).

The aim of our experiments was:

a) *in vitro* selection of saprophytic bacteria with antagonistic activity against INA^+ bacteria,
b) selection of non-ice nucleation active strains screened from isolated antagonistic bacteria,
c) testing the most effective INA^- antagonists against INA^+ *Pseudomonas syringae* on separated apple blossoms in controlled conditions.

MATERIAL AND METHODS

Bacterial strains

INA^+ strain *Ps* CCM 4073 was used as standard in all experiments. This plant pathogenic INA^+ bacterial strain which mean ice nucleation activity (MNT) is expressed at –3 °C in concentration 10^8 CFU ml^{-1} was isolated of caraway (Sykita et al., 1988). INA^+ strain *Ps* CCM 4073 was maintained on nutrient broth-yeast extract (NBY) (Vidaver, 1967) and cultivated on King B medium (KB) (King et al., 1954).

Potential antagonists

Selection of bacterial strains with desirable features (antagonistic activity against INA^+ strain *Ps* CCM 4073, non-pathogenicity, ice nucleation non-activity) was carried out on 71 isolates of *Pseudomonas* sp. These strains were isolated from different host plants, mainly from fruit trees in commercial orchards in the Czech Republic and maintained in collection of department of bacteriology at Research Institute of Crop Production.

Selection of antagonists

Potential antagonistic strains, including INA^+ *Ps* strain CCM 4073, were cultivated on KB agar at 22 ± 2 °C for 48h. Bacterial suspension of INA^+

strain *Ps* CCM 4073 diluted in sterile water was added in NBY medium to reach the final concentration (10^6 CFU ml^{-1}) and poured into Petri dishes. After that potential antagonists were streaked on surface of agar plates in lines and incubated at 22 ± 2 °C. The diameter of inhibition zones were measured after 48–72 hours. The size of inhibition zone corresponds to amount and/or efficiency of antibiotic substances produced by antagonistic strains, resp. difunded to agar (Kokošková, 1993).

The plant pathogenicity of bacterial strains was detected by biochemical (oxidase test) and biological tests (test pathogenicity on tobacco).

Selected antagonistic strains, including INA^+ strain CCM 4073, were cultivated on KB agar at 22 ± 2 °C for 48h before measuring of ice nucleation activity. Suspension of bacterial strains was prepared and diluted to concentration 10^8 CFU ml^{-1} by sterile water. MNT of thirty 10 μl droplets of each strain was measured by Differential Thermal Analysis (DTA) (Burke et al., 1976).

Used antagonistic INA^- strains were isolated from cabbage (*P. sp.* ZE7), tomato (*P. sp.* SP1, *P. sp.* SP2). Strains *P. f.* CCM 2115 and INA^+ strain CCM 4073 were from Czech Collection of Microorganisms.

Preparation of INA^+ strain *Ps* CCM 4073 and antagonistic bacteria for frost tests on blossoms

INA^+ strain *Ps* CCM 4073 was incubated on KB agar for 48h at 22 ± 2 °C for laboratory experiments. Bacterial suspension of INA^+ strain *Ps* CCM 4073 was prepared by dilution with sterile water approximately to concentration 10^6 CFU ml^{-1}. Bacterial suspensions of antagonistic strains were prepared by the same way except they were diluted to concentration 10^8 CFU ml^{-1} for inoculation of apple blossoms.

I. The application scheme of selected INA^- antagonistic bacterial strains on flowering apple shoots

Variant	Time [h]		
	0	24	48
INA^+ *Ps* CCM 4073	–	INA^+ *Ps* CCM 4073	**
INA^- *Ps. sp.* ANTG*	INA^- *Ps. sp.* ANTG*	INA^+ *Ps* CCM 4073	**
Control	sterile water	sterile water	**

*ANTG – antagonistic; ** freezing test on detached blossom

Plant material

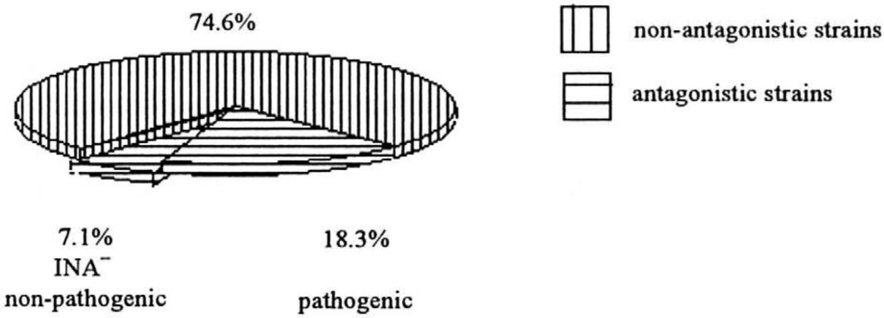
Laboratory experiments were carried out on flowering apple shoots cv. Spartan collected from Research Institute of Crop Protection orchards. Apple shoots were cut in pink flower stage in May, 1994 and maintained in flasks with tap water in controlled conditions (15/12 °C, 70% RH, 12h light period) until full blooming.

Frost tests

Flowering apple shoots were sprayed to runoff with the most effective antagonistic bacterial strains *P. sp.* SP1, *P. sp.* SP2, *P. f.* CCM 2115. Control variant was treated with sterile water. The shoots were maintained under controlled conditions (15/12 °C, 90% RH, 12h light period). Twenty-four hours later each of variants was sprayed to runoff with INA^+ strain *Ps* CCM 4073 (10^6 CFU ml^{-1}), except control, that was sprayed with sterile water. After another 24h under the same conditions, individual variants were exposed to frost stress. Thiry-five flowers of each variant were detached from shoot, to prevent distortion of nucleation temperature due to endogenous ice nuclei in spurs (Bilavčík et al., 1994), and stressed by frost on thermobattery. The thermobattery was composed from 35 measuring and 35 reference thermocouples (Ko, Cu) connected in series into the Line Recorder TZ 4620 (Tesla). Each of measuring thermocouples were put as near as possible to the pistil, which is the most frost sensitive part of flower. The reference thermocouples were free situated in close neighbourhood. The thermobattery filled with detached flowers was placed in the freezer (Marközi MHM-52) and have been frozen at cooling rate approximately 0.34 °C min^{-1} to –15 °C. The freezing events were measured by DTA. The output from thermobattery of 35 flowers was evaluated by positive variation. The curves represents cumulative number of frozen blossoms (100% = all blossoms were frozen).

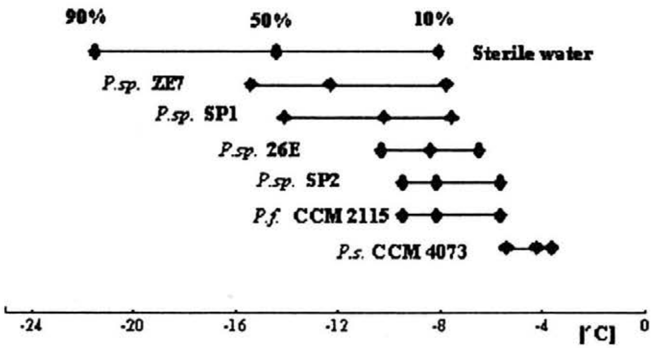
RESULTS AND DISCUSSION

Totally 18 strains of 71 screened pseudomonads were antagonistic against *Ps* CCM 4073, but only 5 of them were non-pathogenic (Fig. 1). The ice nucleation activity of the five selected strains: *P. sp.* SP1, *P. sp.* SP2, *P. f.* CCM 2115, *P. sp.* ZE7 and *P. sp.* 26E is showed in Fig 2. Low ice nucleation activity of all selected strains (below –8 °C) was in contrary to findings of



1. Percemtual proportion of 71 investigated bacterial strains according to their antagonistic, pathogenic and ice nucleation features was evaluated. We selected 25.4% strains producing antibiotic substances which inhibited INA^+ strains *Ps* CCM 4073 *in vitro*. Only 7.1% of them were INA^- non-pathogenic antagonistic strains, which were used in other experiments

Gross et al. (1983) that detected from 30 to 90% INA^+ bacteria from the total amount of isolated bacteria from fruit trees in orchards in May. Our results could be possibly influenced by different collection time. Because for example, Olive and McCarter (1988) found the population of INA^+ bacteria fluctuating from 0 to 1.7×10^5 CFU g^{-1} green tissue during one year. It is known that production of bacteriocins depends on many factors, for example kind of nutrient medium, layer of agar, laboratory temperature, stage of bacterial cultures, time of storage and kind of maintained medium (Vidaver, 1983; Beer et al., 1984). Low frequency, only 5, of selected antagonists resp. strains producing antibiotic substances, which inhibited

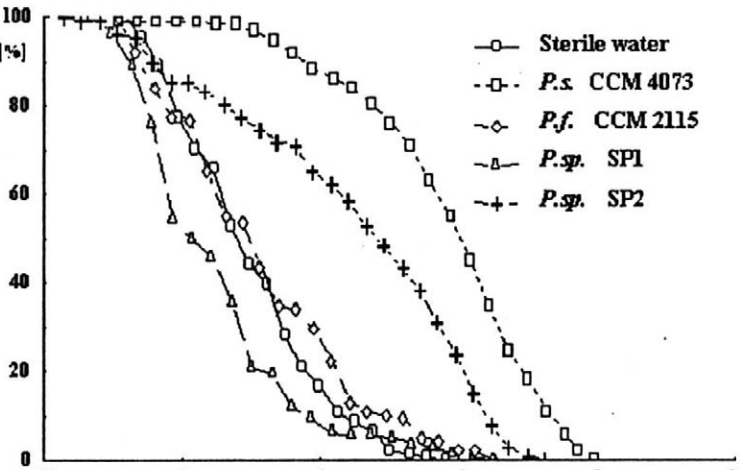


2. The ice nucleation activity of isolated non-pathogenic INA^- antagonistic bacterial strains. The bacterial strains were diluted to 10^8 CFU ml^{-1} in sterile water. Ice nucleation activity of thirty 10 μl droplets of each strain was measured by DTA

INA$^+$ *Ps* CCM 4073 *in vitro*, could rely on longer time of storage of potential antagonists in laboratory conditions before their screening (Kokošková, 1995).

Frost treatments on apple flowers were conducted on three isolates *P. sp.* SP1, *P. sp.* SP2, *P. f.* CCM 2115. Strains *P. sp.* ZE7 and *P. sp.* 26E were excluded from experiments because of their relatively low antagonistic ability *in vitro*.

Mean ice nucleation temperature of detached flowers sprayed with sterile water was –7.2 °C Flowers treated with INA$^+$ strain CCM 4073 showed MNT 3.9 °C MNT of flowers treated with three selected strains were –5.2 °C (*P. sp.* SP2), and –7.0 °C (*P. f.* 2115) and –7.9 °C (*P. sp.* SP1) (Fig. 3.) Similarly Proebsting and Gross (1988) masured MNT at –4.0 °C of apple flowers detached from trees sprayed with INA$^+$ bacterial strain (1.7 x 10^8 CFU ml^{-1}). The shift of freezing of flowers treated with INA$^-$ antagonistic strain *P. sp.* SP1 close to –8 °C was unexpectedly high, although the strain *P. sp.* SP1 did not show the highest antibiosis *in vitro*. On the other hand one of the most promising antagonistic strains *P. sp.* SP2 lowered the MNT of



3. Relative frequency of frozen blossoms [%] sprayed with isolated non-pathogenic INA$^-$ antagonistic bacterial strains *P. sp.* SP1, *P. sp.* SP2, *P. f.* CCM 2115, sterile water and INA$^+$ strain *Ps* CCM 4073. The concentration of non-pathogenic INA$^-$ antagonistic bacterial strains was 10^8 CFU ml^{-1}and *Ps* CCM 4073 10^6 CFU ml^{-1}. The output from thermobattery of 35 flowers was evaluated by positive variance. The curves represents cumulative number of frozen blossoms (100% = all blossoms were frozen)

treated flowers only to –5.2 °C. These results support the idea of Lindow (1988) that *in vitro* selected antagonistic strains need not exhibit the same level of antagonistic features as *in vivo* on plant surfaces. Lindow and Connel (1984) obtained similar results. They found that almond flowers during –3 °C frost in orchard were 50% less damaged due to reduction of INA^+ bacteria by antagonistic bacteria or bactericides. In contrary to Andrews et al. (1986) set out that not only control of INA^+ bacteria is sufficient enough in frosts protection of flowers below –5 °C, because of endogenous ice nuclei intrinsic to plant tissues. According to Andrews et al. (1986) the INA^+ bacteria can caused frost damage during mild spring frosts. If the temperature of flowers colonised by INA^+ bacteria is lower than spur temperature, the ice formation due to exogenous ice nuclei can occur at higher temperature then ice formation caused by endogenous ice nuclei in spurs. Such conditions can formed during a clear night, when the heat loss of flowers is much higher than the heat loss of spurs.

In accordance with our laboratory results the most effective INA^- antagonistic strain *P. sp.* SP1 reducing INA^+ bacterial population of *Ps* CCM 4073 lowered the ice nucleation of apple flowers from –3.9 to –7.9 °C. But the endogenous ice nuclei localised in spur part of flowering apple shoot (Bilavčík, Zámečník, 1995) shift the ice nucleation temperature approximately to –4.9 °C and cause following freezing of flowers.

However, frost protection of apple flowers by biological control agents of INA^+ bacterial population is limited through endogenous ice nuclei associated with plant tissues, during radiative spring frosts, when the conditions allow to decrease the temperature of tender flowers few degrees lower then spur temperature due to their mass differences, the effect of antagonistic bacteria could reach much greater benefit in frost protection.

Literature

ANDREWS, P. K., PROEBSTING, E. L., Jr., GROSS, D. C. (1986): Ice nucleation and supercooling in freeze-sensitive peach and sweet cherry tissues. J. Amer. Soc. Hort. Sci., *111*: 232–236.

ASHWORTH, E. N., WISNIEWSKI, M. E. (1991): Response of fruit tree tissues to freezing temperatures. In: Abstracts Conf. Breeding fruit crops for cold climates., 85th ASHS Annual Meeting/33rd CSHS Annual Meeting East Lansing, Mich. 1988, Hort. Sci., *26*: 501–504.

BEER, S. V., RUNDLE, J. R., NORELLI, J. L. (1984): Recent progress in the development of biological control for fire blight. A review. Acta Hort., *151*: 195–201.
BILAVČÍK, A., ZÁMEČNÍK, J. (1996): The ice nucleation in plants: A limitation of frost survival of apple flowers. In: Abstr. NJF Sem.: New biological approaches to understand and improve winter survival of plants. April 11. 13. 1996, Koldkaergard near Aarhus, Denmark.
BILAVČÍK, A., ZÁMEČNÍK, J., KOKOŠKOVÁ, B. (1994): Decrease of frost damage of apple tree blossoms by antagonistic bacteria to INA^{+} bacteria. In: 7th Inter. Congr. Bacteriol. and Appl. Microbiol. Divis. Prague, Czech Republic, July 3.–8., 1994: 258.
BURKE, M. J., GUSTA, L. V., QUAMME, H. A., WEISER, C. J., LI, P. H. (1976): Freezing and injury in plants. Annu. Rev. Plant Physiol., *27*: 507–528.
DOSTÁLEK, J. (1957): Zmrzání a přechlazování květů ovocných dřevin. Rostl. Výr., *8*: 983–998.
GROSS, D. C., CODY, Y. S., PROEBSTING, E. L., Jr., RADAMAKER, G. K., SPOTTS, R. A. (1983): Distribution, population dynamics, and characteristic of ice nucleation-active bacteria in deciduous fruit tree orchards. Appl. Environ. Microbiol., *46*: 1370–1379.
KOKOŠKOVÁ, B. (1993): Účinnost antagonistických bakterií a chemikálií proti bakteriím *Erwinia amylovora* v podmínkách *in vitro*. Ochr. Rostl., *29* :31–39.
KOKOŠKOVÁ, B. (1995): Biologická ochrana proti původci spály růžovitých, *Erwinia amylovora*. [Dissertation.] 184p.
LINDOW, S. E. (1983a): Methods of preventing frost injury caused by epiphytic ice-nucleation-active bacteria. Plant Dis., *67*: 327–333.
LINDOW, S. E. (1983b): The role of bacterial ice nucleation in frost injury to plants. Ann. Rev. Phytopatol., *21*: 383–384.
LINDOW, S. E., CONNELL, J. H. (1984): Reduction of frost injury to almond by control of ice nucleation active bacteria. J. Amer. Soc. Hort. Sci., *109*: 48–53.
LINDOW, S. E., HIRANO, S. S., BARCHET, W. R., ARNY, D. C., UPPER, C. D. (1982): Relationship between ice nucleation frequency of bacteria and frost injury. Plant Physiol., *70*: 1090–1093.
LINDOW, S. E. (1988): Lack of correlation of *in vitro* antibiosis with antagonism of ice nucleation active bacteria on leaf surfaces by non-ice nucleation active bacteria. Phytopathology, *78*: 444–450.
OLIVE, J. V., McCARTER, S. M. (1988): Occurrence and nature of ice nucleation-active strains of *Pseudomonas syringae* on apple and peach trees in Georgia. Plant Disease, *72*: 837–847.
PROEBSTING, E. L., Jr., GROSS, D. C. (1988): Field evaluations of frost injury to deciduous fruit trees as influenced by ice nucleation-active *Pseudomonas* syringae. J. Amer. Soc. Hort. Sci., *113*: 498–506.
SIKYTA, B., HEGEROVÁ, H., KŮDELA,V. (1988): Czech Patent, 1875–1888.
STRANG, J. G., LOMBARD, P. B., WESTWOOD, M. N., WEISER, C. J. (1980): Effect of duration and rate of freezing and tissue hydratation on 'Bartlett' pear buds, flowers, and small fruit. J. Amer. Soc. Hort. Sci., *105*: 102–107.

VIDAVER, A. K. (1967): Synthetic and complex media for the rapid detection of fluorescence of phytopathogenic pseudomonads: Effect of the carbon source. Appl. Microbiol., *15*: 1523–1524.
VIDAVER, A. K. (1983): Bacteriocins: The lure and the reality. Plant. Dis., *67*: 471–475.

Received February 4, 1997

Pokles mrazového poškození květů jabloní po aplikaci antagonistických bakterií

Zaměřili jsme se na selekci antagonistických bakterií redukujících populace ledově nukleačně aktivních (INA^+) bakterií *Pseudomonas syringae* na květech jabloní s cílem jejich možného využití v protimrazové ochraně ovocných dřevin během vegetace. Bylo testováno 71 bakteriálních kmenů, z nichž přibližně 25 % produkovalo antibiotické substance, které inhibovaly růst INA^+ kmenu *Ps* CCM 4073 v podmínkách *in vitro*. Vybrané antagonistické kmeny byly nepatogenní a také ledově nukleačně neaktivní (INA^-). Tři vybrané INA^- antagonistické kmeny s nejvyšší antibiózou *in vitro* byly testovány na oddělených kvetoucích výhonech jabloně odrůdy Spartan v řízených podmínkách. Květy ošetřené antagonistickými kmeny mrzly při teplotách –5,2 až –7,9 °C, zatímco květy ošetřené INA^+ kmenem *Ps* CCM 4073 mrzly už při teplotě –3,9 °C. Nejefektivnější INA^- antagonistický kmen *P. sp.* SP1 snižoval teplotu podchlazení o 4 °C a tím omezoval mrazové poškození oddělených jabloňových květů. Avšak květy spojené s brachyblasty mrzly v průměru při teplotě –4,3 °C. Zjistilo se, že teplota mrznutí květů byla závislá i na existenci vnitřních ledových krystalizačních jader, nacházejících se v dřevní části výhonů jabloně. Ledové krystaly šířící se z výhonů do květů způsobovaly mrazová poškození a snižovaly tak částečně účinnost INA^- antagonistických bakteriálních kmenů. Přesto byl pozitivní účinek aplikovaných INA^- antagonistických bakterií velmi výrazný. Lze předpokládat, že obzvláště během jarních radiačních mrazíků, když teplota rašících květů je nižší než teplota výhonu, mohou antagonisté významně omezit poškození rostlin mrazem.

ledově nukleační aktivita; *Pseudomonas syringae*; protimrazová ochrana; biologická kontrola

---

Contact address:

Ing. Blanka Kokošková, CSc., Výzkumný ústav rostlinné výroby
odbor rostlinolékařství, 161 06 Praha 6-Ruzyně, Česká republika
tel.: 00 420 2 360 851, fax: 00 420 2 365 229, e-mail: kokoskova@library.vurv.cz

Ochr. Rostl., *33*, 1997 (2) : 113–123

SUSCEPTIBILITY OF KOCHIA (*Kochia scoparia* s. l.) TO SOME HERBICIDES

Daniela CHODOVÁ, Jan MIKULKA

Research Institute of Crop Production – Division of Agroecology, Prague, Czech Republic

Abstract: Some herbicides and their mixtures were tested for their effects on kochia (*Kochia scoparia* s. l.), which has spread in areas along railroad tracks. Plants were grown under defined conditions or in a screened enclosure in containers under natural conditions. Seeds were collected in the areas of railroad stations and sown into containers, or plants taken at railroad stations were directly set into them. Herbicides were applied to kochia plants at different developmental stages. Herbicide efficacy was expressed as percent of killed plants. Applications to plants grown from seeds under defined conditions resulted in 100% efficacy of herbicide Arsenal (250 g of the active ingredient imazapyr) at a dose of 4 l/ha within 40 days from treatment. Herbicide Grodyl 75 WG (75 g of amidosulfuron as a.i.) gave 18% efficacy at a dose of 80 g/ha within 40 days. In the screened enclosure, Arsenal application at a dose of 4 l/ha caused within 6 weeks 96% mortality of plants treated at the stage of seedlings up to the height of 3 cm, while 83% efficacy was observed if plants were treated at the height of 3–10 cm. There were a few very young solitary plants of kochia that survived repeated applications of Arsenal at a dose of 4 l/ha. The herbicide Bladex (500 g of cyanazine as a.i.) had 100% efficacy within 7 days at a dose of 3 l/ha on plants 3–10 cm high, and within 21 days at a height of 10–20 cm. The mixture of herbicides Arsenal + Bladex at a dose of 4 + 3 l/ha and at half that dose showed 100% efficacy of control within 7 days when kochia was treated at the height of 3–10 cm, and at the height of 10–20 cm within a fortnight. Herbicides Grodyl 75 WG and Shorty (90 g of imazethapyr as a.i. + 10 g of imazapyr as a.i.) were not effective at all. To control kochia efficiently it should be treated at younger developmental stages. Applications should be repeated on locations with a high density of plants or great seed reserves in the soil. The experimental results have demonstrated very good efficacy of Bladex and the mixture Arsenal + Bladex to control kochia.

Kochia scoparia s. l.; susceptibility to herbicides; imazapyr; cyanazin; amidosulfuron

Changes in the spectrum of weed species in weed communities occur continuously. But the intensity of changes is different at various times, and each change has its own causes. On farm land, they are evoked by changes in the intensity of crop production, by changes in technologies or cultivars, and by many other factors. Weed species that can adapt to new conditions more readily are propagated quickly and are spreading in the environs. On the other hand, weed species that do not adapt well are gradually disappearing from weed communities. Widespread and repeated applications of some herbicidal preparations resulted in the formation of populations resistant to herbicides with a specific mechanism of action (Chodová, Mikulka, 1990).

Changes in weed communities also occur on nonagricultural land. Very interesting weed communities are settled in areas along railroad tracks, around railroad stations and in surrounding areas. Railroad traffic brings numerous weed species from neighbouring countries to this country. Their introduction and subsequent distribution on farm land are risky.

Our surveys have shown that kochia (*Kochia scoparia* s.l.) has become widely distributed in areas along the railroad tracks and stations (railroad stations Bubny, Vysočany, Vršovice, Nymburk, Kolín, Čáslav). Hejný et al. (1973) indicated ports and points of transshipment on the Elbe river, grain silos and oil crop stores as potential sources of such introduction.

The kochia is an annual plant from the Chenopodiaceae family; it was originally an ornamental plant that then got established as a weed on arable land (Guttieri et al.,1995). Its stalk can be as high as 1 m. The plant is partly self-pollinated, with green flowers, it is propagated exclusively by seeds. Flowers appear 8–12 weeks after germination, seeds in soil remain dormant for up to three years (Thill et al., 1991).

It is one of the most important dicotyledonous weeds in the USA and in Canada that are controlled by chlorsulfuron applications in grain crops. The existence of a biotype resistant to herbicides from the group of imidazolinones and sulfonylureas was demonstrated (Primiani et al., 1990; Stallings et al., 1995). The above groups of herbicides inhibit the enzyme acetolactate synthase (Devine et al., 1991). The latter enzyme has been modified in the resistant biotype in such a way that it is less susceptible to the above herbicides (Saari et al., 1990, 1994). A weed resistant to one herbicide remains susceptible to herbicides with another mechanism of action (Friesen et al., 1993).

Pursuant to guidelines of the Ministry of Agriculture (1995) and according to foreign experience (Torstensson, Lindholm, 1988; Arsenović et al., 1991) the herbicide Arsenal is recommended as suitable for weed control along railroad tracks.

The objective of the first study on kochia was to specify a growth stage appropriate for applications and testing of herbicides or their mixtures for the control of this weed. We also tried to find whether there was not a resistant biotype in the population of the plants included in our tests until now.

MATERIAL AND METHODS

Experimental plants

Plants of kochia for trials were collected by two procedures. Entire plants with seeds were collected in the areas of railroad stations (Kolín, Bubny, Vysočany, Vršovice, Libeň) in fall 1995, dried in a laboratory and seeds were husked. Young plants 2–5 cm high with roots were collected in the areas of railroad stations in May 1996, wrapped up in microtene bags, and on the same day planted into 10 x 10 cm plastic containers with soil. Plants 2–5 m in height were collected. The plants were kept in a screened enclosure where they rooted successfully within about a week.

Plant growing

Plants were grown after seeding in plastic containers and placed either in a growth chamber under defined conditions: photoperiod 10 h light, 14 h darkness, temperature 18–20 °C, humidity 35%, light intensity 23 W/m^2 in the period December–March, or in a screened enclosure in the period May to October together with the young transplants from areas of railroad stations. The plants were watered from below.

Plants were treated with herbicides at several growth stages: seedlings (i.e. about 1 cm in height) up to the height of 3 cm, 3–10 cm, 10–20 cm, 20–50 cm, respectively.

Plant number per container before treatment was reduced to 10, and to five in the oldest plants 20–50 cm high. Twenty plants were treated within one replication in the growth chamber, while in the screened enclosure each treatment consisted of 50 plants with three replications.

Plant treatment

Herbicide	Active ingredient [g per l of chemical]
Arsenal	250 imazapyr
Bladex 50 SC	500 cyanazin
Grodyl 75 WG	75 amidosulfuron
Shorty	90 imazethapyr + 10 imazapyr

The plants were treated with a hand sprayer at an amount of 50 ml of water per 1 m^2 to apply the herbicide doses indicated in the tables. Besides recommended doses, lower concentrations or several times higher ones were used. Plants without any herbicide applications were grown as controls. Effects on kochia were evaluated by counting the number of killed plants and these expressed in percent of the total to show the level of efficacy.

RESULTS AND DISCUSSION

Table I shows the results of trials with kochia grown in a growth chamber under defined conditions.

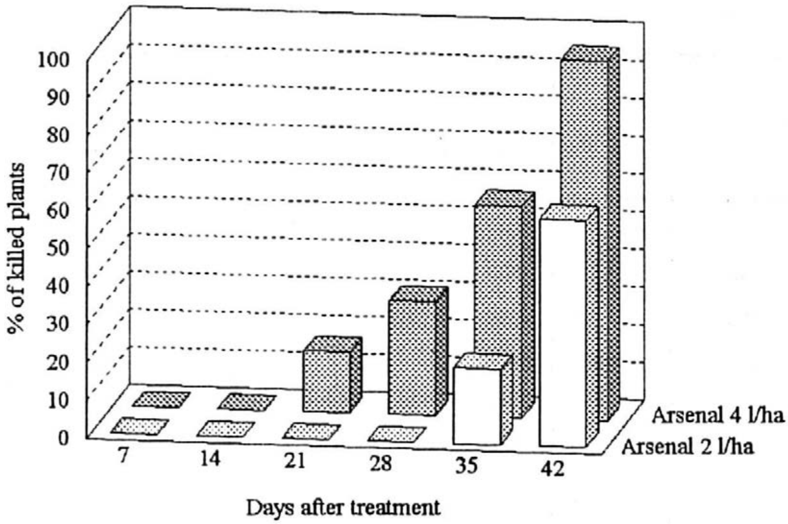
I. The efficacy of herbicides Arsenal and Grodyl 75 WG in the control of *Kochia scoparia* s. l. expressed as the percentage of killed plants

Herbicide	Dose per ha	Percentage of killed plants – days after treatment								
		7	11	13	15	18	21	30	38	40
Arsenal	4 l	0	0	0	7	28	42	64	64	100
	8 l	0	0	0	25	42	42	70	70	100
	16 l	0	0	0	32	45	50	75	100	100
	24 l	0	0	0	37	37	66	88	100	100
Grodyl 75 WG	20 g	0	0	0	0	0	0	10	10	10
	40 g	0	0	0	0	0	15	15	15	15
	80 g	0	0	0	18	18	18	18	18	18

Plants were at the age of four weeks after seeding, 3–10 cm in height on the date of spraying

The herbicide Arsenal at a dose of 4 l/ha killed all plants within 40 days. The speed of action was proportionate to increasing herbicide concentrations. Herbicide Grodyl 75 WG was found ineffective (18% efficacy), after application the plants were of lower habit than the control, dark green in color without any signs of injury.

Fig. 1 and 2 show the effects of Arsenal applications on plants grown from seeds under natural climatic conditions in a screened enclosure (plants were 2 weeks and 4 weeks old, respectively, at the time of treatment).



1. Effects of Arsenal applications on *Kochia scoparia* s. l. at weekly intervals after treatment. Plant age 2 weeks after seeding on the date of application, seedlings up to 3 cm in height

Efficacy of Arsenal at a dose of 4 l/ha reached 96% in the control of plants up to 3 cm in height. There were a few solitary live plants left, green in color, but with retarded growth. A second Arsenal application at the same dose of killed most of these plants, only in one case did five such plants survive. The latter were left in containers until they produced seeds that will be used in further tests to confirm or exclude resistance. As indicated by foreign information (Friesen et al., 1993), the resistance level of kochia populations from Canada and Kansas to sulfonylureas and imidazolinones was different. Saari et al. (1994) were of the opinion that weed populations were present in a mixture of resistant and sensitive types under field conditions.

Arsenal applications to plants 3–10 cm in height resulted in 83% efficacy of the chemical in 6 weeks. The efficacy at a dose of 2 l/ha was not satisfactory enough, at 60–70%.

The results of trials with kochia plants grown under natural conditions and treated with other herbicides and mixtures of herbicides are presented in Table II (after seeding) and in Tables III and IV (after transplanting from a railroad station).

II. The effects of herbicide applications on *Kochia scoparia* s. l. plants expressed as the percentage of killed plants

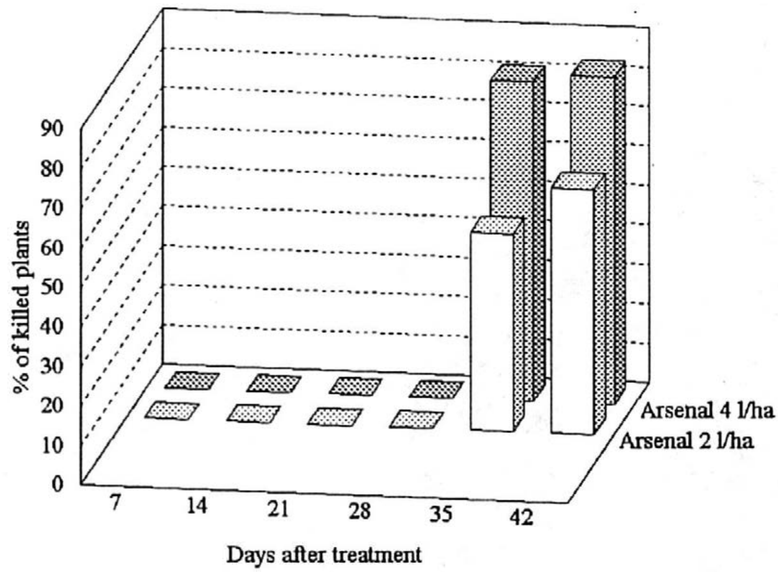
Herbicide	Dose per ha	Percentage of killed plants days after treatment					
		7	14	21	28	35	42
Bladex	3 l	100	100	100	100	100	100
Arsenal + Bladex	2 + 1.5 l	100	100	100	100	100	100
Arsenal + Bladex	4 + 3 l	100	100	100	100	100	100
Grodyl 75 WG	80 g	0	0	0	0	0	0
Shorty	0.5 l	0	0	0	0	0	0
	1 l	0	0	0	0	0	0
	2 l	0	0	0	0	0	0

Plants were at the age of four weeks, 3–10 cm in height on the date of plant spraying

Herbicide Bladex and its mixtures with Arsenal at a dose of 4 + 3 l/ha and at half that dose 2 + 1.5 l/ha when applied to plants 3–10 cm high showed 100% efficacy as soon as within a week after treatment. The herbicide Grodyl 75 WG, even at a dose of 80 g/ha, and Shorty at a dose of 2 l/ha were completely ineffective, the plants produced flowers.

Fig. 3 shows the effects of herbicides Bladex, Arsenal and Shorty on kochia plants.

Herbicide Shorty at a dose of 2 l/ha had no effect on plants 10–20 cm high at the time of application, while the efficacy of Arsenal at a dose of 4 l/ha was only 30% after 6 weeks. There was 100% efficacy within 21 days after



2. Effects of Arsenal applications on *Kochia scoparia* s. l. at weekly intervals after treatment. Plant age 4 weeks after seeding on the date of application, plant height 3–10 cm

treatment with Bladex, and in 14 days following the use of a mixture of the latter and Arsenal. A mixture of these herbicides at half the dose also resulted in 100% efficacy.

III. The effects of herbicide applications on *Kochia scoparia* s. l. plants expressed as the percentage of killed plants

Herbicide	Dose per ha	Percentage of killed plants – days after treatment					
		7	14	21	28	35	42
Bladex	3 l	0	0	100	100	100	100
Arsenal + Bladex	2 + 1.5 l	0	100	100	100	100	100
Arsenal + Bladex	4 + 3 l	0	100	100	100	100	100
Arsenal	4 l	0	0	0	0	0	30
Shorty	2 l	0	0	0	0	0	0

Plants were two weeks after transplanting from the railroad station area on the date of spraying, 10–20 cm in height

Symbol herbicide: 1 – Arsenal 4 l/ha; 2 – 1.5 L Bladex 1.5 l/ha; 2 – 3 L Bladex 3 l/ha; 3 – Arsenal + Bladex 4 l + 3 l/ha; 4 – Shorty 2 l/ha; K – control (right at the top)

3. The effects of herbicide applications on *Kochia scoparia* s. l., treatment four weeks after seeding. The photo was taken 12 days after treatment

Applications of herbicide Arsenal did not kill plants that were 20–50 cm in height at the time of spraying, even at a concentration of 16 l/ha. Plant leaves were lighter in color in comparison with the control, only the oldest leaves were withered. The mixture of Arsenal + Bladex at a dose of 4 + 3 l/ha resulted in 30% mortality of treated plants (the shortest were about 20 cm high).

An important factor for successful control of kochia plants is an appropriate plant age. Herbicide Arsenal at a dose of 4 l/ha, which was found ineffective in some cases under practical conditions, showed 96–100% or 83% efficacy, respectively, when applied to plants at younger developmental stages (up to 3 cm or up to 10 cm high), while 100% efficacy was achieved on plants up to 10 cm in height when grown under defined conditions. Plant density is also important for practical kochia control as shown by our own experience. If the number of individuals per unit area is large, some plants may not be covered by the treatment due to the mode of leaf arrangement on the plants.

Since, as shown by our observations, kochia seeds can germinate in containers under natural conditions all year round, and due to the assumed great reserve of kochia seeds at the places of its distribution, sprayings should be repeated under practical conditions. Hence the use of a herbicide with longer residual effects seems to be required. Herbicide Bladex and the mixture of Arsenal + Bladex showed very good efficacy in the control of kochia plants up to a height of about 20 cm.

References

ARSENOVIĆ, M., ŽIVANOVIĆ, M., ŠOVLJANSKI, R. (1991): Weed control on railways in Yugoslavia. In: Brighton Crop Prot. Conf., Weeds: 1159–1164.

DEVINE, M. D., MARLES, M. A. S., HALL, L. M. (1991): Inhibition of acetolactate synthase in susceptible and resistant biotypes of *Stellaria media*. Pest. Sci., *31*: 273–280.

FRIESEN, L. F., MORRISON, I. N., RASHID, A., DEVINE, M. D. (1993): Response of a chlorsulfuron – resistant biotype of *Kochia scoparia* to sulfonylurea and alternative herbicides. Weed Sci., *41*: 100–106.

GUTTIERI, M. J., EBERLEIN, CH. V., THILL, D. C. (1995): Diverse mutations in the acetolactate synthase gene confer chlorsulfuron resistance in Kochia biotypes. Weed Sci., *43*: 175–178.

HEJNÝ, S., JEHLÍK, V., KOPECKÝ, K., KROPÁČ, Z., LHOTSKÝ, M. (1973): Karanténní plevele Československa. Praha, Academia: 156 s.

MIKULKA, J., CHODOVÁ, D. (1990): Metody diagnostiky rezistentních biotypů plevelů vůči herbicidům a systém jejich regulace. Praha, ÚVTIZ: 54 s.

PRIMIANI, M. M., COTTERMAN, J. C., SAARI, L. L. (1990): Resistance of *Kochia scoparia* to sulfonylurea and imidazolinone herbicides. Weed Technol., *4*: 169–172.

SAARI, L. L., COTTERMAN, J. C., THILL, D. C. (1994): Resistance to acetolactate synthase inhibiting herbicides. In: POWLES S. B., HOLTUM, J. A. M. (Eds.): Herbicide Resistance in Plants. London, Lewis Publ.: 58 s.

STALLINGS, G. P., THILL, D. C., MALLORY-SMITH, C. A., SHAFII, B. (1995): Pollen – mediated gene flow of sulfonylurea resistant *Kochia scoparia*. Weed Sci., *43*: 195–202.

THILL, D. C., MALLORY-SMITH, C. A., SAARI, L. L., COTTERMAN, J. C., PRIMIANI, M. M., SALADINI, J. L. (1991): Sulfonylurea herbicide resistant weeds: discovery, distribution, biology, mechanism and management. In: Herbicide resistance in weeds and crops. Oxford: 115–128.

TORSTENSSON, L., LINDHOLM, O. (1988): Use of herbicides on the Swedish state railways. In: Weeds and weed control. Uppsala: 1–8.

MZeČR – ÚKZÚZ (1996): Seznam registrovaných prostředků na ochranu rostlin. Praha: 184 s.

Received January 15, 1997

Citlivost *Kochia scoparia* s. l. vůči vybraným herbicidům

Byl zkoušen účinek vybraných herbicidů a jejich kombinací na bytel metlovitý (*Kochia scoparia* s. l.), který se rozšířil na železnici, a který by mohl být následně rozšířen na ornou půdu a stejně jako v zahraničí se stát plevelem, který způsobuje rozsáhlé ekonomické ztráty.

Rostliny jsme pěstovali za definovaných podmínek nebo ve vegetační síti za přirozených povětrnostních podmínek v kontejnerech po výsevu semen sebraných z pozemků nádraží. Souběžně jsme rostliny v nejmladší vývojové fázi přesazovali přímo z prostředí nádraží do kontejnerů.

Rostliny jsme ošetřovali v různých fázích vývoje. Účinek herbicidů jsme hodnotili počtem uhynulých rostlin v procentech rostlin před ošetřením.

Při pěstování rostlin ze semen za definovaných podmínek byl účinek herbicidu Arsenal (250 g ú. l. imazapyru) v dávce, která odpovídá 4 l/ha, za 40 dní po ošetření 100%. Rychlost působení herbicidu byla úměrná zvyšující se koncentraci. Herbicid Grodyl 75 WG (75 g ú. l. amidosulfuronu) vykázal v dávce 80 g/ha za 40 dní velmi slabý 18% účinek.

Ošetření herbicidem Arsenal za přirozených povětrnostních podmínek v dávce 4 l/ha působilo za šest týdnů úhyn 96 % rostlin ošetřených ve fázi klíčních až do výše 3 cm (dva týdny po výsevu semen). Na rostliny ošetřené čtyři týdny po výsevu semen při výšce 3–10 cm měl Arsenal po šesti týdnech 83% účinek. Ojediněle přežily rostliny i opakovaný postřik Arsenalem dávkou 4 l/ha. Pro potvrzení rezistence je nutné testovat další rostlinný materiál. Při výskytu rostlin *Kochia scoparia* rezistentních vůči imazapyru se totiž v rámci jedné populace vždy jedná o populaci smíšenou, tj. s výskytem rezistentních i citlivých jedinců.

Herbicid Bladex (500 g ú. l. cyanazinu) vykázal za sedm dní 100% účinek v dávce 3 l/ha, jestliže byly rostliny ošetřeny čtyři týdny po výsevu semen, stejně tak kombinace herbicidů Arsenal + Bladex v dávce 4 + 3 l/ha i v dávce poloviční.

Velmi dobrého účinku bylo dosaženo i po aplikaci herbicidu Bladex a kombinace Arsenal + Bladex na rostliny za dva týdny po přesazení z prostředí nádraží, kdy měly výšku 10–20 cm. Bladex 3 l/ha měl 100% účinek za tři týdny po ošetření, kombinace s herbicidem Arsenal již za dva týdny v obou použitých dávkách.

U rostlin starších, které měly výšku 20–50 cm, se účinek herbicidu Arsenal ani v dávce 16 l/ha neprojevil, kombinace Arsenal + Bladex (4+3 l/ha) měa účinek pouze 30%, a to na rostliny s nejnižší výškou (asi do 20 cm).

Herbicid Grodyl 75 WG i při námi použité nejvyšší dávce 80 g/ha stejně jako herbicid Shorty (90 g ú. l. imazethapyr + 10 g ú. l. imazapyr) v dávce 2 l/ha byly na *Kochia scoparia* s.l. , pěstované za přirozených povětrnostních podmínek neúčinné.

Pro úspěšnou regulaci je nutné rostliny *Kochia scoparia* s. l. ošetřovat v nejmladší vývojové fázi. V místech, kde je velká hustota rostlin, nebo kde je velká zásoba semen v půdě je nutné postřik opakovat. Z výsledků pokusů vyplývá velmi dobrý účinek herbicidu Bladex a kombinace Arsenal + Bladex na *Kochia scoparia* s. l.

Kochia scoparia s. l.; citlivost vůči herbicidům, imazapyr; cyanazin; amidosulfuron

---

Contact address:

RNDr. Daniela Chodová, CSc., Výzkumný ústav rostlinné výroby
odbor agroekologie, 161 06 Praha 6-Ruzyně, Česká republika
tel.: 00 42 2 360 851, fax: 00 420 2 365 229

COST
European Cooperation
on Scientific and
Technical Research

RICP
Research Institute
of Crop Production
Prague -Ruzyně

The organizing committee from Cost action 817 looks forward to your participation in the conference

"APPROACHES TO IMPROVING DISEASE RESISTANCE TO MEET FUTURE NEEDS: Airborne pathogens of wheat and barley "

11–13 November 1997, Prague, Czech Republic

The preliminary schedule of the conference:

November 11 – afternoon:	Opening of the conference Relevance of virulence survey for practical breeders
November 12 – morning:	Deployment of race specific resistance
afternoon:	Breeding strategies for partial resistance
November 13 – morning:	How to assess level for partial resistance Closing of the conference

Sessions will be opened by invited speakers.

Accommondation: Single room – Price 750 Kč/night
Double room – 500 Kč/night/bed

Registration fee: 250 DEM

The registration fee includes Proccedings (containing abstracts of all papers and posters) and the Conference Dinner.

Deadline for preliminary registration is 31 May 1997. Those who submit the preliminary registration form will receive detailed information on the programme and the final registration form during June 1997.

Official language of the conference is English.

The preliminary registration form, as well as all correspondence should be addressed to:

Renata Hanušová
Research Institute of Crop Production
CZ-161 06 Prague 6-Ruzyně

Phone: 00 420 2 360 851
Fax: 00 420 2 365 228
E-mail: hanusova@genbank.vurv.cz

Organizing committee: Renata Hanušová, Pavel Bartoš – Czech Republic
Rosemary Bayles – United Kingdom
Rients E. Niks – The Netherlands
Helge Skinnes – Norway

Ochr. Rostl., *33*, 1997 (2) : 125–133

GERMINATION AND SEED VIABILITY IN A DANDELION, *TARAXACUM OFFICINALE* AGG*

Zdenka MARTINKOVÁ, Alois HONĚK

Research Institute of Crop Production – Division of Phytomedicine
Prague, Czech Republic

Abstract: The rate and final percentage of germination was investigated in seeds of a dandelion (*Taraxacum officinale* agg.) from a population at Praha-Ruzyně. Seeds colected in May were kept either at room conditions (24 ± 2 °C, 40% RH) or buried in the field at 20 cm depth. The germination under 4h photophase was recorded after 2 to 621 days from seed collection. Physiological time to 50% germination was similar at 15 and 25 °C (125 and 100 day degrees above 0 °C threshold) but much slower at 35 °C (812 day degrees). Percentage of germinating seeds at 25 °C decreased little during 1.5 years storage at room conditions while a large proportion of field buried seeds lost germination. Under room conditions decreasing germination was due to mortality of slowly germinating seeds (after 6 days at 25 °C) while under field conditions the mortality was non-selective. The rates of seed mortality differred from those reported by earlier studies. This variation could be caused by variation between microspecies of *T. officinale* agg.

dandelion; seed; germination; survival; soil seedbank; temperature

The biology of seeds and germination in *Taraxacum officinale* agg. has been subject of many studies. This interest is practical: The establishment of seedlings is an important problem in cultures where bare ground surfaces remain exposed to seed rain, in intensive fruit orchards, tree nurseries, and garden cultures of ornamental plants. Dandelion seedlings may appear also in grasslands and permanent cultures when crop cover becomes incomplete.

Dandelion seeds lack primary dormancy and could germinate immediately after maturation. This species is known to form a short-term persistent soil seed bank, i.e. seeds persist for more than one year but usually less than five (Grime et al., 1981, 1990; Burnside et al., 1996). The differences in ger-

* This work was supported by grant No. 513/95/0681 of the Grant Agency of the Czech Republic.

mination rate of freshly collected seeds and seeds stored under different environmental conditions, as well as patterns and causes of seed mortality in time are little known. Another factor that may vary seed biology of dandelion is the polytypic character of the *Taraxacum officinale* agg. The studies revealed that *Taraxacum* section Ruderalia with the species *Taraxacum officinale* Weber in Wiggers consists of a number of micro-species, of which at least 150 was described from central Europe (Dostál,1989). The taxonomic effects on seed biology are not fully appreciated.

In this study we investigate survival and germination of seeds stored at room conditions and try to determine intrinsic causes of mortality. We compare the results with recently published information from Canada (Letchamo, Gosselin, 1996).

MATERIAL AND METHODS

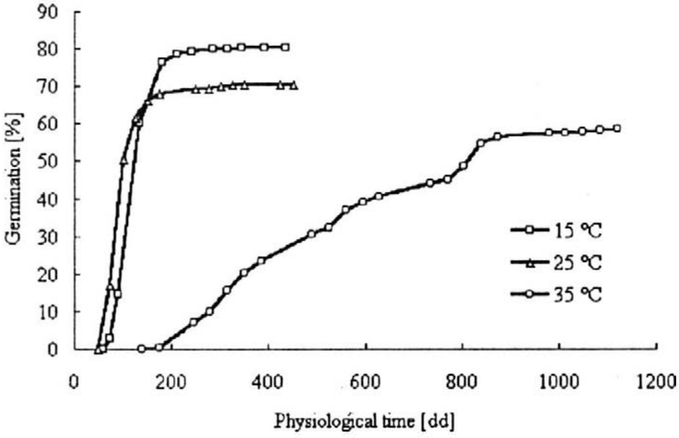
The seeds of a population of *Taraxacum* spp. Section Ruderalia were collected on a small persistent population of plants growing on a meadow at Prague-Ruzyně, on May 10, 1995 and May 21, 1996. The seeds were then stored dry at room temperature of 24 ± 2 °C and 40% RH. Germination experiments were established 2, 6, 24, 37, 80, 122, 171 days (material of 1996) and 499 and 548 days (material of 1995) after seed collection. Seed samples of 1995 material were also buried in the field, on June 16, 1995, 37 days after collecting. The material was divided into lots of about 1500 seeds, which were wrapped into pieces of nylon fabric and buried at 20 cm depth. The seeds were exhumed on November 1 and December 1, 1995, then in monthly intervals starting from February 1, 1996.

Germination experiments were established at 25 °C and 4D : 20L photoperiod. Hundred seeds were placed on petri dish of 10 cm diameter, on a moist filter paper, in 5 or 10 replicates in each germination experiment . The germination was established daily, for 35 days from the beginning of the experiment. The experiment with effect of constant temperatures of 15, 25, and 35 °C on germination rates was established on January 20, 1997, with 1995 seed material stored for 621 d at room conditions. To compare the effect of temperature, the time to germination was expressed as physiological time, number of day-degrees (dd) above 0 °C threshold. The threshold was set at

0 °C since field germination occurs at 2 °C (Fisjunov, 1984) and 50% of maximum germination was observed at rather low temperature of 7 °C (Grime et al., 1981).

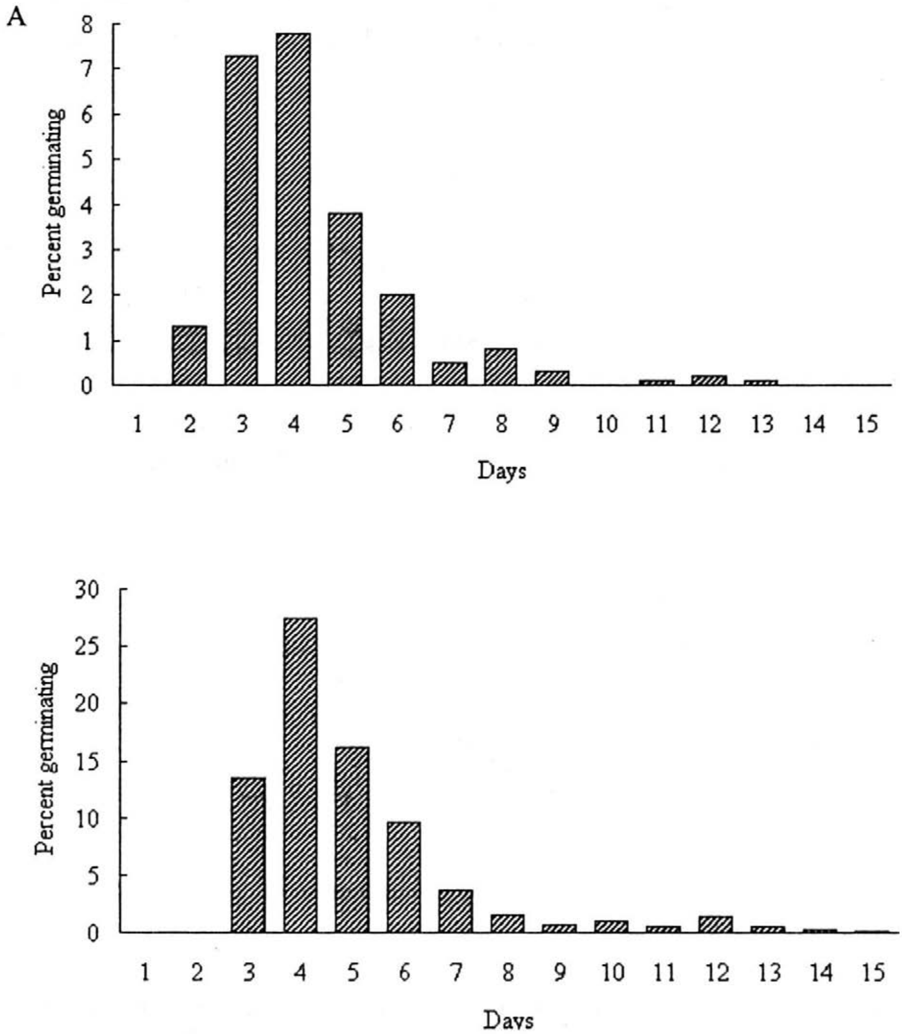
RESULTS

Temperature affected the rate of germination (Fig. 1). There was little difference at medium temperatures of 15 and 25 °C where 50% germination occurred at 125 and 100 dd from the start of germination while a high temperature of 35 °C decreased the rate of germination substantially and 50% germination occurred only after 812 dd. The final percentage of germinating seeds decreased with temperature from 80.3 to 70.5% and 58.6% in 15, 25 and 35 °C, respectively.



1. Cumulative percentage of germination in relation to physiological time, day-degrees (dd) above 0 °C threshold, elapsed from the beginning of germination experiments, at 15, 25 and 35 °C

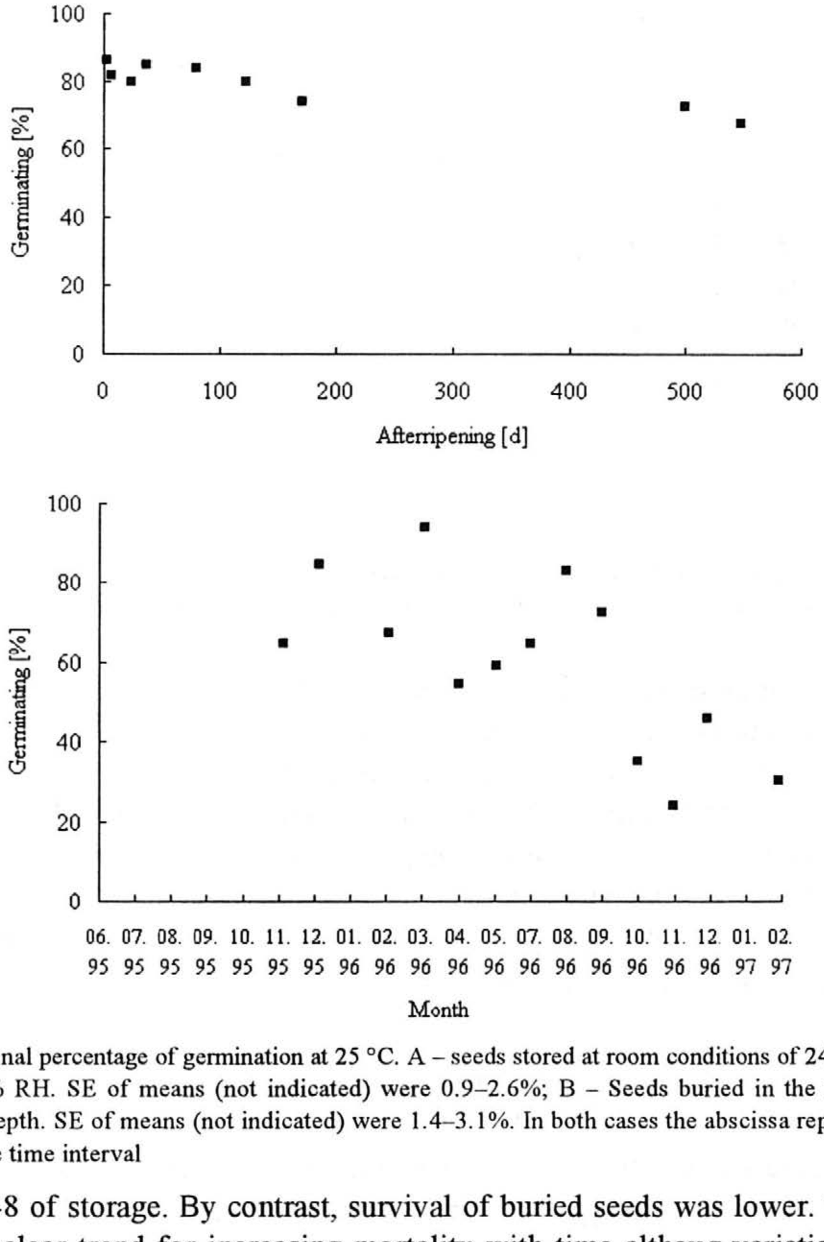
At 25 °C the rate of germination was essentially similar during the first six days, regardless of the length of seed storage at room conditions (Fig. 2A). The average cumulative percentage of germinating seeds on day 6 was 66.8 ± 2.2% (range 63.2–70.6%). The germination rate of seeds buried in the soil for 513 and 598 d was essentially similar to seeds stored at room conditions (Fig. 2B) although final germination rates were small, 33.0% and 30.6%, respectively. The slightly earlier peak of germination in buried seeds was caused by starting germination experiments with seeds which were im-



2. The distribution of time to germination at 25 °C. A – seeds stored at room conditions of 24 ± 2 °C and 40% RH for materials stored 2–548 days (pooled data); B – seeds buried in the field for 513 and 598 days (pooled data)

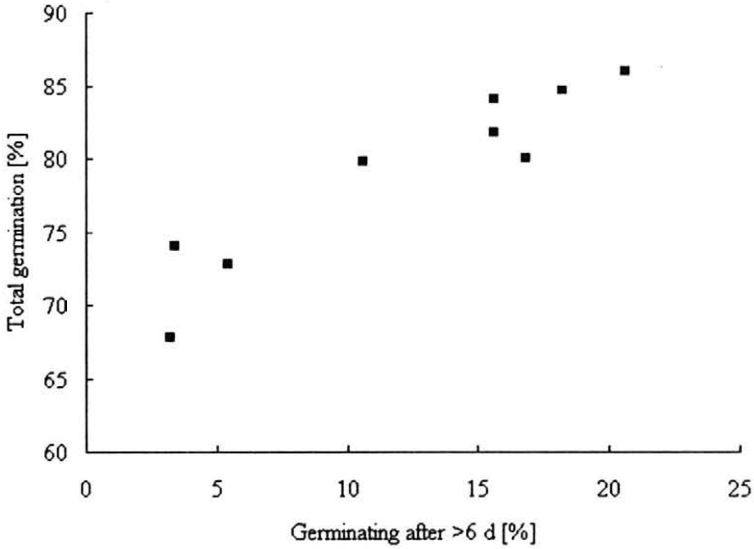
bibed already in the soil, before the start of the germination experiment. The modal germination length was in both cases 4 d from the beginning of the experiment.

The survival, final proportion of germinating seeds, decreased little with time during the storage of dry seeds at room conditions (Fig. 3A). The cumulative percentage of germination decreased from 86.0% on day 2 to 67.8% on



3. The final percentage of germination at 25 °C. A – seeds stored at room conditions of 24 ± 2 °C and 40% RH. SE of means (not indicated) were 0.9–2.6%; B – Seeds buried in the field at 20 cm depth. SE of means (not indicated) were 1.4–3.1%. In both cases the abscissa represents the same time interval

day 548 of storage. By contrast, survival of buried seeds was lower. There was a clear trend for increasing mortality with time althoug variation between samples was greater than in room stored seeds (Fig. 3B). The germination was between 58–94% until the autumn of the second year of burial (1996). Thereafter, from October 1996 (the second year of burial) final germination decreased to 24–46%.

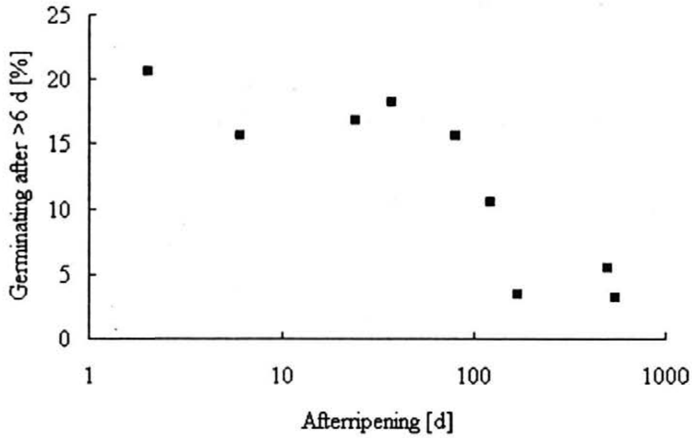


4. The regression of the final percentage of germinating seeds on percentage of seeds whose germination time was > 6 days ([total % germinating] = 0.86 [% germinating after > 6 days] + + 68.5)

The causes of decrasing total germination percentage were analysed during storage at room conditions. The final percentage of germination was directly proportionate to the fraction of seeds that germinated after > 6 days ($r^2 = 0.879$, $df = 7$, $p < 0.001$) (Fig. 4). The proportion of seeds with retarded germination decreased with the length of storage ($r^2 = 0.734$, $df = 7$, $p < 0.005$) (Fig. 5). Thus the high germination rates were caused by slowly germinating seeds and mortality of these poor seeds caused the decrease of germination percentage in materials stored under room conditions for a long time.

DISCUSSION

Most results were congruent with traditional knowledge on dandelion seed and germination biology. The initial germination percentage of freshly harvested seeds was high, although not 100% (Letchamo, Gosselin, 1996). Cyclical induction of secondary seed dormancy may appear in weeds which participate in early stages of sucession on arable land (Bewley, Black, 1982; Baskin, Baskin, 1985). In dandelion which germinates in the spring we would suppose the induction of dormancy during summer



5. The regression of the percentage of seeds with germination time > 6 days on the length of afterripening, storage period at room conditions of 23 ± 2 °C and 40% RH ([% germinating after > 6 days] = –6.94 [log days of afterripening] + 24.3)

and autumn months. We did not establish the induction of secondary seed dormancy in buried seeds, neither in the first year (the year of collection) nor in the second year (the year after) of the burial. As expected, persistence of soil bank of *Taraxacum officinale* agg. was low and seed mortality became to increase rapidly beginning with the autumn of the second year of burial. However, during the vegetation season of the second year (the year following the one in which the seeds developed) the seeds buried in the field soil retained a high germination capacity and their germination percentage at 25 °C was nearly equal to fresh seeds. The seed reserves of the previous year are equal in their capacity to establish dandelion populations as are the populations originating from the current seed rain. However, old seeds should remain on, or should be moved near the soil surface since dandelion seeds germinate from a maximum of 4–5 cm depth (Fisjunov, 1984). Germination at 20 cm depth would be fatal and, in fact, no germination was observed in the buried samples. This was unlike seeds of several other weed species that readily germinated in fatal depths (Honěk, Martinková, in prep.)

The difference against the recent study of Letchamo and Gosselin (1996) is longer viability of seeds under room conditions. This variation could indicate the importance of variation between dandelion populations (or micro-species) which may vary in the intrinsic longevity.

This study also provided an insight into the mechanism of seed mortality. The longevity of stored seeds generally depends on temperature and humidity during storage period (Roberts, Ellis, 1989). Individual seeds, however, may differ in pre-dispositions for survival. During 1.5 years of storage at rooom conditions the decrease of germination percentage was caused by mortality of poor seeds whose inferiority became manifest by prolonged germination period (> 6 days). By contrast, the mortality in the open concerned also intrinsically vigorous seeds. Environmental factors killed a fraction of seeds which would have survived under room conditions.

Acknowledgement

We thank Mrs. Ludmila Kreslová and Mrs. Věra Poláková for excellent technical assistance.

References

BASKIN, J. M., BASKIN, C. C. (1985): The annual dormancy cycle in buried weed seeds: a continuum. Bioscience, *35*: 492–498.

BEWLEY, J. D., BLACK, M. (1982): Physiology and Biochemistry of Seeds. Vol. 2. Berlin - Heidelberg - New York, Springer.

BURNSIDE, O. C., WILSON, R. G., WEISBERG, S., HUBBARD, K. G. (1996): Seed longevity of 41 weed species buried 17 years in eastern and western Nebraska. Weed Sci., *44*: 74–86.

DOSTÁL, J. (1989): Nová květena ČSSR. Praha, Academia.

FISJUNOV, A. V. (1984): Sornye rastenia. Moskva, Kolos.

GRIME, J. P., MASON, G., CURTIS, A.V., RODMAN, J., BAND, S. R., MOWFORTH, M. A. G., NEAL, A. M., SHAW, S. (1981): A comparative study of germination characteristics in a local flora. J. Ecol., *69*: 1017–1059.

GRIME, J. P., HODGSON, J. G., HUNT, R. (1990): The abridged comparative plant ecology. London, Chapman and Hall.

LETCHAMO, W., GOSSELIN, A. (1996): Light, temperature and duration of storage govern the germination and emergence of *Taraxacum officinale* seed. J. Hortic. Sci., *71*: 373–377.

ROBERTS, E. H., ELLIS, R. H. (1989): Water and seed survival. Ann. Bot., *63*: 39–52.

Received March 12, 1997

Ochr. Rostl., *33*, 1997 (2): 125–133

Klíčení a životnost nažek pampelišky lékařské, *Taraxacum officinale* agg.

Byla sledována rychlost klíčení a celkové procento klíčivosti u populace nažek pampelišky lékařské (*Taraxacum officinale* agg.) z Prahy-Ruzyně. Nažky sebrané v květnu byly skladovány v suchu v pokojových podmínkách (24 ± 2 °C, 40 % RH), nebo byla v polních podmínkách zakopány do půdy do hloubky 20 cm. Pokusy s klíčením byly prováděny po 2 až 621 dnech od data sběru, při fotoperiodě se 4 h fotofází. Fyziologická doba potřebná k vyklíčení 50 % nažek byla podobná při teplotě 15 a 25 °C (125 a 100 denních stupňů nad prahovou teplotou 0 °C), avšak mnohem delší při teplotě 35 °C (812 denních stupňů). Procento klíčivých nažek (při teplotě klíčení 25 °C) během osmnáctiměsíčního skladování při pokojových podmínkách pokleslo jen málo, zatímco v půdě v polních podmínkách velká část nažek ztratila klíčivost na podzim druhého roku po zakopání. V pokojových podmínkách byla příčinou snížení klíčivosti mortalita nažek s nízkou rychlostí klíčení (> 6 dní ve 25 °C) zatímco v polních podmínkách byla mortalita neselektivní. Vzrůst mortality nažek v čase se lišil od dříve publikovaných údajů, pravděpodobně vlivem ekologických rozdílů mezi materiály nažek nebo taxonmických rozdílů mezi mikrospeciemi agregace *T. officinale*.

pampeliška lékařská; nažky; klíčivost; přežívání; půdní zásoba nažek; teplota

---

Contact address:

RNDr. Alois Honěk, CSc. Výzkumný ústav rostlinné výroby
odbor rostlinolékařství, 16106 Praha 6-Ruzyně, Česká republika
tel.: 00 420 2 360 851, fax: 00 420 2 365 228

INSTITUTE OF AGRICULTURAL AND FOOD INFORMATION
Slezská 7, 12056 Praha 2, Czech Republic
Fax: + 42 2 24 25 79 39

In this institute scientific journals dealing with the problems of agriculture and related sciences are published on behalf of the Czech Academy of Agricultural Sciences. The periodicals are published in the Czech or Slovak languages with summaries in English or in English with summaries in Czech or Slovak.

Subscription to these journals be sent to the above-mentioned address

Journal	Number of issues per year	Yearly subscription in USD Europe	overseas
Rostlinná výroba (Plant Production)	12	170,-	177,-
Živočišná výroba (Animal Production)	12	170,-	177,-
Zemědělská ekonomika (Agricultural Economics)	12	170,-	177,-
Lesnictví – Forestry	12	170,-	177,-
Veterinární medicína (Veterinary Medicine – Czech)	12	132,-	138,-
Potravinářské vědy (Food Sciences)	6	76,-	80,-
Zemědělská technika (Agricultural Engineering)	4	51,-	53,-
Ochrana rostlin (Plant Protection)	4	51,-	53,-
Genetika a šlechtění (Genetics and Plant Breeding)	4	51,-	53,-
Zahradnictví (Horticultural Science)	4	51,-	53,-

Ochr. Rostl., *33*, 1997 (2) : 135–142

INSECTICIDAL ACTIVITY OF SUBSTANCES OF PLANT ORIGIN AGAINST STORED PRODUCT INSECTS*

Irma KALINOVIĆ, Julijo MARTINČIĆ, Vlatka ROZMAN, Vlado GUBERAC

University of Josip Juraj Strossmayer in Osijek, Faculty of Agriculture, Osijek, Croatia

Abstract: The aim of these experiments was to study the insecticidal activity of different aromatic plants against *Sitophilus granarius* (L.) and *Acanthoscelides obtectus* Say. In laboratory conditions *A. obtectus* was most effectively controlled by an oil extract of *Lavandula officinalis*. Less effective was dried dust of *Origanum vulgare, Laurus nobilis, Thymus vulgaris* and *Rosmarinus officinalis*. For the control of *S. granarius*, the most effective was dried dust of *L. nobilis*. Under store house conditions the best insecticidal efficacy against *S. granarius* in milling wheat was shown by dust of *R. officinalis*, while in seed wheat an oil extract of *L. nobilis* was best. None of the investigated preparations of plant origin had a negative influence on seed wheat germination.

aromatic plants; insecticidal effect; stored pests; mercantile wheat; seed wheat; germination

The protection of stored agricultural granular products (beans, wheat) against harmful insects is throughout the world and in Croatia carried out mostly with chemical insecticides, which may leave harmful residues in food and pollute the human environment. Lately, there has been research on alternative substances with insecticidal and/or repellent activity on harmful insects in granular agricultural products. The purpose of our investigations was to find such non-pesticide preparations, like some aromatic plant species, for use against the most important stored product insects. Tests would involve laboratory and commercial storage conditions, and the influence of the preparations on the germination of seeds.

* The paper was supported by the Croatian Ministry of Science, Croatian Enterprises Agros Osijek, Institute of Agriculture in Osijek, Osječanka Osijek and Žitar Donji Miholjac.

The possibility to use different plants as insecticides and/or repellents, as in traditional protectants of stored products, is investigated all over the world. The first systematic study of the insecticidal properties of the plant *Acorus calamus* against stored product pests in small native granaries in India was by Subramanian (1942). Till today, the activity of aromatic plants on more than 30 species of stored insects and mites have been investigated, with different results (Daniel, Smith, 1990; Dunkel et al., 1991; Hu et al., 1993; Schmidt, 1991; Lungshi et al., 1994).

The investigation of Regnaoult-Roger and Hamraoui (1993) with *Acanthoscelides obtectus* Say. adults (bean weevil) showed significant insecticidal effect of aromatic plants like *Origanum serpyllum, Thymus vulgaris* L., *T. serpyllum, Satureia hortensis, Rosmarinus officinalis* L., *Mentha piperita* and *Tilia cordata*. The toxicity of essential oils extracted from aromatic plants to the stored product pests *Rhizopertha dominica* F., *Oryzaephilus surinamensis* L., *Tribolium castaneum* Herbst and *Sitophilus oryzae* L. have been studied with the conclusion that the sensitivity of a species to the same oil is quite different (Shaayaa et al., 1991). In Croatia, so far we have had poor results in the first investigations of insecticidal and/or repellent influence of some aromatic plants (*Laurus nobilis* L., *Rosmarinus officinalis, Thymus vulgaris)* against stored product insects [*R. dominica, Sitophilus granarius* (L.), *A. obtectus*] (Kalinović, Ilić, 1995).

MATERIALS AND METHODS

Investigations were carried out in laboratory and in floor store house (locality Osijek). Tested plants were *Rosmarinus officinalis, Laurus nobilis, Thymus vulgaris, Origanum vulgare* L.. *and Lavandula officinalis* (as dried dust and oil extract).

Laboratory test (temperature 23–25 °C; moisture level 65–75%; photoperiod 12h light, 12h dark)

Biological materials

I. *Phaseolus vulgaris* L. – kidney bean (1 g dry plant material or 0,1ml oil extract per 1 kg beans)

Acanthoscelides obtectus – bean weevil adult

II. *Triticum vulgare* L. – milling wheat (1g dry plant material or 0,1ml oil extract per 1 kg wheat)

Sitophilus granarius – wheat weevil adult

Twenty beans and 20 bean weevils or 20 wheat grains and 20 wheat weevils were put in Petri dishes, in eight repetitions for each treatment.

Test in a store house (temperature 4–7 °C; moisture level 70–75%; moisture of wheat 14%; photoperiod 12h light, 12h dark)

Biological materials

I. *Triticum vulgare* – milling wheat (37g dry plant material or 3.7ml oil extract per 5 kg wheat in a paper bag)

II. *Triticum vulgare* – seed wheat (37g dry plant material 3.7ml oil extract per 5 kg wheat in a paper bag)

Sitophilus granarius – 10 adults were put in a small wire cage

Each treatment was investigated in four repetitions.

Seed wheat germination was tested in laboratory conditions by standard methods on filter paper (Pravilnik o kvaliteti ..., 1987).

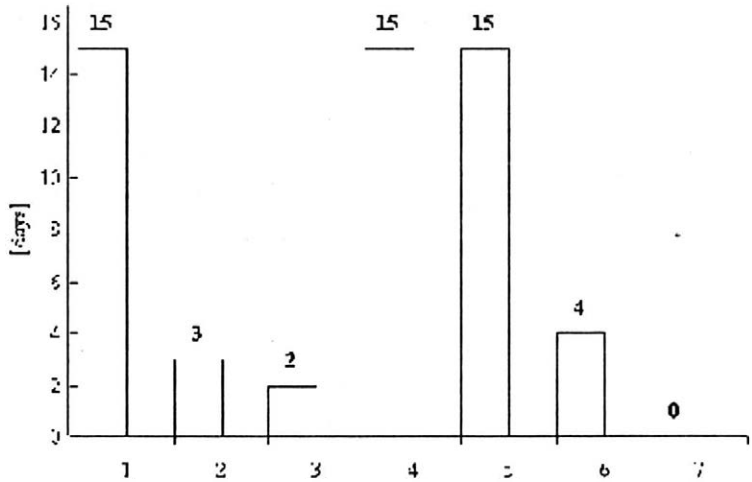
The results were tested by variation analysis and an Lsd-test.

RESULTS AND DISCUSSION

Laboratory test

For the control of *Acanthoscelides obtectus* in *Phaseolus vulgaris* (Fig. 1) the most effective was an oil extract of Lavandula officinalis (1 ml/1 kg, total mortality within 24 hours). Less effective was dust of *L. officinalis* and *Origanum vulgare* (1 g/1 kg, total mortality after 48 and 72 h), and dust of *Laurus nobilis, Thymus vulgaris* and *Rosmarinus officinalis* (total mortality after 15 days). According to the laboratory investigations of Regnault-Roger and Hamraoui (1993), dried *O. serpyllum, T. vulgaris* and *Satureia hortensis* showed a significant insecticidal effect on *A. obtectus* adults in bean.

In the test to control *Sitophilus granarius* in wheat, *Triticum vulgare,* (Fig. 2), dust of Laurus nobilis was most effective on milling wheat (1g/1 kg, total mortality during 26 days). Less effective was dust *of R. officinalis, T. vulgaris* and *L. officinalis*, where total mortality was attained during



1 – *Laurus nobilis* – dust; 2 – *Lavandula officinalis* – dust; 3 – *Lavandula officinalis* – oil extract; 4 – *Thymus vulgaris* – dust; 5 – *Rosmarinus officinalis* – dust; 6 – *Origanum vulgare* – dust; 7 – control

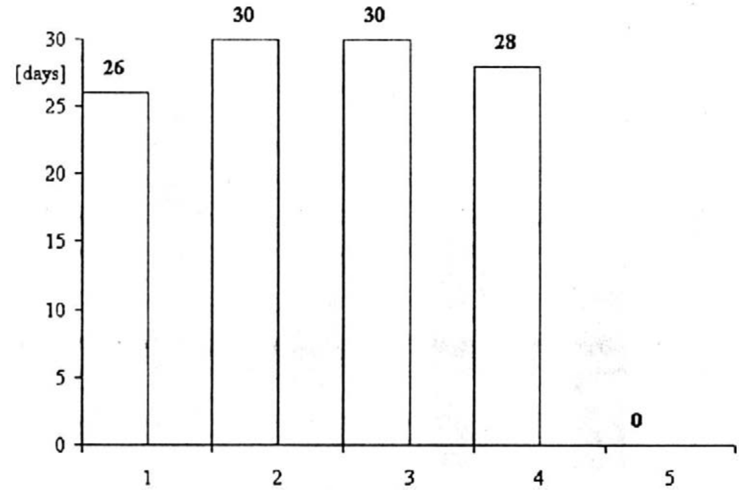
1. Exposure to achieve 100% mortality of *Acanthoscelides obtectus* Say – Laboratory test

28–30 days of exposure. Similar results were obtained during the first investigations in Croatia (Kalinović, Ilić, 1995).

Test in store house

In store house conditions against *S. granarius* in milling wheat (Fig. 3), the most effective was an extract of *L. officinalis* (3,7 ml/5 kg, total mortality during 12 h) and dust of *R. officinalis* (37 g/5 kg, total mortality after 48 h exposure). Dust of *L. nobilis* (37 g/5 kg) and oil extract of *R. officinalis* (3,7 ml/ 5kg) against *S. granarius*, showed similar results (total mortality after 96 h). Other tested preparations, like dust of *L. officinalis* and *T. vulgaris,* and oil extract of *Laurus nobilis* and *T. vulgaris* where less effective (total mortality during 144 and 168 h).

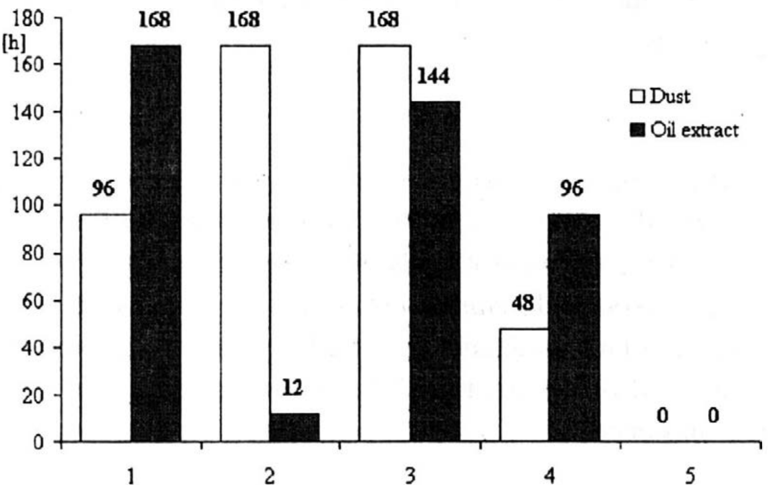
For the control of *S. granarius* in seed wheat, the most effective was an oil extract of *L. nobilis* (3,7 ml/5 kg, with total mortality during 72 hours). Oil extracts of other investigated plants (*L. officinalis, T. vulgaris* and *R. officinalis*) were less effective (total mortality during 96 and 144 hours). The dusts of these plants were the least effective for the control of *S. granarius* (total mortality after 120 and 168 hours of exposure).



1 – *Laurus nobilis* – dust; 2 – *Lavandula officinalis* – dust; 3 – *Thymus vulgaris* – dust; 4 – *Rosmarinus officinalis* – dust; 5 – control

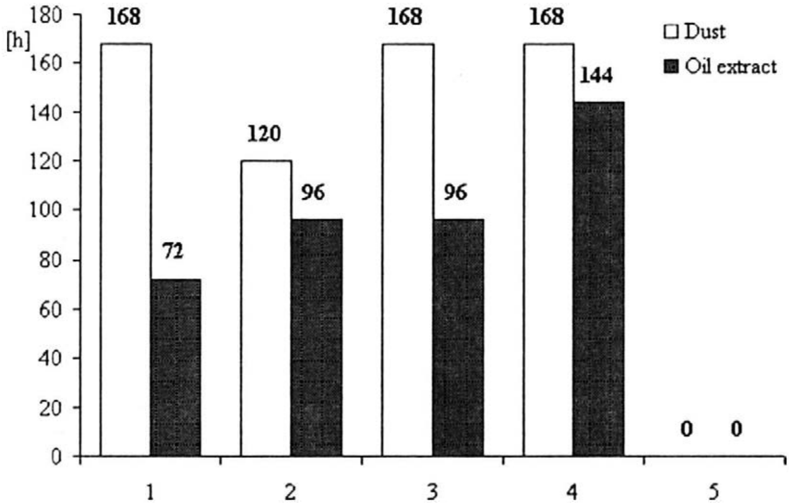
2. Exposure to achieve 100% mortality of *Sitophilus granarius* (L.) – Laboratory test

All investigated preparations were tested for their effect on germination of wheat seeds, but none had a negative influence on germination and rootlet length.



1 – *Laurus nobilis*; 2 – *Lavandula officinalis*; 3 – *Thymus vulgaris*; 4 – *Rosmarinus officinalis*; 5 – control

3. Exposure to achieve 100% mortality of *Sitophilus granarius* (L.) in milling wheat – Test in store house



1 – *Laurus nobilis*; 2 – *Lavandula officinalis*; 3 – *Thymus vulgaris*; 4 – *Rosmarinus officinalis*; 5 – control

4. Exposure to achieve 100% mortality of *Sitophilus granarius* (L.) in seed wheat – Test in store house

These tests about the insecticidal properties of different preparations from plants, which are native to southern Croatia, will be continued in the future for final results and for the recommendation for the control of harmful insects in stored agricultural products in practice.

Conclusion

Rosmarinus officinalis, *Laurus nobilis*, *Thymus vulgaris*, *Origanum vulgare* and *Lavandula officinalis*, in dried dust or oil extract form, had insecticidal properties for the control of the most important stored product pests – *Sitophilus granarius* and *Acanthoscelides obtectus*. The different efficacy of plant origin substances against the same insect in milling wheat and seed wheat is influenced by different capatibility for the absorption the vapor of investigated substances.

References

DANIEL, S. H., SMITH, R. H. (1990): The repellent effect of neem (*Azadirachta indica* A. Juss.) oil and its residual efficacy against *Callosobruchus maculatus*

(Coleoptera: Bruchidae) on cowpea. In: Proc. 5th Int. Conf. Stored Prod. Protect., Bordeaux, II: 1589–1598.

DUNKEL, F., WEAVER, D., PUYVELDE, L., CUSKER, J. L., SERUGENDO, A. (1990): Population suppression effects of Rwandan medical plant *Tatrademia riparia* (Hochst) Codd. (Lamiaceae) on stored grain and bean insects. In: Proc. 5th Int. Conf. Stored Prod. Protect., Bordeaux, III:1609–1612.

HU, M., KLOCKE, J. A., CHIU, S., KUBO, I. (1993): Response of five insects species to a Botanical Insecticide *Rhodojaponin*. III. J. Econom. Entomol., *86*: 706–711.

KALINOVIĆ, I. (1994): Insekticidi biljnog podrijetla. In: Zbor. radova Zaštita uskladištenih poljoprivrednih proizvoda, ZUPP 94: 129135.

KALINOVIĆ, I., ILI, V. (1995): Mogućnost suzbijanja kukaca u skladištima insekticidima biljnog porijekla. In: Zbor. sažetaka 39. Seminara iz zaštite bilja, Opatija, XVIII: 19.

LUNGSHI, L., XIAOWEI, ZHANG, YIGUAN, G. (1994): The influence of temperature and modified atmosphere on effectiveness of *Lavandula angustifolia* Mill. oil for controlling *Tyrophagus putrescetiae*. In: Proc. 6th Int. Working Conf. Stored Prod. Protect., *2*: 817–818.

REGNAULT-ROGER, C., HAMRAOUI, A. (1993): Efficiency of plant from South of France used as traditional protectants of *Phaseolus vulgaris* L. against bruchid *Acanthoscelides obtectus* Say. J. Stored Prod. Res., *29*: 259–264.

SHAAYAA, E., RAVID, U., PASTER, N., JUVEN, B., LISMAN, U., PISSAREV, V. (1991): Fumigant toxicity of essential oil against four major stored-product insects. J. Chem. Ecol., *713*: 499–504.

SCHMIDT, G. H., RISHA, E. M., EL-NAHAL, A. K. M. (1991): Reduction of progeny of some stored product *Coleoptera* by vapours of *Acorus calamus* oil. J. Stored Prod. Res., *27*: 121–127.

SUBRAMANIAN, T. V. (1942): *Acorus calamus* – the sweetflag – a new indigenous insecticide for the household. Ind. J. Ent., *4*: 238.

Pravilnik o kvaliteti sjemena poljoprivrednog bilja (1987): Službeni list br. 47: 1153–1185.

Received October 21, 1996

Účinek insekticidů rostlinného původu proti hmyzu v uskladněných produktech

Cílem těchto pokusů bylo zkoumat účinek insekticidů různých aromatických rostlin *proti Sitophilus granius* (L.) a *Acanthoscelides obtectus* Say. V laboratorních podmínkách byl proti *A. obtectus* nejúčinnější olejný extrakt z rostlin druhu *Lavandula officinalis*. Méně účinný byl suchý prášek z rostlin druhu *Origanum vulgare*, *Laurus nobilis*, *Thymus vulgaris* a *Rosmarinus officinalis*. K *hubení S. granarius* byl nejúčinnější prášek z *L. nobilis*. Při skladování merkantilu pšenice vykazoval nejlepší insekticidní účinky prášek z rostlin druhu *R. officinalis*, u osiva pšenice olejný extrakt z *L. nobilis*. Žádný z přípravků rostlinného původu neměl negativní vliv na klíčivost obilek pšenice.

aromatické rostliny; insekticidní účinnost; skladištní škůdci; merkantilní pšenice; osivo pšenice; klíčivost

This paper was presented at the XX International Congress of Entomology, Firenze, Italy, August 25–31, 1996.

Contact address:

Prof. Dr. Sc. Irma Kalinović, University of Josip Juraj Strossmayer in Osijek Faculty of Agriculture, 31000 Osijek, Trg Sv. Trojstva 3, P.O.B. 117, Croatia
tel: + 385 31 124 333, fax: + 385 31 128 017

Ochr. Rostl., *33*, 1997 (2) : 143–149

CHARAKTERIZACE *Fusarium* spp. POMOCÍ RAPD MARKERŮ*

Characterization of *Fusarim* spp. using RAPD Markers

Jaroslav SALAVA, Markéta HÁJKOVÁ, Petra SOMMEROVÁ

Research Institute of Plant Production, Prague, Czech Republic

Abstract: Sixteen species of *Fusarium* sp. and an isolate of *Pseudocercosporella herpotrichoides* and *Gaeumannomyces graminis* (Table I) from the Collection of Fungi at RICP in Prague, were analyzed by random amplified polymorhic DNA (RAPD) assay. Using 25 synthetic decamers, informative bands for all *Fusarium* spp. including two isolates of *F. culmorum* and for the isolates of *P. herpotrichoides* and *G. graminis* were obtained (Fig. 1). Two isolates of *F. equiseti* could not be distinquished by their RAPD profiles. These results agreed with other reports which showed many genetic differences in the genus *Fusarium* (Möller et al., 1994; Yli-Mattila et al., 1996). DNA fingerprints generated in this study will be applied in the fungal collection.

Fusarium spp.; diagnostics; DNA; PCR

Abstrakt: Dvacet pět náhodných primerů o délce deset bází bylo použito k diferenciaci 16 druhů z rodu *Fusarium*, dvou izolátů *F. culmorum* a *F. equiseti* a jednoho izolátu *Pseudocercosporella herpotrichoides* a *Gaeumannomyces graminis*. Získáné RAPD markery umožnily charakterizovat všechny druhy *Fusarium* sp., izoláty *F. culmorum* a izoláty *P. herpotrichoides* a *G. graminis*. Izoláty *F. equiseti* se nepodařilo odlišit. Výhodou této metody je její rychlost, spolehlivost a to, že nevyžaduje klonování a sekvenování houbové DNA. Výsledky jsou porovnány s literárními údaji a zmíněno je také jejich využití.

Fusarium spp.; diagnostika; DNA; PCR

Houby rodu *Fusarium* patří po celém světě mezi důležité patogeny řady zemědělských plodin. Jejich negativní působení lze rozdělit do dvou skupin: 1. snižují výnos pěstovaných rostlin (Parry et al., 1995), 2. produkují to-

* Financováno z výzkumného úkolu MZe ČR RE 5535.

xiny, které po konzumaci napadeného materiálu působí negativně na zdraví člověka a hospodářských zvířat (Snijders, 1990; Wong et al., 1995).

Přesná diferenciace různých druhů rodu *Fusarium* je důležitá při vedení jejich sbírek a při studiu jejich škodlivosti. Identifikace *Fusarium* spp. je v současnosti prováděna především podle jejich morfologických odlišností.

Metoda RAPDs (Random Amplified Polymorphic DNAs) byla vyvinuta nezávisle dvěma laboratořemi (Welsh, McClelland, 1990; Williams et al., 1990) a je založena na hodnocení genetické variability jedním náhodným primerem. Znalost specifické sekvence není nutná a amplifikace genomové DNA je započata v cílových místech, které se nacházejí v celém genomu. Polymorfní fragmenty jsou výsledkem variability v počtu vhodných míst pro navázání primeru.

Výhodami DNA diagnostiky je obrovský polymorfismus DNA. DNA markery nejsou ovlivněny podmínkami vnějšího prostředí, pro analýzy je zapotřebí pouze malé množství materiálu a v krátké době je možné zpracovat velké množství vzorků.

Cílem této práce bylo ověřit vhodnost použití techniky RAPD u rodu *Fusarium* a nalezení RAPD markerů pro diferenciaci jednotlivých druhů rodu *Fusarium*.

MATERIÁL A METODY

Houbový materiál

Všechny monosporové izoláty *Fusarium* spp. a izoláty *Pseudocercosporella herpotrichoides* a *Gaeumannomyces graminis* (tab. I) pocházely ze sbírky fytopatogenních hub oddělení mykologie VÚRV Praha-Ruzyně. Tyto izoláty byly získány na rostlinách pšenice v letech 1988 až 1995. Taxonomické zařazení jednotlivých druhů rodu *Fusarium* bylo provedeno srovnáním s materiály získanými ze Sbírky mikroorganismů v Brně. Houby byly pro izolaci DNA pěstovány podle postupu, který publikovala Fassatiová (1979).

Izolace houbové DNA

DNA byla izolována metodou, kterou publikovaly Pažoutová a Nováková (1993). Houbové mycelium bylo rozetřeno v tekutém dusíku na jemný

I. Seznam použitých izolátů *Fusarium* spp. a dalších patogenů obilovin - List of isolates of *Fusarium* spp. and other cereal pathogens investigated in the presented study

Označení[1]	Druh[2]	Izolát[3]
A	*F. culmorum* (W.G.Sm.) Sacc.	I
B	*F. avenaceum* (Fr.) Sacc.	
C	*F. equiseti* (Corda) Sacc.	I
D	*F. oxysporum* f. sp. *pisi* (Hall) Raillo	
E	*F. poae* (Peck) Wollenw.	
F	*F. solani* (Mart.)	
G	*F. culmorum* (W.G.Sm.) Sacc.	II
H	*F. redolens* Wollenw.	
M	*F. equiseti* (Corda) Sacc.	II
Q	*F. moniliforme* Sheldon	
R	*F. heterosporum* Nees	
S	*F. graminearum* Schwabe	
T	*F. acuminatum* (syn. *F. aloes* Kalchb. Cooke)	
U	*F. lateritium* Nees	
V	*F. sambucinum* Fuck.	
W	*F. fuscum* (Bon.) Sacc. (syn. *F. chlamydosporum* var. *fuscum*)	
X	*F. tricinctum* (Corda) Sacc.	
Y	*Microdochium nivale* (Sr.) Samuels et Hallett [*F. nivale* (Fr.) Ces.]	
AA	*Gaeumannomyces graminis* (Sacc.) Arx et Olivier	
AB	*Pseudocercosporella herpotrichoides* (Fron) Deighton	

[1]code; [2]species; [3]isolate

prášek. DNA byla extrahována 3,5 ml extrakčního pufru (2% Triton X-100, 1% SDS, 0,25M NaCl, 0,1M Tris-HCl pH 7,8 a 0,1M EDTA pH 8,2). Po 15 minutách promíchávání na třepačce byl přidán stejný objem směsi fenolu a chloroformu, obsah zkumavek byl protřepán a centrifugován (3 000 rpm, 15 min). Vodná fáze byla přenesena do nové zkumavky, spojena s 0,7 objemu izopropanolu a vzniklá sraženina byla centrifugována za stejných podmínek jako v předchozím případě. Vzniklý sediment byl rozpuštěn v 3 ml TE pufru (pH 8,2) s 10 μl roztoku RNasy A (10 mg/ml) a zkumavky byly inkubovány 30 až 40 min v 37 °C. Následně byly vzorky dvakrát extrahovány 3 ml chloroformu a centrifugovány 15 min při 3 000 rpm. Supernatant byl vysrážen 0,1 objemu 4M LiCl a dvěma objemy 96% etanolu. Sediment byl osušen na vzduchu a rozpuštěn v TE pufru (pH 7,4). Obsah DNA ve vzorcích byl stanoven spektrofotometricky.

Podmínky amplifikace DNA

Amplifikační reakce byly prováděny v objemu 50 µl. Každá reakce obsahovala jednu jednotku *Taq* DNA polymerasy (Promega), 50mM KCl, 10mM Tris-HCl pH 9,0 při 25 °C, 0,1% Triton X-100 (Promega 10x Thermophilic Buffer Magnesium Free), 1,5mM $MgCl_2$ (Promega), 0,1mM každý dNTP (Promega), 2 µM primer a přibližně 50 ng houbové DNA.

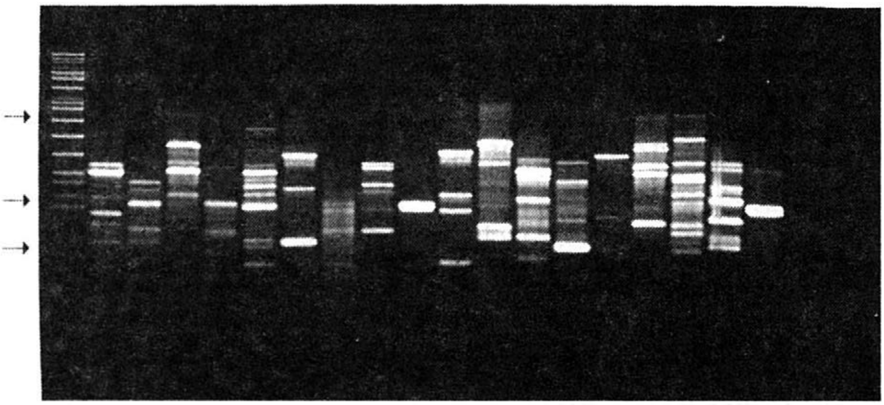
K amplifikacím bylo použito celkem 25 náhodných primerů o délce 10 nukleotidů (Genosys Biotechnologies, Inc. a Operon Technologies, Inc.).

Čtyřicet cyklů amplifikace bylo uskutečněno v PCR termocykleru Techne Progene. Každý cyklus sestával z denaturace (20 s při 94 °C), přichycení primeru (1 min při 36 °C) a prodlužování primeru (1 min při 72 °C). V závěrečném cyklu byl poslední krok prodloužen na 9 min, aby byly dosyntetizovány chybějící úseky fragmentů. Ke změně teplot mezi jednotlivými segmenty cyklů s výjimkou přechodu přichycení primeru/prodlužování primeru byla použita její nejvyšší možná rychlost (60 °C za 1 min). Mezi přichycením primeru a jeho prodlužováním se teplota měnila rychlostí 12 °C za 1 min. Produkty reakcí byly rozděleny v 1,5% agarózovém gelu (Sigma) při 3,5 V/cm. Vizualizace PCR produktů byla provedena jejich fluorescencí v UV světle po obarvení etidium bromidem. Všechny amplifikace byly opakovány jednou nebo dvakrát (v případech s rozdílnými profily v předchozích amplifikacích).

VÝSLEDKY A DISKUSE

Všech 25 použitých primerů poskytlo produkty amplifikace. V závislosti na kombinaci primer a izolát bylo získáno 1 až 13 fragmentů o velikosti 0,2 až 3,5 kb.

Získané RAPD markery nám umožnily odlišit všech 16 druhů rodu *Fusarium*. Jednotlivé *Fusarium* spp. bylo možné odlišit se všemi použitými



1. Analýza RAPD s primerem CS15 (5′ AACACATGCC 3′). Dráha M je Raoul žebřík (Appligene). Šipky na pravé straně obrázku označují fragmenty 3609, 1050 a 375 párů bází dlouhé. Ostatní dráhy jsou: 1 – *Fusarium. culmorum*, I; 2 – *F. avenaceum*; 3 – *F. equiseti*, I; 4 – *F. oxysporum* f. sp. *pisi*; 5 – *F. poae; 6 – F. solani*; 7 – *F. redolens*; 8 – *F. moniliforme;* 9 – *F. heterosporum*; 10 – *F. graminearum*; 11 – *F. acuminatum*; 12 – *F. lateritium*; 13 – *F. sambucinum*; 14 – *F. tricinctum; 15 – F. fuscum*; 16 – *F. nivale*; 17 – *Gaeumannomyces graminis;* 18 – *Pseudocercosporella herpotrichoides* – RAPD analysis with primer CS15 (5′ AACACATGCC 3′). DNA fragments were fractionated on a 1.5% agarose gel and stained with ethidium bromide. Lane M is the Raoul marker (Appligene). The white arrows on the side mark fragments 3609, 1050 and 375 base pairs long. The other lanes are: 1 – *Fusarium culmorum*, I; 2 – *F. avenaceum*; 3 – *F. equiseti*, I; 4 – *F. oxysporum* f. sp. *pisi*; 5 – *F. poae;* 6 – *F. solani*; 7 – *F. redolens*; 8 – *F. moniliforme*; 9 – *F. heterosporum*; 10 – *F. graminearum;* 11 – *F. acuminatum*; 12 – *F. lateritium*; 13 – *F. sambucinum*; 14 – *F. tricinctum*; 15 – *F. fuscum*; 16 – *F. nivale*; 17 – *Gaeumannomyces graminis;* 18 – *Pseudocercosporella herpotrichoides*

primery. Jedinou výjimku tvořily druhy *F. culmorum* a *F. graminearum* s primerem CS15 (obr. 1). Schilling et al. (1996) však uvádějí, že *F. culmorum* a *F. graminearum* jsou si velice blízce příbuzné.

Dva izoláty *F. culmorum* se podařilo odlišit pouze jedním (OPA10) z dvaceti pěti použitých primerů, což bylo způsobeno malým polymorfismem na úrovni DNA u tohoto druhu (Möller et al., 1994). To potvrdily i následné pokusy, ke kterým jsme použili 12 izolátů *F. culmorum* (Salava, nepublikováno).

Dva izoláty *F. equiseti* se nám nepodařilo odlišit ani jedním primerem. Příčiny toho lze hledat v nízkém počtu použitých primerů (Kresovich et al., 1992) a dále v tom, že u tohoto druhu nebyly po sběru izolátů provedeny žádné testy pro jejich diferenciaci (K r á t k á , osobní sdělení).

Izoláty *Gaeumannomyces graminis* a *Pseudocercosporella herpotrichoides* nebyl problém odlišit mezi sebou a od všech *Fusarium* spp. ani jedním použitým primerem.

DNA izolovaná z různých kultur stejných izolátů *F. culmorum*, *F. equiseti* a *F. solani* poskytovala stejné výsledky RAPD analýz.

Nejčastěji uváděný problém techniky RAPDs, kterou je špatná reprodukovatelnost jejich bandů a profilů (D e v o s, G a l e, 1992), se v naší práci neprokázal. Přes 98 % získaných fragmentů DNA bylo reprodukovatelných. Rozdíly v relativním množství některých fragmentů byly pravděpodobně způsobeny kompeticí fragmentů o primery, protože když k tomuto jevu došlo a nějaký band byl amplifikován ve větším množství, jiné bandy měly sníženou intenzitu. Všechny majoritní fragmenty DNA byly opakovaně amplifikovány.

Při optimalizaci PCR protokolu jsme zjistili, že největší vliv na výsledky měla koncentrace Mg^{2+} iontů v reakční směsi. Reprodukovatelné výsledky jsme dostali pouze při 1,5 až 2,5mM koncentraci Mg^{2+}. Ostatní faktory ovlivňující PCR (množství primeru, dNTP, *Taq* polymerasy a templátové DNA, teplota přichycení primeru 38 °C a zařazení jednoho cyklu s delší denaturací před ostatní cykly), které jsme měnili v rozmezí hodnot, které uvedl S a m e c (1993), neměly žádný vliv na získané výsledky nebo měly vliv pouze na výtěžnost reakcí .

Získané DNA markery budou využívány při vedení kolekce *Fusarium* spp. ve VÚRV Praha-Ruzyně a pro identifikaci neznámých vzorků patogenních hub.

Vzhledem k získaným výsledkům lze říci, že metodu RAPDs můžeme doporučit k charakterizaci jednotlivých druhů *Fusarium* sp.

L i t e r a t u r a

DEVOS, K. M., GALE, M. D. (1992): The use of random polymorphic DNA markers in wheat. Theor. Appl. Genet., *84*: 567–572.

FASSATIOVÁ, O. (1979): Plísně a vláknité houby v technické mikrobiologii. Praha, SNTL: 20.

KRESOVICH, S., WILLIAMS, J. G. K., McFERSON, J. R., ROUTMAN, E. J., SCHAAL, B. A. (1992): Characterization of genetic identities and relationship of *Brassica oleracea* L. via amplified polymorhic DNA assay. Theor. Appl. Genet., *85*: 190–196.

MÖLLER, E. M., SCHILLING, A. G., GEIGER, H. H., BAHNWEG, G. (1994): Identification and characterization of genotypes of cereal-pathogenic *Fusarium* species by means of RAPD markers. Z. Mykol., *60*: 239–252.

PARRY, D. W., JENKINSON, P., McLEOD, L. (1995): *Fusarium* ear blight (scab) in small grain cereals - a review. Plant Pathol., *44*: 210–214.

PAŽOUTOVÁ, S., NOVÁKOVÁ, M. (1993): Molekulárně genetické metody v taxonomii vláknitých hub – Návody. Praha, MBÚ AV ČR a PřF UK.

SAMEC, P. (1993) : DNA polymorphism and RAPD technology. Genet. a Šlecht., *29*: 291–320.

SCHILLING, A.G., MÖLLER, E. M., GEIGER, H. H. (1996): Molecular differentiation and diagnosis of the cereal pathogens *Fusarium culmorum* and *F. graminearum*. Sydowia, *48*: 71-82.

SNIJDERS, C. H. A. (1990): Fusarium head blight and mycotoxin contamination of wheat: a review. Neth. J. Plant Pathol., *96*: 187–198.

WELSH, J., McCLELLAND, M. (1990): Fingerprinting genome using PCR with arbitrary primers. Nucleic Acid Res., *18*: 7213–7218.

WILLIAMS, J. G. K., KUBELIK, A. R., LIVAK, K. J., RAFALSKI, J. A., TINGEY, S. V. (1990): DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Res., *18*: 6531–6535.

WONG, L. S. L., ABRAMSON, D., TEKAUZ, A., LEISLE, D., McKENZIE, R. I. H. (1995): Pathogenicity and mycotoxin production of *Fusarium* species causing head blight in wheat cultivars varying in resistance. Can. J. Plant Sci., *75*: 261–267.

YLI-MATTILA, T., PAAVANEN, S., HANNUKKALA, A., PARIKKA, P., TAHVONEN, R., KARJALAINEN, R. (1996): Isoenzyme and RAPD-PCR analyses of *Fusarium avenaceum* strains from Finland. Plant Pathol., *45*: 126–134.

Došlo 28. 2. 1997

Kontaktní adresa:

Dr. Ing. Jaroslav Salava, Výzkumný ústav rostlinné výroby,
161 06 Praha 6-Ruzyně, Česká republika
tel.: 00 420 2 360 851, l. 350, fax: 00 420 2 365 228
e-mail: salava@genbank.vurv.cz

Ústav zemědělských a potravinářských informací, Praha

Studijní informace 1997

řada Rostlinná výroba

Tyto publikace shrnují nejnovější poznatky a hlavní trendy z oboru rostlinná výroba.

Klír J.: Setrvalé zemědělství	40 Kč
Šimon J.: Zemědělství v marginálních oblasech	40 Kč
Pikula J.: Bojujeme proti plevelům	40 Kč
Flohrová A.: Vápník a jeho význam pro půdu a rostliny	35 Kč
Blechtová A.: Význam organické hmoty v půdě	40 Kč
Mikula P.: Pěstování brambor	40 Kč

Uvedené publikace si můžete objednat na adrese:

Ústav zemědělských a potravinářských informací
Slezská 7
120 56 Praha 2

Ochr. Rostl., *33*, 1997 (2): 151–158

NEMATÓDY ČEĽADE LONGIDORIDAE VO VINOHRADOCH NA SLOVENSKU – GEOGRAFICKÉ ROZŠÍRENIE*

Nematodes of the Family Longidoridae in the Vineyards of Slovakia – Geographical Distribution

Marta LIŠKOVÁ

Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovak Republic

Abstract: A total of 13 species of nematodes of the family Longidoridae were detected from 133 vineyard localities of Slovakia: *Longidorus elongatus, L. euonymus, L. juvenilis, L. picenus, L. raskii, Longidorus* sp., *Paralongidorus maximus, Xiphinema diversicaudatum, X. italiae, X. pachtaicum, X. simile, X. taylori* and *X. vuittenezi*. From the nematodes – vectors of grapevine virus diseases the species *L. elongatus, P. maximus, X. diversicaudatum* and *X. italiae* were observed. The nematodes occurred in 74% of localities and in 60% of soil samples (266 soil samples were investigated). This paper also presents the soil and climatic aspects of the occurrence of individual nematode species according to the vineyard areas.

nematodes; Longidoridae; virus vectors; geographical distribution; grapevine; Slovakia

Abstrakt: Na 133 lokalitách vinohradov na Slovensku bol zistený výskyt a geografické rozšírenie 13 druhov nematódov čeľade Longidoridae: *Longidorus elongatus, L. euonymus, L. juvenilis, L. picenus, L. raskii, Longidorus* sp., *Paralongidorus maximus, Xiphinema diversicaudatum, X. italiae, X. pachtaicum, X. simile, X. taylori* a *X. vuittenezi*. Celkove sa nematódy vyskytovali na 74 % lokalít a v 60 % pôdnych vzoriek (z 266 vyšetrených). Z nematódov – vektorov vírusových ochorení viniča boli zistené *L. elongatus, P. maximus, X. diversicaudatum* a *X. italiae*. V práci sú zhodnotené pôdne a klimatické aspekty výskytu zistených druhov nematódov podľa jednotlivých vinohradníckych oblastí.

nematódy; Longidoridae; vektory vírusov; rozšírenie; vinič; Slovensko

Fytopatologicky dôležitú zložku mikroedafónu pôdy v rámci ekosystému vinohradu tvoria ektoparazitické nematódy čeľade Longidoridae, zahŕňajúce rody *Longidorus, Paralongidorus* a *Xiphinema*. Niektoré druhy týchto ne-

* Táto práca je súčasťou grantového projektu č. 2/1365, podporovaného Slovenskou grantovou agentúrou VEGA.

matódov sú z medzinárodného hľadiska zaradené medzi karanténne organizmy. Sú to tie druhy nematódov, ktoré okrem priameho poškodzovania koreňov prenášajú karanténne druhy vírusových ochorení viniča. Z tohto dôvodu sa týmto nematódom v celosvetovom meradle venuje veľká pozornosť tak z hľadiska ich geografického rozšírenia, ako aj biológie, škodlivosti, prenášania vírusov, ochrany voči nim a podobne. Doposiaľ bol experimentálne dokázaný prenos vírusových ochorení viniča u 9 druhov nematódov čeľade Longidoridae z Európy a stredozemskej oblasti (Brown et al., 1993; Jones et al., 1994). Aj keď sa nematódy čeľade Longidoridae vo vinohradoch Slovenska sporadicky študovali už od 70. rokov (Mali, Vanek, 1971, 1972; Lišková, 1980; Lišková et al., 1992), až výsledky analýzy rozsiahleho helmintologického materiálu v posledných rokoch umožňujú komplexnejšie zhodnotenie ich rozšírenia a dovoľujú spracovanie jednoduchého prehľadného identifikačného kľúča na ich determináciu.

V tejto práci je zhodnotené geografické rozšírenie jednotlivých druhov nematódov čeľade Longidoridae podľa jednotlivých vinohradníckych oblastí.

MATERIÁL A METÓDY

V priebehu rokov 1991–1996 sme zo 133 lokalít všetkých vinohradníckych oblastí odobrali 266 priemerných vzoriek pôdy. Pôda bola odoberaná z koreňovej sféry viniča spolu s korienkami, vždy z viacerých krov, z nej bola odobraná priemerná vzorka o hmotnosti minimálne 0,5 kg. Hĺbka odberu pôdy bola 20–40 cm, za sucha aj okolo 60 cm. Nematódy sme z pôdy izolovali sitovou premývacou metódou, ktorú publikovali Brown a Boag (1988), fixovali sme ich v 4% formalíne a identifikovali z trvalých glycerínových preparátov.

VÝSLEDKY

Geografické rozšírenie jednotlivých druhov nematódov je zachytené na obr. 1 (rody *Longidorus* a *Paralongidorus*) a obr. 2 (rod *Xiphinema*), frekvencia a prevalencia jednotlivých druhov sú vyhodnotené v tab. I. Nematódy čeľade Longidoridae (rody *Longidorus*, *Paralongidorus* a *Xiphinema*) sa vyskytovali na 99 lokalitách (frekvencia = 74 %) a v 160 vzorkách (prevalencia = = 60 %). Identifikovaných bolo 13 druhov nematódov. Z rodu *Longidorus* boli zistené druhy *L. elongatus*, *L. euonymus*, *L. juvenilis*, *L. picenus*,

I. Frekvencia a prevalencia nematódov čeľade Longidoridae vo vinohradoch na Slovensku – The frequency and prevalence of nematodes of the family Longidoridae from the vineyards of Slovakia

Druhy[1]	Lokality[2]		Vzorky[3]	
	počet pozitívnych[4]	frekvencia[5] [%]	počet pozitívnych	prevalencia[6] [%]
Longidorus spp.				
L. elongatus	2	1,5	3	1,1
L. euonymus	2	1,5	2	0,7
L. juvenilis	4	3,0	8	3,0
L. picenus	1	0,7	1	0,4
L. raskii	1	0,7	1	0,4
Longidorus sp.	1	0,7	1	0,4
Paralongidorus spp.				
P. maximus	5	3,7	5	1,9
Xiphinema spp.				
X. diversicaudatum	1	0,7	1	0,4
X. italiae	1	0,7	5	1,9
X. pachtaicum	4	3,0	6	2,2
X. simile	20	15,0	27	10,1
X. taylori	24	18,0	36	13,5
X. vuittenezi	73	54,9	120	45,1

[1]species of nematodes; [2]localities; [3]soil samples; [4]number of positive localities; [5]frequency; [6]prevalence

L. raskii a *L.* sp. doteraz neidentifikovaný, z rodu *Paralongidorus* to bol *P. maximus*, z rodu *Xiphinema* sú to *X. diversicaudatum*, *X.italiae*, *X. pachtaicum* (pôvodne *X. mediterraneum*), *X. simile*, *X. taylori* (pôvodne *X. brevicolle*) a *X. vuittenezi*. Z týchto druhov vektormi vírusov na viniči sú *L. elongatus* (prenáša vírus krúžkovitosti maliny – Raspberry ringspot virus a vírus čiernej krúžkovitosti rajčiaka – Tomato black ring virus), *P. maximus* (vírus krúžkovitosti maliny), *X. diversicaudatum* (vírus mozaiky arábky – Arabis mosaic virus) a *X. italiae* (roncet viniča – Grapevine fanleaf virus). Aj druh *X. vuittenezi* je viacerými autormi pokladaný za vektor roncetu vírusu (Rüdel, 1980; Brown, Taylor, 1987), avšak doteraz chýba exaktné potvrdenie tohto prenosu.

Výskyt a rozšírenie jednotlivých druhov longidoridných nematódov podľa vinohradníckych oblastí a niektorých ekologických faktorov:

Druhy rodu *Longidorus*

L. elongatus (de Man, 1876) Thorne et Swanger, 1936 – vyskytuje sa v Malokarpatskej oblasi (lokalita Myslenice) a v severnej časti Hlohovecko-Trnavskej oblasti (Piešťany). Tento druh sa na Slovensku vyskytuje predovšetkým v piesočnatých pôdach na aluviálnych naplaveninách alebo viatych pieskoch a z jeho častého výskytu na ovocných drevinách a plantážach drobného ovocia na Záhorí (Lišková, 1995) je možné predpokladať jeho rozšírenie aj vo vinohradoch Skalicko-Záhorskej oblasti.

L. euonymus Mali et Hooper, 1973 – bol zistený na východnom okraji Lučenecko-Rimavskej oblasti (lokalita Hostice) a v Skalicko-Záhorskej oblasti (Šaštín-Stráže). Vyskytuje sa tak v hlinitých pôdach na vápenatých sprašových pokryvoch, ako aj v piesočnatých pôdach na naviatych pieskoch. Jeho výskyt sa môže očakávať aj v Kráľovsko-Chlmeckej oblasti či Podunajskej rovine, kde sa často vyskytuje na ornej pôde (Lišková, Planderová, 1995).

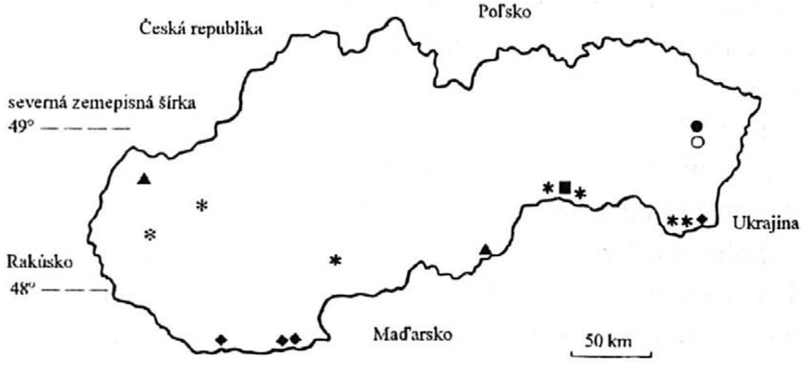
L. juvenilis Dalmasso, 1969 – vyskytuje sa len v najteplejšej a najjužnejšej časti Podunajskej roviny (lokality Moča a Zlatná na Ostrove), na piesočnatých pôdach alúvia Dunaja a na východnom Slovensku v Kráľovsko-Chlmeckej oblasti (Malý Horeš) v pôdach na naviatych pieskoch.

L. picenus Roca, Lamberti et Agostinelli, 1984 a *L. raskii* Lamberti et Agostinelli, 1993 – tieto dva druhy sme zistili spoločne v zmiešanej populácii len na jedinej lokalite – Brekov (najsevernejšia časť Michalovsko-Sobraneckej oblasti), kde sa nematódy vyskytovali v ílovito-hlinitých pôdach na vápencovom podklade.

Longidorus sp. – doposiaľ neopísaný druh sa vyskytuje vo vinohradoch južného okraja Slovenského krasu (Turniansko-Moldavská oblasť). Výskyt tohto druhu je viazaný na pôdy na vápencovom podklade.

Druhy rodu *Paralongidorus*

P. maximus (Bütschli, 1864) Siddiqi, 1964 – je rozšírený podobne ako predošlý druh predovšetkým v Turniansko-Moldavskej oblasti, ale aj vo vinohradoch v Hontianskych Tesároch (Hontiansko-Levická oblasť), opäť však len v pôdach na vápencovom substráte. Okrem týchto oblastí sme ho v poslednom období identifikovali aj na viatych pieskoch Kráľovsko-Chlmeckej oblasti.



∗ *L. elongatus* ▲ *L. euonymus* ◆ *L juvenilis*
● *L. picenus* ○ *L. raskii* ■ *L.* sp.
✱ *P. maximus*

1. Nematódy čeľade Longidoridae – rody *Longidorus* a *Paralongidorus* v rizosfére viniča na Slovensku – Nematodes of the family Longidoredae – genus *Longidorus* a *Paralongidorus* in the rhizosphere of grapevine in Slovakia

Druhy rodu *Xiphinema*

X. diversicaudatum (Micoletzky, 1927) Thorne, 1939 – tento druh bol zistený iba v Malokarpatskej oblasti (lokalita Myslenice), v ľahkých hlinito-piesočnatých pôdach s prímesou skeletu. Aj keď ide o ojedinelý nález tohto druhu na viniči, jeho výskyt je dôležitý z hľadiska prenosu vírusu mozaiky arábky.

X. italiae Meyl, 1953 – vyskytuje sa spoločne s druhom *L. juvenilis* v najjužnejšej a najteplejšej oblasti Podunajskej roviny (lokalita Moča) v ľahkých piesočnatých pôdach na piesočnato-štrkovitých terasách Dunaja. Obidva spomenuté druhy sú rozšírené v stredozemskej oblasti, a preto predpokladáme, že boli pôvodne zavlečené na naše územie z južných oblastí Európy. *X. italiae* je vektorom roncetu viniča.

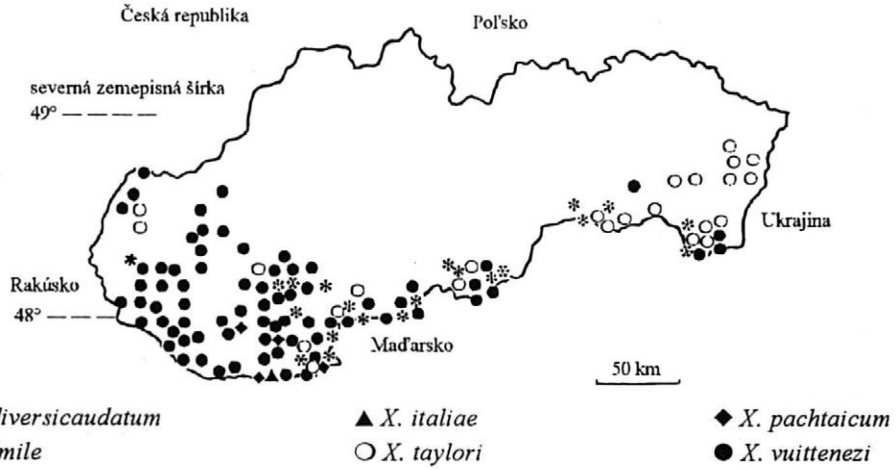
X. pachtaicum (Tulaganov, 1938) Kirjanova, 1951 – sa vyskytuje v nivných a lužných pôdach vinohradníckych oblastí Podunajskej roviny (lokality Moča, Kravany a Zlatná na Ostrove) a hnedozemiach na vápenatých sprašiach (Rubáň), niekedy v zmiešaných populáciách s morfologicky veľmi príbuzným druhom *X. simile* (napr. v Bajtave v Novozámocko-Štúrovskej oblasti).

X. simile Lamberti, Choleva et Agostinelli, 1983 – výskyt tohto druhu nadväzuje geograficky severnejšie na výskyt druhu *X. pachtaicum*. Bol zistený predovšetkým na pôdach na sprašových pokryvoch, prípadne neogénnych sedimentoch (Nitriansko-Vrábeľská a Lučenecko-Rimavská oblasť), ale aj na pôdach južného okraja Sloveského krasu (Turniansko-Moldavská oblasť) či Východoslovenskej roviny (Kráľovsko-Chlmecká oblasť – lokalita Viničky).

X. taylori Lamberti, Ciancio, Agostinelli et Coiro, 1991 – sa vyskytuje na Záhorí, potom od Malých Karpát smerom na východ a juh (najmä Podunajská oblasť) akoby výskyt tohto druhu absentoval, avšak od Nitriansko-Vrábeľskej a východu Novozámocko-Štúrovskej oblasti sa vyskytuje vo všetkých vinohradníckych oblastiach Slovenska, končiac na východe Michalovsko-Sobraneckou oblasťou. Najčastejší je však v pôdach na vápencoch Slovenského krasu (Turniansko-Moldavská oblasť), vápenatých sprašiach a sprašových hlinách (Novozámocko-Štúrovská, Nitriansko-Vrábeľská a Michalovsko-Sobranecká oblasť).

X. vuittenezi Luc, Lima, Weischer et Flegg, 1964 – o tomto druhu podľa našich skúseností možno povedať, že „v oblastiach kde končia vinohrady, tam končí aj geografické rozšírenie *X. vuittenezi* na Slovensku". Je to najrozšírenejší druh vyskytujúci sa na viac ako 50 % lokalít a približne v 50 % pôdnych vzoriek. Zistili sme ho takmer na 75 % lokalít, ktoré boli pozitívne na výskyt longidoridných nematódov, a aj v tom istom percente pozitívnych vzoriek. Okrem pôd na vápencoch, kde sme ho nezistili (južný okraj Slovenského krasu a Sobranecko), je tento druh rozšírený vo všetkých vinohradníckych oblastiach Slovenska.

Celkove sme zistili, že nematódy rodov *Longidorus* a *Paralongidorus*, aj keď počtom druhov približne rovnako zastúpené ako rod *Xiphinema*, (porovnaj obr. 1 a 2), sa vyskytovali skôr len na jednotlivých lokalitách a neboli „plošne" rozšírené, ako je to u niektorých druhov rodu *Xiphinema* (najmä najrozšírenejšie druhy *X. simile, X. taylori* a *X. vuittenezi*). Z hľadiska patogénneho vplyvu, predovšetkým prenosu vírusov, dôležitým zistením je častý výskyt dvoch až štyroch druhov nematódov čeľade Longidoridae. Príkladom môže byť Malokarpatská oblasť (Myslenice), kde sa vyskytuje *X. diversicaudatum*– vektor vírusu mozaiky arábky spoločne s druhom *L. elongatus*, ktorý na viniči prenáša vírus krúžkovitosti maliny a vírus čiernej krúžkovitosti rajčiaka. Ďalším príkladom je spoločný výskyt kombinácie štyroch druhov *L. juvenilis*, *X. italiae*, *X. pachtaicum* a *X. vuittenezi* na lokalite Moča (Podunajská oblasť), aj keď z týchto druhov je doteraz



2. Nematódy čeľade Longidoridae – rody *Xiphinema* v rizosfére viniča na Slovensku – Nematodes of the family Longidoredae – genus *Xiphinema* in the rhizosphere of grapevine in Slovakia

potvrdeným vektorom len *X. italiae* (prenáša roncet viniča). Abundancia jednotlivých druhov nematódov bola rôzna (1–192 exemplárov na 500 g pôdy) a kolísala podľa jednotlivých lokalít aj v rámci jedného druhu. Z fytopatologického aspektu aj nízka abundancia môže mať negatívny dosah, pretože už prítomnosť jedného exemplára nematóda – vektora vírusov v 100 cm^3 pôdy vytvára predpoklady pre prenos vírusu (Bird, 1989).

Všetky získané údaje dopĺňajú a rozširujú doterajšie poznatky o longidoridných nematódoch vo vinohradoch na území Slovenska. Z fytopatologického hľadiska pre vinohradnícku prax je dôležitý aktuálny prehľad rozšírenia jednotlivých druhov (predovšetkým vektorov vírusov) a ich spätosť s niektorými klimatickými a pôdnymi faktormi, resp. konkrétnymi oblasťami či lokalitami. Získané výsledky môžu byť základom pre ďalšie sledovanie tak priameho patologického vplyvu, ako aj štúdium prenosu vírusových ochorení viniča nematódmi. Keďže aj vo vinohradoch na Slovensku sa nematódy – vektory vírusov vyskytujú, pre vinohradnícku prax zostáva naďalej jediným ekologicky prijateľným opatrením dodržiavanie karanténnych opatrení – predovšetkým výsadba zdravého bezvírusového materiálu a správna agrotechnika. Pri obnove vinohradov je potrebné odstrániť všetky zvyšky starých koreňov viniča a dodržiavať 4–5ročný „úhor“ s pestovaním strukovín, ďatelinovín, kukurice a ďalších plodín, ktoré nie sú vhodnými hostiteľmi tejto skupiny nematódov.

Literatúra

BIRD, G. W. (1989): Avoidance and management of nematode problems in tree fruit production in Michigan. Mich. St. Univ. Ext. Bull. E–801-R: 8–9.

BROWN, D. J. F., BOAG, B. (1988): An examination of methods used to extract virus-vector nematodes (Nematoda : Longidoridae and Trichodoridae) from soil samples. Nematol. medit., *16*: 93–99.

BROWN, D. J. F., DALMASSO, A., TRUDGILL, D. L. (1993): Nematode Pests of Soft Fruits and Vines. In: EVANS, K., TRUDGILL, D. L., WEBSTER, J. M.: Plant Parasitic Nematodes in Temperate Agriculture. Wallingford, CAB Int.: 684 s.

BROWN, D. J. F., TAYLOR, C. E. (1987): Comments on the occurrence and geographical distribution of longidorid nematodes in Europe and the Mediterranean region. Nematol. medit., *15*: 333–373.

JONES, A. T., BROWN, D. J. F., McGAVIN, W. J., RÜDEL, M., ALTMAYER, B. (1994): Properties of an unusual isolate of raspberry ringspot virus from grapevine in Germany and evidence for its possible transmission by *Paralongidorus maximus*. Ann. appl. Biol., *124*: 282–300.

LIŠKOVÁ, M. (1980): Nematodofauna rizosféry *Vitis vinifera L.* na východnom Slovensku. Biolologia, *35*: 357–366

LIŠKOVÁ, M. (1995): Faunistic and ecological notes on Xiphinema (Nematoda : Dorylaimida) in Slovakia. Biologia, *50*: 125–131.

LIŠKOVÁ, M., PLANDEROVÁ, M. (1995): Nematodes – plant virus vectors on potato stands in Slovakia. Poľnohospodárstvo, *41*: 199–210.

LIŠKOVÁ, M., VALOCKÁ, B., SABOVÁ, M. (1992): Nematódy – vektory vírusov vo vinohradoch na Slovensku.Vinohrad, Bratislava, *10*: 146–147

MALI, V. R., VANEK, G. (1971): *Xiphinema vuittenezi* a probable vector of some grapevine viruses in Czechoslovakia. In: Plant Virology. Proc.7th Conf. Czechoslov. Pl. Virol., High Tatras: 353–361.

MALI, V. R., VANEK, G. (1972): Nematodes of the family Longidoridae (Thorne) Meyl and their role in Czechoslovakian vineyards. Biologia, *27*: 841–852.

RÜDEL, M. (1980): *Xiphinema vuittenezi* (Nematoda:Dorylaimida) – Virusüberträger bei Reben? Weinwissenschaft, *35*: 177–194.

Došlo 10. 3. 1997

Kontaktní adresa:

Ing. Marta Lišková, CSc., Slovenská akadémia vied, Parazitologický ústav SAV Hlinkova 3, 040 01 Košice, Slovenská republika

tel.: 00 421 95 633 14 11, fax: 00 421 95 633 14 14, e-mail:pausav@linux1.saske.sk

Ochr. Rostl., *33*, 1997 (2) : 159–160

ŽIVOTNÍ JUBILEA

Prof. Ing. Josef Zvára, CSc., pětašedesátníkem

Dne 31. 3. 1997 se dožil šedesáti pěti let prof. Ing. Josef Zvára, CSc., významný fytopatolog a vynikající pedagog.

Narodil se v Býkovicích na okrese Benešov. Po absolvování reálného gymnázia studoval v letech 1951–1955 na Vysoké škole zemědělské v Praze. Stal se po-sluchačem specializovaného studia ochrany, což předznamenalo jeho další profesní zaměření.

Po absolvování vysoké školy nastoupil jako šlechtitelský asistent se zaměřením na zemědělskou fytopatologii na Šlechtitelskou stanici Slapy u Tábora. Zde se také začal výzkumně zabývat chorobami brukvovitých rostlin a pícních plodin, které zůstaly středem jeho zájmu i v dalším období.

V roce 1960 přešel na Katedru rostlinné výroby Provozně ekonomické fakulty Vysoké školy zemědělské v Českých Budějovicích, která se zde v té době začínala budovat. Pod jeho vedením se postupně začalo formovat také oddělení ochrany rostlin.

V roce, kdy se dožívá 65 let, má za sebou 37 let úspěšné činnosti vysokoškolského učitele. Zajišťuje přednášky, praktická cvičení a semináře předmětů ochrana rostlin, zemědělská fytopatologie a integrovaná ochrana rostlin. Působil také na zahraničních univerzitách jako „guest lecturer". Byl vedoucím více než 120 diplomových prací a školitelem 12 aspirantů, resp. doktorandů. Jako docent v oboru ochrana rostlin se habilitoval v roce 1973. Profesorem byl jmenován v roce 1990.

Prof. Zvára zastával řadu vedoucích funkcí. Byl vedoucím Katedry rostlinné výroby, proděkanem Zemědělské fakulty a v letech 1991–1993 děkanem této fakulty Jihočeské univerzity.

Je předním fytopatologickým vědeckým pracovníkem specializujícím se na choroby pícnin, obilnin a brukvovitých rostlin. Publikoval 56 původních vědeckých prací. Je spoluautorem tří celostátních učebnic a dvou titulů skript. Podílí se na činnosti „International clubroot working group".

Prof. Zvára si získal vážnost u svých studentů, spolupracovníků a kolegů nejen pro své hluboké odborné znalosti, ale i svědomitost, starostlivost, vyrovnanost a skromnost.

Do dalších let přejeme jubilantovi hodně zdraví, osobní pohody a další pracovní úspěchy.

Redakční rada časopisu Ochrana rostlin

Nejvýznamnější publikace prof. Ing. J. Z v á r y , CSc.

ZVÁRA, J. (1970): Metoda umělé infekce brukvovitých rostlin houbou *Plasmodiophora brassicae* Wor. Ochr. Rostl., *8*: 293–300.

ZVÁRA, J. (1974): Patogenita různých izolátů houby *Plasmodiophora brassicae* Wor. na brukvovitých (Brassicaceae). Ochr. Rostl., *10*: 49–58.

ZVÁRA, J. (1981): Influence of soil acidity on the infection of eith *Brassica* species with club root (*Plasmodiophora brassicae* Wor.). In: Sbor. Mezinár. Symp. Fyziologie a ekologie fytopatogenních hub, Praha: 138–141.

ZVÁRA, J. (1984): Příspěvek k integrované ochraně pšenice před braničnatkou plevovou (*Septoria nodorum*). In: Sbor. II. věd. Konf. Ochr. Rostl., Piešťany: 128–132.

ZVÁRA, J. a kol. (1990): Problematika biologické ochrany jabloní před strupovitostí (*Venturia inaequalis*). In: Sbor. věd. Konf. Alternativní zemědělství rostlinné produkce, Praha: 64–70.

Instructions for authors

Manuscripts in duplicate should be addressed to: RNDr. Marcela Braunová, Ústav zemědělských a potravinářských informací, Slezská 7, 120 56 Praha 2, Czech Republic.

Manuscript should be typed with a wide margin, double spaced on standard A4 paper. Articles on **floppy disks** are particularly welcome. Please indicate the editor programme used.

Text

Full research manuscript should consist of the following sections: Title page, Abstract, Keywords, a short review of literature (without "Introduction" subtitle), Materials and Methods, Results, Discussion, References, Tables, Legends to figures. A title page must contain the title, the complete name(s) of the author(s), the name and address of the institution where the work was done, and the telephone, fax and e-mail numbers of the corresponding author. The Abstract shall not exceed 120 words. It shall be written in full sentences and should comprise base numerical data including statistical data. As a rule, it should not give an exhaustive review of literature. In the chapter Materials and Methods, the description of experimental procedures should be sufficient to allow replication of trials. Organisms must be identified by scientific name. Abbreviations should be used if necessary. Full description of abbreviation should follow the first use of an abbreviation. The International System of Units (SI) and their abbreviations should be used. Results should be presented with clarity and precision. Discussion should interpret the results. It is possible to combine Results and Discussion in one section. References in the text to citations comprise the author's name and year of publication. If there are more than two authors, only the first one should be named in the text, followed by the phrase "et al.". References should include only publications quoted in the text. They should be listed in alphabetical order under the first author's name, citing all authors, full title of an article, abbreviation of the periodical, volume number, year, first and last page numbers.

Tables and Figures

Tables, figures and photos shall be enclosed separately. The text must contain references to all these annexes. Figures should be referred solely to the material essential for documentation and for the understandig of the text. Duplicated documentation of data in figures and tables is not acceptable. All illustrative material must be of publishing quality. Figures cannot be redrawn by the publisher. All figures should be numbered. Photographs should exhibit high contrast. Both line drawings and photographs are referred to as figures. Each figure should contain a concise, descriptive legend.

Offprints: Forty offprints of each paper are supplied free of charge to the author.

Authors have full responsibility for the contents of their papers. The board of editors of this journal will decide on paper publication, with respect to expert opinions, scientific importance, contribution and quality of the paper.

OBSAH - CONTENTS

Vědecký časopis OCHRANA ROSTLIN ◆ Vydává Česká akademie zemědělských věd – Ústav zemědělských a potravinářských informací, Praha ◆ Redakce: Slezská 7, 120 56 Praha 2, tel.: 02/251 098, fax: 02/242 53 9 38, e-mail: fofo@uzpi.cz ◆ Sazba a tisk: ÚZPI Praha ◆

Rozšiřuje Ústav zemědělských a potravinářských informací, referát odbytu, Slezská 7, 120 56 Praha 2