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THE USE OF PCR AMPLIFICATION IN DETERMINING THE TOXIGENIC POTENTIAL OF *FUSARIUM SAMBUCINUM* AND *F. SOLANI* ISOLATED FROM POTATO TUBERS WITH SYMPTOMS OF DRY ROT

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Abstract

Fusarium sambucinum and *F. solani* are the main causes of the dry rot of potato tubers. PCR amplification was used to (i) confirm the taxonomy of 28 isolates of *F. sambucinum* and 24 isolates of *F. solani* which had been isolated from various potato crops in Poland and identified on the basis of their morphology *in vitro*, and (ii) study the potential of *F. sambucinum* and *F. solani* to produce trichothecene mycotoxins. All *F. sambucinum* isolates, but none of *F. solani* isolates, showed the presence of the *Tri*5 gene which encodes for production of trichothecenes. *Fusarium sambucinum* isolates did not have the potential to produce the B-type trichothecenes, the group that includes deoxynivalenol (DON) and nivalenol (NIV). Chemical analyses of potato tubers inoculated with *F. sambucinum* showed the presence of the A-type trichothecenes: monoacetoxyscirpenol (MAS) and diacetoxyscirpenol (DAS).

Key words: potato, *Fusarium sambucinum*, *F. solani*, mycotoxins, *Tri*5 gene, PCR

Introduction

A demand for increased supplies of high quality potatoes for both direct consumption and the food industry has been observed recently. Poland has been the main producer of potatoes in Europe but has insufficient storage capacity. The crop is stored mostly in soil-covered clamps. Such a method of storage favours the spread of diseases. Dry rot of tubers is one of the most common fungal diseases of potatoes. The group of fungi isolated from tubers with dry rot includes most frequently *F. sambucinum* (*F. sulphureum*), *F. solani* (*F. coeruleum*), *F. oxysporum, F. avena-*

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ceum, F. culmorum and *F. equiseti*. Their pathogenicity towards potatoes varies in different parts of the world and so the designation of individual species as the main cause of disease is difficult. Also, dry rot of tubers is very rarely caused by one pathogen. It usually results from a mixed infection caused by more than one *Fusarium* species (Boyd and Tickle 1972, Stachewicz et al. 1978, Latus-Ziętkiewicz 1993).

Fusarium solani var. *coeruleum* is considered to be the main cause of dry rot of potato tubers in many European countries, including Germany (Langerfeld 1970), Sweden (Olofsson 1976) and Finland (Seppänen 1981). *Fusarium sambucinum* is recognized as the main cause of the disease in France (Tivoli and Jouan 1981), Hungary (Hornok 1982) and Italy (Logrieco et al. 1987). *Fusarium sambucinum*, followed by *F. solani*, are considered to be the main cause of the disease in Poland (Wojciechowska-Kot et al. 1983, Kapsa 1993, Kurzawińska 1995).

Damage from the *Fusarium* fungi results not only from the reduced tuber crop and its quality, but also from the production of secondary metabolites which are toxic to humans and animals (Latus-Ziętkiewicz 1993, Sveeney and Dobson 1999).

Considering the risks from infection of potato tubers by species of *Fusarium*, the objective of this study was to evaluate isolates of *F. sambucinum* and *F. solani* collected from different potato crops and locations in Poland from the point of view of their potential to produce mycotoxins, including trichothecenes. The toxigenic potential of selected isolates of *Fusarium* was confirmed by inoculation of potato tubers which were then analysed to determine mycotoxin concentrations.

Materials and methods

Isolation of fungi and identification by morphology

Fungi from potato tubers collected in 14 locations (Kujawsko-pomorskie, Lubelskie, Mazowieckie, Pomorskie, Zachodniopomorskie, Wielkopolskie provinces) with dry rot symptoms were isolated according to Kurzawińska (1994) and identified on the basis of their morphology according to Nirenberg (1995) and Hering and Nirenberg (1995).

Extraction of DNA and molecular identification

Single-spore cultures of *F. sambucinum* and *F. solani* which had been isolated from rotting tubers were cultured on 2% potato dextrose agar (PDA; 40 g filtered white potatoes, 20 g agar, 100 mg dihydrostreptomycin sulphate, 100 mg penicillin G, 1 l distilled water, pH 7) for four days at 20°C. Four discs of agar (5 mm diameter) with *Fusarium* mycelium were cut from the edge of a colony and transferred to 60 ml liquid potato dextrose broth in a 250 ml flask. Flasks were shaken for five–six days at 20°C (150 rot/min). Mycelium was collected into 2 ml tubes and frozen. Total DNA was extracted with cetyl trimethylammonium bromide

(CTAB), phenol, chlorophorm and ethanol (Doyle and Doyle 1990). DNA concentration was measured with a spectrophotometer. DNA was diluted to a final concentration of 100 μg/l and frozen (–30°C). *Fusarium sambucinum* DNA was amplified by PCR using species-specific primers with *Taq* PCR Core Kit (Qiagen, USA) (Table 1).

Each 12.5 μl PCR mixture consisted of 3.9 μl H_2O, 2.5 μl 5× buffer Q, 1.25 μl 10× buffer, 0.5 μl $MgCl_2$ (final concentration 1 mM/μl), 0.25 μl dNTP mix (final concentration 200 μM/μl each), 0.75 μl primer FSF1 (0.6 pM/μl), primer 0.75 μl FSR1 (0.6 pM/μl), 0.1 μl *Taq* polymerase (0.5 unit), 2.5 μl DNA (final concentration 20 ng/μl).

The PCR amplification was run on a Uno II thermocycler (Biometra, Germany) in 30 cycles using cycling conditions presented in Table 2. The PCR products were checked by electrophoresis of 5 μl of product in a 1.4% agarose gel containing ethidium bromide (0.5 μg/ml) to stain the DNA. The gel was visualized immediately after the run using an ultraviolet light box. Pictures were taken with a digital camera using the program Biocapt (Vilbert Lourmat, France).

Table 1

Primers used in the PCR reaction

For detection	Primer	Primers (5' – 3')	Literature
F. sambucinum	FSF1 FSR1	5'-ACATACCTTTATGTTGCCTCG-3' 5'-GGAGTGTCAGACGACAGCT-3'	Mishra et al. 2003
*Tri*5	HA*Tri*/F HA*Tri*/R	5'-CAGATGGAGAACTGGATGGT-3' 5'-GCACAAGTGCCACGTGAC-3'	Edwards et al. 2001
*Tri*13DON	*Tri*13F *Tri*13DONR	5'-CATCATGAGACTACTTGTAGTTTGG-3' 5'-GCTAGATCGATTGTTGCATTGAG-3'	Chandler et al. 2003
*Tri*13NIV	*Tri*13NIVF *Tri*13R	5'-CCAAATCCGAAAACCGCA-3' 5'-TTGAAAGCTCCAATGTCGTG-3'	

Table 2

PCR conditions

Step	Temperature (°C)	Kind of test	Time (min)
Initial denaturation	94		2
Denaturation	94		1
Annealing	60.5	*F. sambucinum*	1
	57.7	*Tri*5	1
	61.0	*Tri*13DON	1
	61.4	*Tri*13NIV	1
Elongation	72		2
Final extension	72		5
Cooling	4		–

Molecular analyses of the mycotoxigenic potential of *Fusarium*

The potential for isolates of *F. sambucinum* and *F. solani* to produce trichothecenes was determined by analyses with the specific primers HA*Tri*/F and HA*Tri*/R, which amplify the trichodiene synthase gene (*Tri*5) coding the potential for trichothecene production. The type of trichothecenes produced was determined using the specific primers *Tri*13F and *Tri*13DONR, and *Tri*13NIVF and *Tri*13R, which amplify genes coding the potential to produce deoxynivalenol (DON) and nivalenol (NIV), respectively. The PCR amplification was done using the conditions presented in Table 2.

Pathogenicity tests

Potato tubers of cultivar 'Bard' were inoculated with *F. sambucinum* and *F. solani*. Five isolates of each *Fusarium* species which had been sampled from different potato crops in Poland were used (Table 3).

Inoculation was carried out according to Latus-Ziętkiewicz (1993). Healthy, medium-sized potato tubers were surface-disinfected with sodium hypochlorite (NaOCl; 1% available chlorine) for 3 min. Cylindrical cores (5 mm diameter × 10 mm deep) were removed from the potato tissue using a cork borer. Discs of SNA (Nirenberg 1976; 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose, 20 g agar, 1 l distilled water) with 14-day-old *Fusarium* culture was placed in the hole and was covered with the core of tissue. A sterile disc of SNA was placed in a hole in control tubers. The tubers were incubated at 14°C in 95% relative humidity (RH). Each treatment had five replicates. *Fusarium* pathogenicity was evaluated after incubation for 30 days. The diameter and depth of the rot were measured, after cutting the tuber in half, in each of five replicates. The infection rate was calculated from an average diameter + depth in combination diminished by an average diameter + depth in the control.

Table 3

Origin of isolates used in the artificial inoculation of tubers

Isolate code		Cultivar	Locality	Province
F. sambucinum	*F. solani*			
F_{SA}-07-41	F_S-07-41	'Vineta'	Zawidz Mały	Mazowieckie
F_{SA}-06-31	F_S-06-31	'Asterix'	Radostowo	Pomorskie
F_{SA}-06-13	F_S-05-21	'Bartek'	Osiny	Lubelskie
F_{SA}-05-42	F_S-05-37	'Dorota'	Zamarte	Kujawsko-pomorskie
F_{SA}-05-34	F_S-05-34	'Bryza'	Koło	Wielkopolskie

Chemical detection of mycotoxins

Potato tubers which had been inoculated with one of two *F. sambucinum* isolates (F_{SA}-05-42 or F_{SA}-05-34) were analysed chemically for concentration of mycotoxins, including B-type trichothecenes (deoxynivalenol, nivalenol). Detection of mycotoxins was carried out with high-performance liquid chromatography tandem mass spectrometer (HPLC-MS/MS) assay at the Division of Physiology and Toxicology, Department of Experimental Biology, Kazimierz Wielki University, Bydgoszcz, Poland.

Results

28 isolates of *F. sambucinum* and 24 isolates of *F. solani* were obtained from potato tubers of 20 cultivars with dry rot symptoms, which had been sampled in 14 localities in Poland. Single-spore cultures of each isolate were prepared for molecular and chemical analyses.

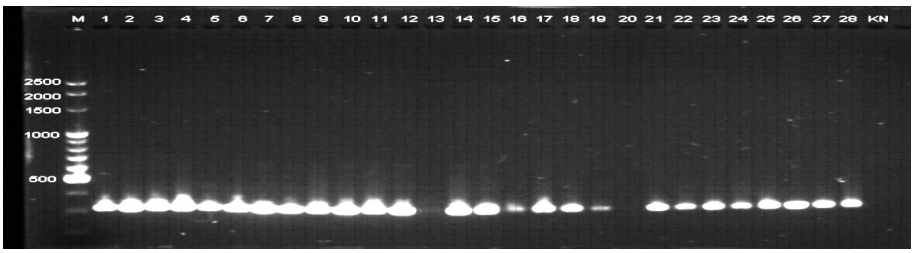
The fungi were identified by morphological and molecular methods. The molecular analysis with the species-specific primers confirmed the taxonomy of fungi evaluated by morphology.

PCR amplification of the fungal DNA with species-specific primers FSF1 and FSR1 designed from the nrDNA sequence variability found within the ITS region yielded a product of 315 bp in the 26 *F. sambucinum* isolates. Two independent PCR amplifications of rDNA from two other *F. sambucinum*-like isolates (No 13 and No 20) yielded no product specific for *F. sambucinum*, and thus did not support the identification based on morphology (Phot. 1).

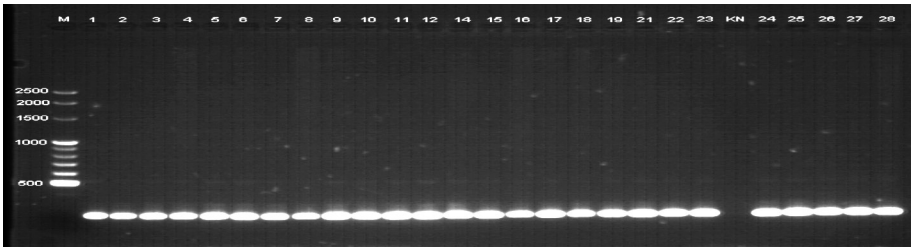
Primers HA*Tri*/F and HA*Tri*/R amplified the *Tri*5 gene in 26 isolates of *F. sambucinum* (Phot. 2) but did not amplify any genes in the 24 isolates of *F. solani*.

The PCR amplification of *F. sambucinum* DNA yielded products of 260 bp, which indicate the presence of the *Tri*5 gene coding the potential for trichothecene production. The PCR amplification of *F. solani* rDNA yielded no products, demonstrating that the sequence of the *Tri*5 gene was absent.

Primers *Tri*13F and *Tri*13DONR, and primers *Tri*13NIVF and *Tri*13R did not amplify any genes in *F. sambucinum* isolates studied. The PCR amplification did not yield any of the expected products of 282 bp (which would have suggested poten-



Phot. 1. Molecular detection of *Fusarium sambucinum* with the specific primers (photo by L. Lenc)



Phot. 2. Molecular detection of the *Tri*5 gene in the *Fusarium sambucinum* isolates (photo by L. Lenc)

tial for deoxynivalenol production) and 312 bp (suggesting potential for nivalenol production).

The size of the dry rot in the potato tubers varied and depended on the *Fusarium* species and isolate used for inoculation. The variation in the size of rot caused by *F.*

Table 4

Average size of the tuber rot caused by *Fusarium sambucinum* and *F. solani*

F. sambucinum		*F. solani*	
code	size (cm)	code	size (cm)
F_{SA}-07-41	37.8	F_S-07-41	2.8
F_{SA}-06-31	35.7	F_S-06-31	4.0
F_{SA}-06-13	37.9	F_S-05-21	2.9
F_{SA}-05-42	41.5	F_S-05-37	3.4
F_{SA}-05-34	45.7	F_S-05-34	2.5
Average	40.4	Average	3.1
$LSD_{0.05}$	I = 2.11	I/II = 4.72	II/I = 6.77

Table 5

Mycotoxin concentration in potato tubers inoculated with *Fusarium sambucinum* (ppb)

Toxin	F_{SA}-05-42	F_{SA}-05-34
Monoacetoxyscirpenol	6 404.0	382.0
Diacetoxyscirpenol	427.0	14.8
Deoxynivalenol	nd	nd
Nivalenol	nd	nd
T-2 toxin	nd	nd
HT-2 toxin	nd	nd
3-acetylodeoxynivalenol	nd	nd
15-acetylodeoxynivalenol	nd	nd

nd – not detected.

sambucinum and *F. solani* suggests the presence of variation in pathogenicity of both species (Table 4).

Potato tubers inoculated with one of two *F. sambucinum* isolates (F_{SA}-05-42 or F_{SA}-05-34) and analysed with HPLC-MS/MS assay did not contain any B-type trichothecenes, including deoxynivalenol and nivalenol, but contained low to high concentrations of A-type trichothecenes, including monoacetoxyscirpenol (MAS) and diacetoxyscirpenol (DAS, Table 5). The results obtained from chemical analyses confirm those from PCR amplification of rDNA with primers specific for genes coding the potential for trichothecene production.

The data show that the concentration of mycotoxins produced by *F. sambucinum* in tubers did depend on the size of the tuber rot. *Fusarium sambucinum* (F_{SA}-05-34), which caused advanced tuber rot, produced only a small amount of toxins.

Discussion

Dry rot caused by *Fusarium* is an important disease of potatoes, not only from the economic point of view but also because of the serious hazard to human and animal health. The disease is caused by *Fusarium* species, some of which are known for their toxigenic potential.

Hohn and Desjardins (1992) detected and identified the *Tri*5 gene in *F. sambucinum*. The *Tri*5 gene encodes trichodiene synthase, which catalyses the first step in the trichothecene biosynthetic pathway. Edwards et al. (2001) confirmed by molecular methods (PCR assay) the ability of *F. sambucinum*, as well as *F. sporotrichioides, F. graminearum, F. crookwellense, F. culmorum* and *F. poae*, to produce trichothecenes.

Identification of fungi from the genus *Fusarium*, if based on morphology, needs experience. The small variations in size and shape of characters used for morphotyping do not guarantee the correct taxonomic classification. Molecular methods of identification, particularly PCR amplification of rDNA with species-specific primers, are faster and more reliable. PCR amplification of rDNA with species-specific primers confirmed the taxonomy based on morphology in 26 out of 28 *F. sambucinum* isolates. Two other isolates were similar to *F. sambucinum* only in morphology and were excluded from the further studies.

All *F. sambucinum* isolates and none of the *F. solani* isolates had the potential to produce thrichothecenes. None of *F. sambucinum* isolates had the potential to produce deoxynivalenol, nivalenol, and their derivatives, i.e. 3-, and 15-acetylodeoxynivalenol (3-ADON, 15-ADON) belonging to the B-type trichothecene group.

Results from PCR amplification with the specific primers were confirmed by chemical analyses of the inoculated and diseased potato tubers. The HPCL-MS/MS assay successfully detected, however, low to high concentrations of the trichothecenes A-type group, including monoacetoxyscirpenol. The potential of *F. sambucinum* to produce A-type trichothecenes was not, however, tested by molecular analysis.

This paper seems to present the results of the first molecular studies in Poland to evaluate the potential for toxin production by *Fusarium* species isolated from potato tubers. The results presented support the findings of Latus-Ziętkiewicz (1993), who studied the toxigenic potential of *Fusarium* fungi from potato tubers with dry rot using High Performance Thin Layer Chromatography (HPTLC). She found that all *F. sambucinum* isolates studied produced trichothecenes of the A-type group, including particularly diacetoxyscirpenol, and did not produce trichothecenes of the B-type group. She also reported that *F. solani* does not produce trichothecenes.

Studies on the toxigenic potential of *Fusarium* are not unequivocal and can often be questioned. El-Banna et al. (1984) reported that *F. solani* var. *coeruleum* and *F. sambucinum* can produce A-type trichothecenes (HT-2 toxin) and B-type (DON, ADON, NIV). *Fusarium solani* var. *coeruleum* produced DON in both potato tubers and liquid medium *in vitro*. The DON concentration in potato tubers stored at the same temperature varied. Desjardins and Plattner (1989) claimed that *F. sambucinum* can synthesize A-type trichothecenes, particularly DAS which was produced in higher concentration, and MAS, T-2 toxin and neosolaniol, which were however produced in smaller concentrations. Jeleń et al. (1995) detected A-type trichothecenes (DAS, 15-MAS, 4-MAS) in all potato tubers inoculated with *F. sambucinum*. Concentrations of toxins varied and depended on the isolate used. Ripperger et al. (1975) and Steyn et al. (1978) reported that *F. solani* and *F. sambucinum* produce A-type trichothecenes (DAS) and do not produce B-type trichothecenes.

Some isolates of potentially toxigenic *Fusarium* species may not produce toxins. The individual toxigenic potential depends on the genetical characteristics of the particular isolate (Ward et al. 2002), their habitat and environment, including temperature, moisture and nutrient content of the substrate (Mateo et al. 2002).

Potato tubers infected by *F. sambucinum* are hazardous to human and animal health (Desjardins and Plattner 1989). Contaminated feedstuffs, if consumed, can cause numerous fusariotoxicoses, which are the symptoms resulting from poisoning. Potato tubers of the worst quality and with the dry rot symptoms are often used as animal fodder, mainly for pigs, which are more sensitive to trichothecenes than are other animals. Negative effects occur, even after application of low doses of trichothecenes. Symptoms usually include reduced feed intake and weight loss. Impairment of the immune and blood-vascular systems has also been observed. Toxins may enter the food chain. They may accumulate in tissues of farm animals. If mycotoxin residues are present in animal products (i.e. meat) they pose a threat to human health (Rotter et al. 1992, Rafai et al. 1995).

Conclusions

1. Studies on the biology and metabolic activity of fungi require their correct taxonomic classification. Identification based on morphology should be

confirmed with molecular techniques. Morphological similarity, when only classical methods of identification are used, may lead to artifacts resulted from errors or misrepresentation. The molecular technique used did not confirm the identity of two out of 28 isolates identified as *F. sambucinum* on the basis of morphology.

2. *Fusarium sambucinum* has the potential to produce trichothecenes. PCR amplification with gene-specific primers detected the gene *Tri*5, which encodes trichothecene synthesis, in all *F. sambucinum* isolates. Chemical analyses of potato tubers inoculated with *F. sambucinum* detected a high concentration of monoacetoxyscirpenol (MAS) and low concentration of diacetoxyscirpenol (DAS).

3. *Fusarium solani* had no potential to produce trichothecenes.

4. The *F. sambucinum* isolates studied had no potential to produce trichothecenes of the B-type group (DON and NIV). PCR amplification did not yield any of the expected products of 282 bp (for deoxynivalenol) and 312 bp (for nivalenol).

5. Potato tubers with even small symptoms of dry rot should not be used for feeding farm animals. They may be contaminated with *Fusarium* trichothecene mycotoxins. Potato tuber contamination with trichothecenes is inconsistent with the intensity of the disease symptoms.

Streszczenie

WYKORZYSTANIE METOD MOLEKULARNYCH DO OKREŚLANIA ZDOLNOŚCI TWORZENIA MIKOTOKSYN PRZEZ *FUSARIUM SAMBUCINUM* I *F. SOLANI* POCHODZĄCYCH Z BULW ZIEMNIAKA Z OBJAWAMI SUCHEJ ZGNILIZNY

Wykorzystując metodę PCR, określano przynależność gatunkową patogenów oraz zdolność tworzenia mikotoksyn przez *Fusarium sambucinum* i *F. solani* – główne patogeny powodujące suchą zgniliznę bulw ziemniaka. Materiał pozyskano z różnych rejonów kraju (województw: kujawsko-pomorskiego, pomorskiego, zachodniopomorskiego, warmińsko-mazurskiego, lubelskiego, mazowieckiego). Z 28 badanych izolatów *F. sambucinum* wszystkie miały gen *Tri*5 warunkujący możliwość tworzenia trichotecenów. Dalsze badania wykazały, że izolaty te nie posiadały zdolności tworzenia trichotecenów grupy B – DON i NIV. Analizy chemiczne bulw inokulowanych wybranymi izolatami wykazały obecność trichotecenów grupy A – MAS i DAS. W 24 badanych izolatach *F. solani* nie stwierdzono występowania genu *Tri*5, co oznacza, że nie posiadały one zdolności tworzenia trichotecenów.

Literature

Boyd A.E.W., Tickle J.H., 1972: Dry rot of potato tubers caused by *Fusarium sulphureum*. Plant Pathol. 21, 4: 195.

Chandler E.A., Duncan R.S., Thomsett M.A., Nicholson P., 2003: Development of PCR assays to *Tri*7 and *Tri*13 trichothecene biosynthetic genes, and characterisation of chemotypes of *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium cerealis*. Physiol. Mol. Plant Pathol. 62: 355–367.

Desjardins A.E., Plattner R.D., 1989: Trichothecene toxin production by strains of *Gibberella pulicaris* (*Fusarium sambucinum*) in liquid culture and in potato tubers. J. Agric. Food Chem. 37, 2: 388–392.

Doyle J.J., Doyle J.L., 1990: Isolation of plant DNA from fresh tissue. Focus 12: 13–15.

Edwards S.G., Pirgozliev S.R., Hare M.C., Jenkinson P., 2001: Quantification of trichothecene-producing *Fusarium* species in harvested grain by competitive PCR to determine the efficacies of fungicides against *Fusarium* Head Blight of winter wheat. Appl. Environ. Microbiol. 67: 1575–1580.

El-Banna A.A., Scott P.M., Lau P.Y., Sakuma T., Platt H.W., Campbell V., 1984: Formation of trichothecenes by *Fusarium solani* var. *coeruleum* and *Fusarium sambucinum* in potatoes. Appl. Environ. Microbiol. 47: 1169–1171.

Hering O., Nirenberg H.I., 1995: Differentiation of *Fusarium sambucinum* Fuckel sensu lato and related species by RAPD PCR. Mycopathologia 129: 159–164.

Hohn T.M., Desjardins A.E., 1992: Isolation and gene disruption of the Tox5 gene encoding trichodiene synthase in *Gibberella pulicaris*. Mol. Plant-Microbe Interact. 5: 149–156.

Hornok L., 1982: Dry rot of potato tubers caused by *Fusarium trichothecioides* Wollenw., a fungus newly recorded in Hungary. Acta Phytopathol. Acad. Sci. Hung. 17: 81–83.

Jeleń H.H., Mirocha C.J., Wąsowicz E., Kamiński E., 1995: Production of volatile sesquiterpenes by *Fusarium sambucinum* strains with different abilities to synthesize trichothecenes. Appl. Environ. Microbiol. 61: 3815–3820.

Kapsa J., 1993: Sucha zgnilizna bulw ziemniaka. Ziemn. Pol. 2: 15–17.

Kurzawińska H., 1994: Zbiorowiska grzybów środowiska glebowego z uprawy ziemniaka i ich wpływ na sprawców suchej zgnilizny bulw w zależności od nawożenia azotowego. Zesz. Nauk. AR Krak. Rozpr. Hab. 192.

Kurzawińska H., 1995: Wpływ nawożenia azotowego i okresu przechowywania ziemniaka na występowanie suchej zgnilizny bulw. W: Materiały Ogólnopolskiej Konferencji Naukowej „Nauka Praktyce Ogrodniczej". AR, Lublin: 319–321.

Langerfeld E., 1970: Lagerfaulen an Kartoffeln 1969/1970. Nachrichtenbl. Dtsch. Pflanzenschutzdienstes (Braunschw.) 23: 109–110.

Latus-Ziętkiewicz D., 1993: Toksynotwórczość grzybów rodzaju *Fusarium* powodujących suchą zgniliznę bulw ziemniaka podczas przechowywania. Typescript. Faculty of Food Science and Nutrition, Agricultural University, Poznań.

Logrieco A., Bottalico A., Solfrizzo M., 1987: Toxigenic *Fusarium* species isolated from rotted potato tubers. In: Proceedings of the First European Seminar: *Fusarium* – Mycotoxins, Taxonomy, Pathogenicity. 8–10 Sept. 1987, Warsaw, Poland. Agricultural University, Warsaw: 105–110.

Mateo J.J., Mateo R., Jiménez M., 2002: Accumulation of type A trichothecenes in maize, wheat and rice by *Fusarium sporotrichioides* isolates under diverse culture conditions. Int. J. Food Microbiol. 72: 115–123.

Mishra P.K., Fox R.T.V., Culham A., 2003: Development of a PCR-based assay for rapid and reliable identification of pathogenic *Fusaria*. FEMS Microbiol. Lett. 218: 329–332.

Nirenberg H.I., 1976: Untersuchungen über die morphologische und biologische Differenzierung in der *Fusarium*-Section *Liseola*. Mitt. Biol. Bundesanst. Land- u. Forstwirtsch. Berl.-Dahlem 169.

Nirenberg H.I., 1995: Morphological differentiation of *Fusarium sambucinum* Fuckel *sensu stricto*, *F. torulosum* (Berk. & Curt.) Nirenberg comb. nov. and *F. venenatum* Nirenberg sp. nov. Mycopathologia 129: 131–141.

Olofsson J., 1976. Viktiga sjukdomar i potatislager. Vaxtskyddsnotiser 40: 40–45.

Rafai P., Bata A., Vanyi A., Papp Z., Brydl E., Jakab L., Tuboly S., Tury E., 1995: Effect of various levels of T-2 toxin on the clinical status, performance and metabolism of growing pigs. Vet. Rec. 136: 485–489.

Ripperger H., Seifert K., Römer A., Rulkötter J., 1975: Isolierung von Diacetoxyscirpenol aus *Fusarium solani* var. *coeruleum*. Phytochemistry 14: 2298–2299.
Rotter R.G., Thompson B.K., Trenholm H.L., Prelusky D.B., Hartin K.E., Miller J.D., 1992: A preliminary examination of potential interaction between deoxynivalenol (DON) and other selected *Fusarium* metabolites in growing pigs. Can. J. Anim. Sci. 72: 107–116.
Seppänen E., 1981: *Fusarium* of the potato in Finland. I. On the *Fusarium* species causing dry rot in potatoes. Ann. Agric. Fenn. 20: 156–160.
Stachewicz H., Pett B., Pett B., Kleinhempel D., Brazda G., Effmert M., 1978: Zur Bedeutung der Mischinffektion (*Erwinia carotovora* var. *atroseptica, Fusarium* spp., *Phoma exiqua* var. *exiqua*) bei der Kartoffel. Tagungsber. Akad. Landwirtschaftswiss. DDR 157: 101–111.
Steyn P.S., Vleggaar R., Rabie C.J., Kriek N.P.J., Harington J.S., 1978: Trichothecene mycotoxins from *Fusarium sulphureum*. Phytochemistry 17: 949–951.
Sveeney M.J., Dobson A.D.W., 1999: Molecular biology of mycotoxin biosynthesis. FEMS Microbiol. Lett. 175: 149–163.
Tivoli B., Jouan B., 1981: Importance and pathogenicity of the different *Fusarium* species and varietas causing dry rot in potato tubers. Agronomie 1: 787–794.
Ward T.J., Bielawski J.P., Kistler H.C., Sullivan E., O'Donnell K., 2002: Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic *Fusarium*. Proc. Natl. Acad. Sci. USA 99: 9278–9283.
Wojciechowska-Kot H., Kiszczak E., Markiewicz E., 1983: Patogeniczność izolatów *Fusarium solani* var. *coeruleum* (Sacc.) Both. Zesz. Nauk. AR-T Olszt. Roln. 38: 143–149.

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