INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Mendelova univerzita v Brně

Agronomická fakulta

Transfer výsledků výzkumu prostřednictvím vědeckých publikací

Řešení výzkumných úkolů

Vyhledávání v databázi Web of Science

Jak se píše vědecká publikace?

Sborník z Workshopu konaného dne 5. 12. 2013 v rámci projektu
CZ.1.07/2.4.00/17.0022 Partnerská síť Agronomické fakulty MENDELU s komerční sférou

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Tento projekt je spolufinancován z Evropského sociálního fondu a státního rozpočtu České republiky

Řešení výzkumných úkolů

Výzkumný úkol

Výzkum je často popisován jako aktivní, vytrvalý a systematický proces bádání s cílem objevit, interpretovat nebo přepracovat fakta. Tento intelektuální proces produkuje velké množství teorií, zákonů, popisů chování a umožňuje jejich praktické využití. Slovo výzkum může být použito ve významu celé kolekce informací o daném subjektu a je často spojován s vědou a vědeckými metodami.

Pro řešení výzkumného úkolu je nutné vytvořit tým nejlépe složený ze studentů, mladých vědeckých pracovníků a zkušených vědeckých pracovníků, kde je zajištěn tok informací oběma směry. Tyto informace následně slouží pro řízení řešení výzkumného úkolu a kontrolu dosažených výsledků.

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Řešení výzkumného úkolu

Ústav chemie a biochemie

- Department of Chemistry and Biochemistry, Mendel University in Brno

✓ 72 employees,

✓ More than 350 m^2 of laboratories,

✓ Part of Central European Institute of Technology (CEITEC, Large infrastructural project funded mainly by EU),

✓ Well equipped.

Řešení výzkumného úkolu

Ústav chemie a biochemie

- Equipment

✓ Animal breeding and cell cultivation facilities,

✓ *In vivo* imaging system,

✓ Mass spectrometers,

✓ Capillary electrophoresis with UV-Vis and laser-induced fluorescence detection,

✓ Gel electrophoresis (1D and 2D systems),

✓ Fluorescence and confocal microscope,

✓ High performance liquid chromatographs with electrochemical, UV-Vis, and mass detectors,

✓ Automatic pipetting robot,

✓ Stationary electrochemical instruments and microarray technology.

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Řešení výzkumného úkolu

Projekty

CEITEC (Vrba, Hubálek) CZ.1.05/1.1.00/02.0068	CEITEC- Středoevropský technologický institut, centrum excelentní vědy	5 miliard (1)	2009-2020
FNUSA-ICRC (Provazník) CZ.1.05/1.1.00/02.0123	Fakultní nemocnice u sv. Anny v Brně Mezinárodní centrum klinického výzkumu	3 miliardy (2)	2009-2020
Výzkumné centrum SIX (Hubálek) CZ.1.05/2.1.00/03.0072	Centrum senzorových systémů – SIX Sensor communication		
MAS (Hubálek) 7H10021	MAS. Nanoele		
NanoTeam CZ.1.07/2.3.00/09.0224 (Hubálek)	Budování výzkumného vzdělávání výzkum nanotechnologie		
Research4Industry CZ.1.07/2.4.00/17.0006) (Vrba, CEITC VUT)	Budování a roz výzkumným a		
NANOLABSYS CZ.1.07/2.3.00/20.0148 (Hubálek)	Mezinárodní spo zobrazovacích te		
NanoBioMetalNet CZ.1.07/2.4.00/31.0023 (Hubálek)	Partnerská síť ce bionanotechnolo		
CYTORES GA ČR P501/10/0356 (Suborová)	Studie participace na cytotoxicitě cyt chemorezistentním		
NanoBioTECell GA ČR P102/11/1068 (Provazník)	Nano-elektro-bio- biologické studie		
NanoIR GA ČR P102/13-19947S (Neužil)	New Nanostructu (NanoIR)	(xx)	2015-2019
GA ČR P102/13-18210S (Prášek)	Research of the micro electro mechanical artificial cochlea based on mechanical filter bank	7800 (xx)	2013-2017
GA ČR P102/13-20303P (Chomoucki)	Labelling of proteins with quantum dots and their sensing using microfluidic system	2100 (xxx)	2013-2016
QI91A032 (Krška, NAZVA)	Výběr rezistentních genotypů meruněk k PPV v širší kvalitou plodů	981	2009-2013

MSMT 6215712402 (Beklová, Zrzka)	Veterinární aspekty bezpečnosti a kvality potravin (píše se jeho tento rok)	300 (38)	2013
IRP 3/2013 (Klejdus/Zrzka)	Technologická podpora nově budované infrastruktury pro centrum excelentní vědy na MENDELU	3000 (20)	2013
Postdoci II CZ.1.07/2.3.00/30.0039) (Tomelová, CEITC VUT)	Excelentní mladí vědci na VUT v Brně- postdoci	189421 (21)	2012-2015
Popularizacevut CZ.1.07/2.3.00/35.004) (Bejček, SIX VUT)	Popularizce výsledků VaV VUT v Brně a podpora systematické práce se studenty	45000 (22)	2012-2014
DOC CEITEC.02/2012 (Gumulec)	Application of magnetizable particles in analysis of molecular mechanisms of zinc ions in prostate tumor cells	966 (23)	2012-2015
DOC CEITEC.01/2012 (Šoborová)	Preparation and testing of quantum dots-protein complex as a label for in vitro and in vivo imaging of prions	650 (24)	2012-2015
Postdoci CZ.1.07/2.3.00/30.0017) (Klejdus, CEITEC MENDELU)	Postdoktorandi v oborech biologických věd na MENDELU	(25)	2012-2015

		(12)	
Liga proti rakovině Praha LPR 2012(Ryvolová)	Význam znaku v nádorových onemocněních Studium distribuce znaku v proteomu nádorových buněčných linií a krevního séra	250 (13)	2011-2012
	Centrum pro těžké kovy a metalomický výzkum:	(14)	2011-2016
	plazením uspořádání a proteinů a DNA	600 (15)	2010-2013
	Sciences to Create a New	4.41 M euro (16)	Start Date: 2011-10-01 End Date: 2014-09-30
	ace nových nanomateriálů nálezů	1500 (17)	2011-2015
	st Financial Aid	360 (18)	1.9.2010-31.8.2013
	etod stanovení biogenních výšení zdravotní bezpečnosti	750 (19)	Start Date: 2013-01-01 End Date: 2017-12-31
	a pro odstranění těžkých	120 (xxx)	2013
	ří	120 (xxx)	2013
	ms v buňkách	120 (xxx)	2013
	pro in vivo monitorování kinin	1125 (xxx)	2013
4/2013/FVHE (Heger/Beklová)	Studium interakce estradiolu jako endokrinního disruptoru s DNA	372 (xxx)	2013
20/2013/FVHE (Škochová/Pikula)	Využití kyseliny ethylendiaminotetraoctové (EDTA) při terapii otrav olovem u ptačích embryí	xxx (xxx)	2013

Řešení výzkumného úkolu

Organizace

Řešení výzkumného úkolu

Ústav chemie a biochemie

- Vedoucí ústavu
- ✓ Vedoucí laboratoře
  - ✓ Vedoucí sekce (5 sekcí)
    - ✓ Laboratoř aplikovaných nanobiotechnologií
    - ✓ Laboratoř klinické biochemie, hematologie a imunochemie
    - ✓ Laboratoř nanobioelektrochemie
    - ✓ Laboratoř pokročilých průtokových technik
    - ✓ Laboratoř výpočetní chemie a biochemie
    - ✓ Laboratoř buněčné nanobiologie a nanomedicíny
    - ✓ Laboratoř mikrosenzorů a nanotechnologií
    - ✓ Laboratoř biofyzikální chemie a elektrochemie

Řešení výzkumného úkolu

Ústav chemie a biochemie

	číslo článku	název práce	Journal	Typ	První autor	časová osa	popis časové osy	experimentální plán	speciální číslo	IF		I	II	III	IV	V	VI
2		Seznam článků 2013										Elektroche	HPLC	foto	počítač	MU	forenz
3																	
4																	
5																	
6																	
7																	
8																	
9																	
10	Article																
11												0	0	1	0	0	0
12	E1_13	Preprocessing and classification of electrophoresis gel images using dynamic time warping	Int. J. Electrochem. Sci.	article	Helena Skutková,	1.1.2013	vyšlo		PS2012	3.7							
13	E2_13	SDS-PAGE AS A TOOL FOR SIZE-DEPENDENT SEPARATION OF QUANTUM DOTS	Advanced Material	article	Sona Krizkova, Libo	30.4.2013	11.5.2013		N	13							
14	E3_13	Differential pulse voltammetry heavy metal analysis in blood children with malignant	Acta Biochemica Polonica	article	ina1,2,3, Tomas Eck	20.2.2013		17_2012	N	3.7		0	0	1	0	0	0
15	E4_13	Study of oxidative stress in Staphylococcus aureus bacterial culture treated with silver(I) ions -	Int. J. Electrochem. Sci.	article	Chudobova	30.3.2013		11/2012*; 9/2012*	N	3.7							
16	E5_13	Study of zinc interaction with DNA by using screen printed electrode and spectrometry	Sensors	article	Nejdl	20.3.2013	18.10.2013		N	1.9	http://www.mdpi.com/journal/sensors/special_issues/nanoparticle-sensing						
17	E6_13	Electrochemical Behaviour of Doxorubicin Encapsulated in Apoferritin	Int. J. Electrochem. Sci.		Katerina Tmejova1,	14.4.2013			N	3.7							
18	E7_13	Influence of Magnetic Microparticles Isolation on Adenine Homonucleotides Structure	Materials-MDPI	article	Krejcová	30.1.2013	15.11.2013		N		http://www.mdpi.com/journal/materials/special_issues/magnetic-materials						
19	E8_13	Sensitive flow injection electrochemical analysis CdS quantum dots ssssssssssssss	Int. J. Electrochem. Sci.	article	Dana Dospivova, L	13.3.2013	15.6.2013		N	3.7							
20																	
21	E10_13	The effect of doxorubicin enclosure into different types of liposomes on its electrochemical and fluorescence behaviour	International Journal of Molecular Science	article	Zitka	30.9.2013	22.11.2013		N	1.8							
22	E11_13	Apoferritin Magnetic particles as doxorubicin carriers for anticancer drug delivery	Int. J. Mol. Sci.	article	Blazkova	31.3.2013	28.3.2013		Magnetic Nanop	2.58	http://www.mdpi.com/journal/ijms/special_issues/magnetic-nanoparticles_20						
23	E12_13	ČÁSTICEMI MODIFIKOVANÁ ELEKTRODA V MIKROPRŮTOČNÉM USPOŘÁDÁNÍ PRO ANALÝZU NUKLEOVÝCH KYSELIN OBSAHUJÍCÍCH REPETICE ADENIN	Chemické Listy	article	Skalíčková	13.4.2013			MEROD2013	0.5							
24	E13_13	Serum Metallothioneins in Childhood Tumours – A Potential Prognostic Marker	ational Journal of Molecular Sc	article	Krusenov, J.(1), Krize	30.4.2013	30.5.2013		N	3.5							
25	E14_13	Ion Exchange Chromatographic and Mass Spectrometric Analysis of Cadmium-Phytochelatin(II) Complexes	ational Journal Environmental S	article	Miguel Angel Meri	16.1.2013	19.1.2013		N	1							
26	E15_13	Změny antioxidační vlastnosti v krevním séru prasnic po přídavku dvou koncentrací selenu do krmné dávky	Int. J. Electrochemical Sci.	article	Horky	30.5.2013			N	1							
27	E16_13	Cisplatin-resistant prostate cancer model: differences in antioxidant system, apoptosis and cell cycle	nternational Journal of Oncolog	article	Gumulec	30.5.2013	18.11.2013		N	2							
28	E17_13	Magnetic electrochemical bar code array for point mutation detection in H5N1 neuraminidase gene	Viruses	article	Ludmila Krejcova1,	15.1.2013	17.5.2013		H5N1 influenza	1.5	http://www.mdpi.com/journal/viruses/special_issues/H5N1						
29	E18_13	Synthetic peptide mediated quantum dot-human immunoglobulin bioconjugation	Electrophoresis	article	Libor Janu1, Maja S	30.3.2013	27.3.2013		N	3.3							
30	E19_13	Rychlé metoda pro rozlišení platinových cytostatic a chloridů platiny v odpadních vodách pomocí FIA-ED	Water	article	kominková												

Řešení výzkumného úkolu

Ústav chemie a biochemie

- Experimentální plán

✓ Vedoucí plánu

- ✓ Měření
- ✓ Tvorba obrázků
- ✓ Psaní úvodu
- ✓ Spojení komentáře k výsledkům
- ✓ Diskuze
- ✓ Odeslání
- ✓ Revize

Tento projekt je spolufinancován z Evropského sociálního fondu a státního rozpočtu České republiky

Vyhledávání v databázi Web of Science

Databáze

Databáze, které shromažďují základní bibliografické informace o publikacích v odborných recenzovaných časopisech, mohou sloužit nejen pro hledání výsledků dosažených ve výzkumu a vývoji, ale také pro hodnocení jak vědeckých pracovníků, tak samotných periodik. V tomto příspěvku jsou stručně popsány a diskutovány dnes nejpoužívanější databáze vědeckých publikací a způsoby hodnocení vědecké práce.

Význam citačních databází

Scientometrie je vědeckou disciplínou, která má za hlavní úkol porovnávat a sledovat kvalitu vědecké práce [1-3]. Aby něco takového bylo uskutečnitelné a dosažené výsledky měly vysokou vypovídací hodnotu, je nezbytné shromáždit všechny formy výstupů vědecké práce (především publikace v odborných recenzovaných časopisech) do specializovaných databází. Pro tyto účely byla vytvořena celá řada takových médií, které jsou různě specializovány. Globální povědomí zřejmě získala nejdříve databáze National Public of Health s označením PubMed, kde jsou indexovány časopisy, které mají vztah k biologickým disciplínám. Další významnou globální databází, která vzniká na evropském kontinentu, je databáze Scopus, která si klade za cíl shromáždit informace o vydávaných pracích ze všech oborů. V databázi je možné vyhledávat podle všech důležitých bibliografických ukazatelů, tedy klíčových slov, autora, názvu práce, časopisu, roku. K dispozici jsou služby jako abstrakty publikovaných článků, přímé odkazy na stránky časopisů, kde je práce dostupná a seznam citované literatury. Dále je zde možné nalézt citace publikované práce. Ovšem největší pozornosti mezi databázemi se může pyšnit databáze nazvaná Web of Science, která se svým obsahem a poskytovanými službami snaží dostát svému názvu co nejlépe.

Web of Science

Díky velkému a mohutnému rozvoji databáze Web of Science a jí vydávanému dopadovému faktoru (impact factor, IF) je potřebné se o této databázi zmínit ve

speciálním odstavci [4-6]. IF jako bibliografický indikátor vypracoval se svými spolupracovníky Euegene Garfield na univerzitě Johna Hopkinse na přelomu 50. a 60. let 20. století. V roce 1961 vyšel první svazek Science Citation Index (SCI) jako seznamu časopisů setříděných podle Journal Impact Factor [7-11].

Co potřebuje konečný uživatel všech těchto databází? Odpověď je nasnadě. Existenci jedné databáze, kde by bylo dostupné vše, co bylo uveřejněno v odborných publikacích bez ohledu na zemi jejího vydání. Jen toto umožní zabránit zkoumání již vyzkoumaného, nebo usnadní navázání na práci někoho, kdo ji nemohl dokončit před 20, 50, 100 a více lety.

Scientometrické hodnocení časopisu

Jak se určí impaktový faktor?

V době pravidelného hodnocení pracovního výkonu každého z nás, podle počtu uveřejněných prací, konferencí, přednášek, obhájených diplomantů, doktorandů, sumy impakt faktorů a počtu citací je otázkou zda bude do toho či onoho časopisu psát zkušený a uznávaný odborník. Podle našeho názoru je pak naprosto nezbytné sledovat jasný cíl, a tím je dosažení maximálního impakt faktoru časopisu [12-18]. Otázkou sledování a smyslu těchto scientometrických parametrů se zabývá celá řada výzkumníků a výsledky jsou prezentovány ve specializovaných časopisech [19-21]. Impakt faktor se počítá jako podíl sumy publikovaných prací v předešlých dvou letech a počtu všech citací ve stejném období.

Které časopisy mají nejvyšší impaktové faktory?

Na prvních místech tabulky se objevují tradičně americké časopisy. Velmi zajímavým trendem jsou první místa obsazována skupinou různých mutací časopisu Nature. V roce 2007 byl nejcitovanějším v USA vycházející časopis (lépe asi kniha) s názvem CA-A Cancer Journal for Clinicians, jehož IF je asi nejvyšší v historii databáze (69,026). Již tradičně se na předních příčkách, konkrétně druhé, objevil časopis New England Journal of Medicine s IF 59,589. Pomyslné třetí místo patří ročence Annual Review of

Immunology s IF 47,981. Časopis Nature se nachází na desátém místě s IF 28,751. Podobně multi-oborově orientovaný časopis Science je na čtrnáctém místě s IF 26,372. Obecně lze vysledovat, že hodnoty impaktových faktorů jednotlivých časopisů mírně narůstají. Tento fakt je způsoben řadou okolností, počínaje dobrou redakční prací editorů a zlepšeným přístupem k publikovaným pracím díky elektronizaci databází a iniciativou autorů otevřených článků (Open Access).

Časopisy vydávané a zařazené do databáze ISI v ČR

Česká republika je pro rok 2007 v databázi zastoupena 23 odbornými časopisy z různých oborů výzkumu (Tab. 1). Na počátku roku 2008 byly do databáze ISI znovu zařazeny časopisy Listy cukrovarnické a řepařské a Plant Soil and Environment, jejichž aktuální hodnota IF je zatím nula. Nynější počet ISI indexovaných časopisů vydávaných v České republice není rozhodně konečný a do dalších let by měl narůstat.

Stejně jako loni přesáhly čtyři časopisy hranici IF 1, a to Preslia, Physiological Research, Folia Geobotanica a Folia Parasitologica. Ovšem je třeba zmínit, že hodnoty jejich impaktových faktorů se spíše snížily. Např. Physiological Research zaznamenal pokles IF o 0,5. V celkovém součtu poklesl u výše zmíněných časopisů IF z 6,919 (2006) na 5,702 (2007).

Chemické vědy jsou v databázi stále zastoupeny časopisem Collection of Czechoslovak Chemical Communication a Chemickými listy. Impaktový faktor časopisu Collection of Czechoslovak Chemical Communication je letos přibližně stejný jako v minulém hodnoceném období a dosáhl hodnoty 0,879 (Tab.1).

Tab. 1: Seznam impaktovaných časopisů vydávaných v České republice, které jsou zařazeny v databázi Web of Science.

Název časopisu	Obor	Celkový počet citací 2005/2006	Počet článků (2007)	IF
Preslia	Rostlinná věda	335	23	2,064
Physiological Research	Fyziologie	1445	132	1,505
Folia Geobotanica	Rostlinná věda	574	26	1,133
Folia Parasitologica	Parazitologie	750	39	1,000
Folia Microbiologica	Mikrobiologie	922	89	0,989
Photosyntetica	Rostlinná věda	1291	96	0,976
Collection of Czechoslovak Chemical Communication	Chemie	2600	118	0,879
European Journal of Enthomology	Entomologie	781	95	0,734
Studia Geophysica and Geodetica	Geochemia a geofyzika	370	95	0,733
Acta Veterinaria Brno	Veterinární věda	425	98	0,687
Chemické Listya	Chemie	469	108	0,683
Veterinární Medicína	Veterinární věda	335	67	0,645
Czech Journal of Animal Science	Zemědělství	276	63	0,633
Folia Biologica	Biologie	244	32	0,596
Acta Virologica	Virologie	515	25	0,560
Kybernetika	Počítačová věda	329	68	0,552
Ceramics-Silikaty	Materiálová věda	137	37	0,488
Czech Journal of Food Science	Potravinářství	190	38	0,488
Czechoslovak Journal of Physics	Fyzika	1225	0	0,423
Folia Zoologica	Zoologie	445	47	0,376
Neural Network World	Počítačová věda	113	44	0,280
Czechoslovak Mathematical Journal	Matematika	515	87	0,155
Česká a Slovenská Neurologie a Neurochirurgiea	Neurovědy	24	15	0,037

a Tyto časopisy jsou publikovány především v češtině a slovenštině, ostatní výhradně v angličtině.

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Jak se píše vědecká publikace?

Publikace

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Odeslaná verze

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

How do grass species, seasons and ensilage influence content of mycotoxins in forage?

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Abstract: Mycotoxins are secondary metabolites produced by fungi species having harmful effects on mammals. The aim of the study was to assess the content of mycotoxins in green mass of selected forage grass species during the growing season and at the end of the growing season. Furthermore, an assessment mycotoxin contents in subsequently produced the first cut silages were also evaluated with respect to species used in this study (*Lolium perenne* (cv. Kentaur), *Festulolium pabulare* (cv. Felina), *Festulolium braunii* (cv. Perseus) and mixture of these species with *Festuca rubra* (cv. Gondolin) or/and *Poa pratensis* (Slezanka)). We found that deoxanivalenol mycotoxins, zearalenone and T2 toxin were detected mainly in the green mass of grasses. However, fumonisin and aflatoxin content was below the limit of detection. July and October were the most risky period of mycotoxins occurrence. During cold temperatures in November and December, the occurrence of mycotoxins in green mass declined. Although June was the period with a low incidence of mycotoxins in green silage, content of deoxynivalenol and zearalenone in silages of the first cut exceeded several times

contents determined in their biomass collected directly from the field. Moreover, we observed that preservatives did not prevent mycotoxin production.

Keywords: grass; silage; mycotoxin; environmental factor

1. Introduction

Prerequisite for high-quality fodder is a clean and healthy phytomass. Epiphyte flora of plants consisting of number of microorganisms, include undesirable microorganisms, such as clostridia and fungi [1,2]. Development of microscopic fungi may lead to the formation of mycotoxins [3]. Mycotoxins are secondary metabolites produced by fungi especially *Aspergillus*, *Penicillium* and *Fusarium* [4]. Production of mycotoxins is caused by interactions and reactions of fungi on environmental conditions [5]. Production of mycotoxins is associated with stress caused by extreme weather conditions or damage caused by insects or animals. The occurrence of mycotoxins contaminating silages is associated with failure to silage management practices [6]. Mycotoxins cause serious health problem in the human population, annually increasing incidence of liver cancer caused by aflatoxin, up to 28.2% of cases of liver cancer is caused by the aflatoxins [7]. Mycotoxins have naturally negative impact on livestock. They cause the alterations in hormonal functions, poor feed utilization, lower gain and possibly death. Moreover, some mycotoxins may pass into the milk [8-11]. Preventing the occurrence of mycotoxins in forage should begin in the field, there have been suggested some guidelines and practises. Some of such practices include the use of varieties or hybrids that are adapted to the growing area and resistant to fungal disease [12].

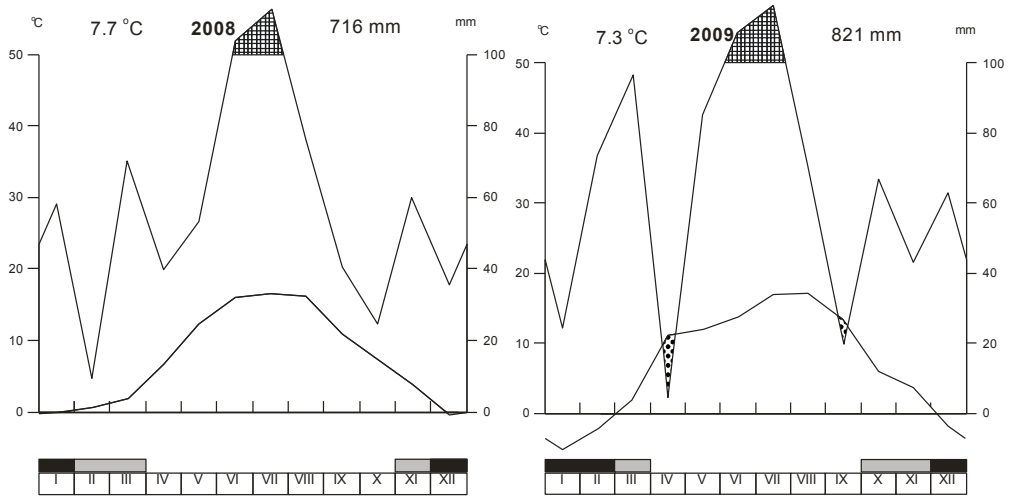
Various grasses are used for grazing and the production of canned feed, however, There are considerable differences among the grass species. *Lolium perenne* includes among the species susceptible to fungal infestation. On the contrary, Festulolium ssp. are being considered as the resistant species [3]. Interspecific hybrids of *Festulolium* ssp. combine the endurance of the *Festuca* sp. family with the high quality of the *Lolium* sp. family. The aim of the study was to assess the content of mycotoxins in green mass of selected forage grass species during the growing season and at the end of the growing season. Furthermore, an assessment mycotoxin contents in subsequently produced the first cut silages were also evaluated with respect to species used in this study (*Lolium perenne* (cv. Kentaur), *Festulolium pabulare* (cv. Felina), *Festulolium braunii* (cv. Perseus) and mixture of these species with *Festuca rubra* (cv. Gondolin) or/and *Poa pratensis* (Slezanka)).

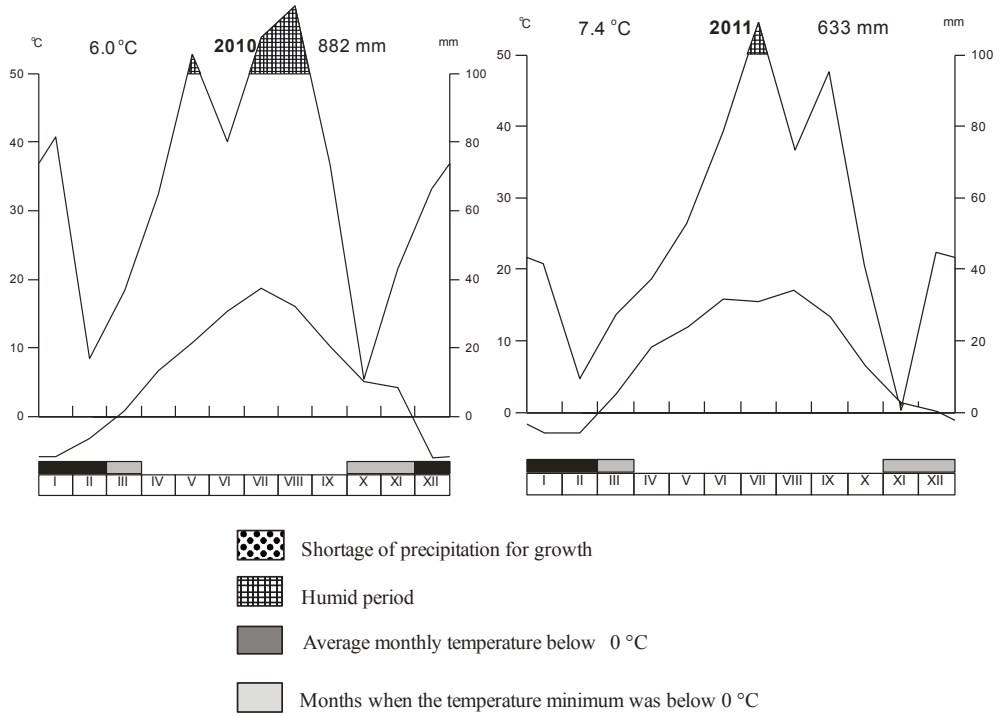
2. Experimental Section

2.1. Plant Material and Cultivation

The small-plot experiment was conducted at the Research Station of Fodder Crops in Vatín, Czech Republic (49°31'N, 15°58'E) and established in 2007 at an altitude of 560 m a.s.l. In 1970–2000, mean annual precipitation was 617 mm and mean annual temperature was 6.9 °C. Precipitation and mean temperature in years of observed are in Fig. 1. Soil type used in our experiments was Cambisol as a sandy-loam on the diluvium of biotic orthogneiss. In the year of observation, the contents of soil nutrients were 89.1 mg kg^{-1} P, 231.6 mg kg^{-1} K, 855 mg kg^{-1} Ca; pH was 4.76. The experimental plots were fertilized with 50 kg ha^{-1} N in the spring (March). Dates of cuts were beginning of June, end of July, and beginning of October, beginning of November and beginning of December. Biomass from the first cut was ensilaging. The experiment was carried out in triplicates. A split plot design was used with plots of 1.5 × 10 m. The plots were harvested by the self-propelled mowing machine with an engagement width of 1.25 m. Harvested area was 12.5 m^2. Stubble height was 0.07 m. The grasses were harvested at the stage of earing.

Figure 1. Precipitations and mean temperatures in years 2008 – 2011 in Research Fodder Station Vatin.





2.2. Green mass and silages preparation

The contents of mycotoxins in green mass and contents of mycotoxins in silages were evaluated. In the evaluation of green mass, species as *Lolium perenne* (cv. Kentaur), *Festulolium pabulare* (cv. Felina), *Festulolium braunii* (cv. Perseus) and mixture of these species with *Festuca rubra* (cv. Gondolin) or/and *Poa pratensis* (Slezanka) was as the first factor taken into the account (Table 1). Pure stands of each species were sown with 30 kg ha^{-1} seeds. Sown of the mixtures was 37.5 kg ha^{-1}. Season with degree beginning of June, end of July, beginning of October, beginning of November and beginning of December was the second evaluated factor. The cumulative effect was observed. In the evaluation of silages type of species was the first factor. Second factor was preservative with degree untreated, chemical ingredient (formic acid, propionic acid, ammonium formate) and biological-enzymatic inoculant (*Enterococcus faecium*, *Lactobacillus plantarum*, *Pediococcus acidilactici*, *Lactobacillus salivarius*, cellulase, hemicellulase, and amylase, with 1x1011 CFU; 10 g t^{-1}). The amount of chemical ingredient was 4 l t^{-1} and the amount of biological additive was 10 g t^{-1}. The assessed grasses were wilted 20 – 30 hours. Biomass was after wilting ensilaged in containers whose diameter and height were 0.15 m and 0.64 m, respectively. The observation

of silages was for three years 2008 (1st harvest year), 2009 (2nd harvest year) and 2010 (3rd harvest year). In the fourth harvest year, silages were not produced due to low yields.

2.3. Mycotoxin determination

Green forage samples and silages dried at 60 °C and homogenized to a particle size of < 1 mm were analysed for content of mycotoxins deoxynivalenol (DON), zearalenone (ZEA), fumonisin (FUM), aflatoxin (AFL) and T2 toxin (T2) by ELISA method according to [13]. The data were processed using the STATISTICA.CZ Version 8.0 (Czech Republic). The results are expressed as means (x). The obtained results were further analysed using the ANOVA and Scheffe test. Graphical representation of cluster analysis was performed.

3. Results and Discussion

3.1. Green mass

In our study, mycotoxins deoxynivalenol (DON), zearalenone (ZEA) and T2 toxin (T2) were mainly detected. The content of fumonisin (FUM) and aflatoxin (AFL) was in the majority of samples below the limit of detection. The lowest DON content in green mass was found at *Festulolium pabulare* as 31.02 ppb (Table 1). On the contrary, the highest content in the green mass was determined at mixture with *Festuca rubra* as 42.15 ppb. Similarly, the low levels of ZEA were determined at the green mass of *Festulolium pabulare*. Due to the high variability of the samples, statistically significant influence of grass species on mycotoxin content was not confirmed. However, it is obvious lower tendency to the occurrence of mycotoxins in *Festulolium pabulare*. This is evidenced by the results of the cluster analysis (Fig. 2). *Festulolium pabulare* stands outside a cluster of other species, particularly in June, October and December.

Table 1. Influence of species, date of cut and year on the content (ppb) of deoxynivalenol (DON), fumonisin (FUM), aflatoxins (AFL), zearalenone (ZEA) and T2-toxin (T2) in green mass of grasses. LOQ…limit of quantification.

Factor	DON	FUM	AFL	ZEA	T2
Species					
Lolium perenne	41.03	<LOQ	<LOQ	17.06	24.80
Festulolium pabulare	31.02	<LOQ	0.07	4.95	24.19

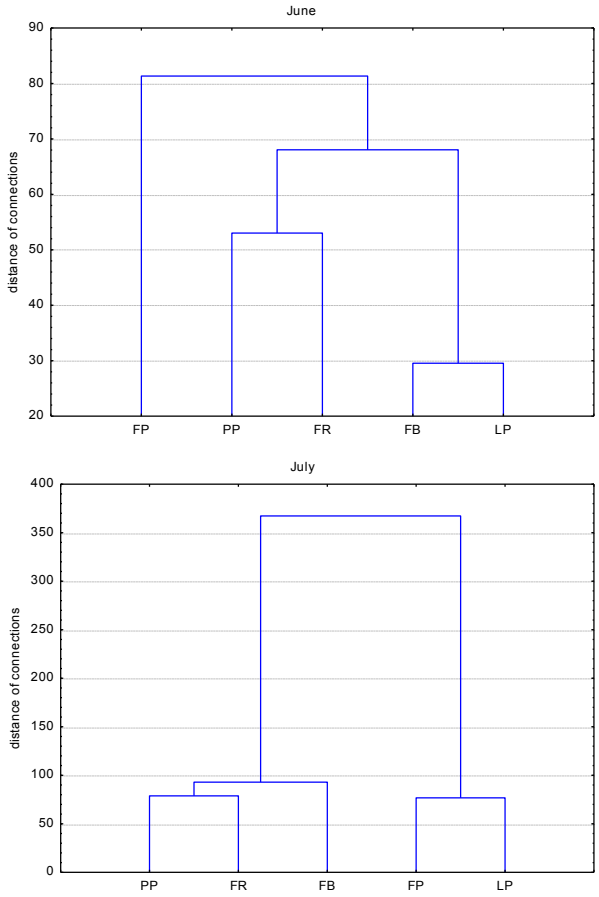
Festulolium braunii	36.98	<LOQ	<LOQ	36.45	24.94
Mixture with *Festuca rubra*	42.15	<LOQ	<LOQ	47.37	30.40
Mixture with *Poa pratensis*	40.19	<LOQ	<LOQ	48.15	29.98
p	0.6347	-	0.5288	0.4581	0.7976
Date of Cut					
Beginning June	16.09^{a}	<LOQ	<LOQ	1.46	24.70
End July	51.90^{b}	<LOQ	0.09	61.18	28.49
Beginning October	41.94^{b}	<LOQ	<LOQ	86.55	36.49
Beginning November	41.58^{b}	<LOQ	<LOQ	1.88	18.25
Beginning December	39.86^{ab}	<LOQ	<LOQ	2.91	26.39
p	0.0004	-	0.0176	0.0045	0.1112
Year					
2008	37.63^{ab}	<LOQ	<LOQ	115.76^{a}	34.89^{ab}
2009	46.28^{a}	<LOQ	0.08	6.15^{b}	48.37^{b}
2010	47.13^{a}	<LOQ	<LOQ	<LOQ^{b}	5.34^{c}
2011	22.06^{b}	<LOQ	0	1.23^{b}	18.87^{ac}
p	0.0019	-	0.0138	0.0000	0.0000

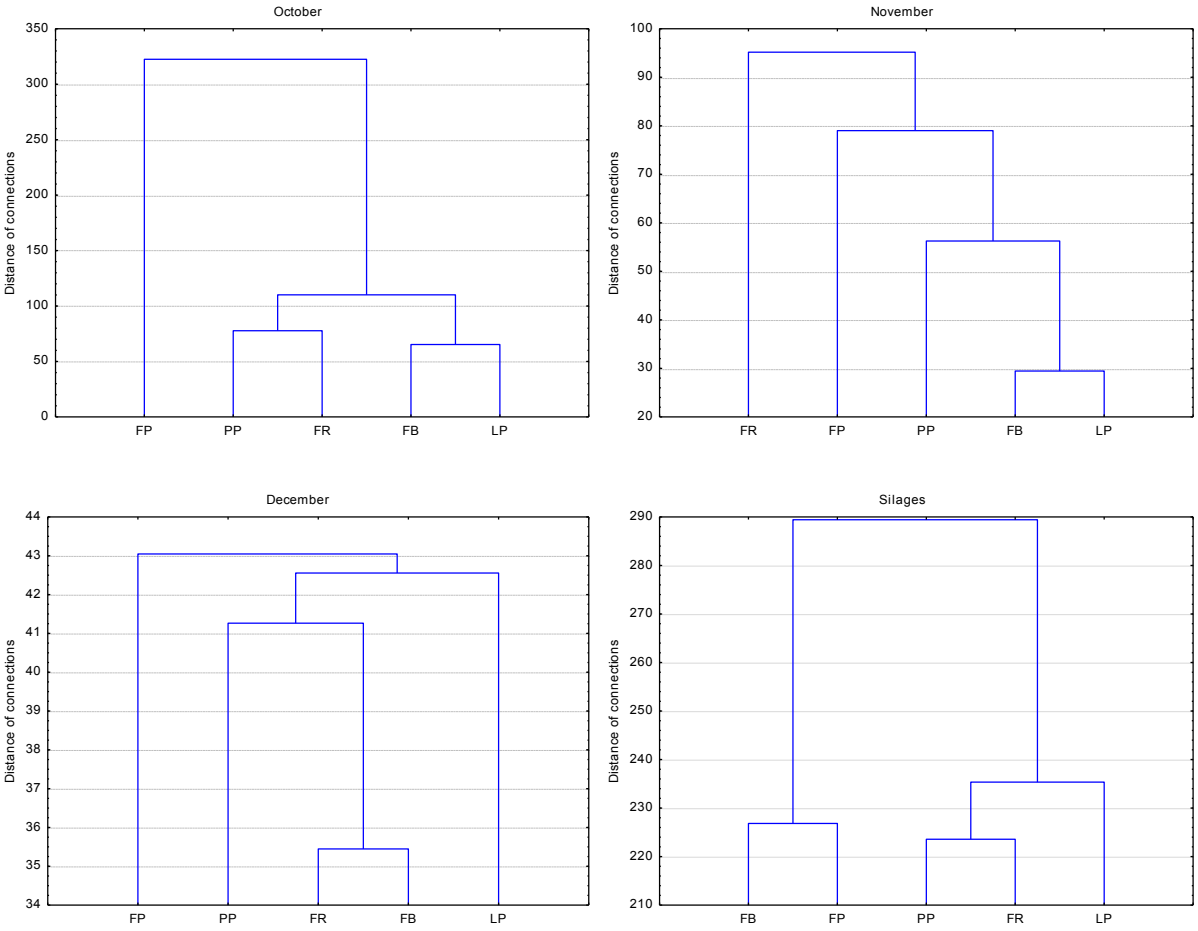
Mean values in the same column with different superscripts (^{a,b,c}) are significant at the P<0.05 level after Scheffe test analysis.

Date of cut influenced (P <0.01), especially the content of deoxynivalenol and zearalenone. Deoxynivalenol content was highest (P <0.05) at the end of July (51.90 ppb). High content retained also in October (41.94 ppb) and November (41.58 ppb). In December there was a decrease to 39.86 ppb. Similarly, high zearalenone (61.18 ppb) content, which culminated in October on the value of 86.55 ppb, was found at the late of July. The population density of filamentous fungi is positively associated with the senescence process of plants [14]. Fodder from November and December had low levels of zearalenone (1.88 and 2.91 ppb,

respectively). Reduction of mycotoxins in forage late autumn and early winter is also evidenced by analysis of T2 toxin. However, the onset of winter would be associated to the death of biomass, as already mentioned above, and the senescent processes would be bound by microscopic fungi capable of producing mycotoxins. One would expect rather more of mycotoxins increase with continued coming autumn and winter. The fact, however, was the opposite. Low temperatures reduce the risk of mycotoxins (mean annual temperature was 6.9 °C, Fig. 1). Denijs et al., Engels and Krämer, and Behrendt et al. also found the influence of not only biotic, but also abiotic factors on the production of mycotoxins [14-16]. Higher levels of mycotoxins can be determined in the winter months as shown by Goliński et al. [17]. Fodder from the beginning of June is generally characterized by low levels of mycotoxins, especially this is evident ($P < 0.01$) for deoxynivalenol and zearalenone.

Figure 2. Cluster analysis of evaluated species.





LP = *Lolium perenne*, FP = *Festulolium pabulare*, FB = *Festulolium braunii*, FR = mixture with *Festuca rubra*, PP = mixture with *Poa pratensis*

The interannual variability of the average content of deoxynivalenol, zearalenone and T2 toxin is significant ($P < 0.01$). In the case of deoxynivalenol, there is an obvious difference ($P < 0.05$), especially between 2010 and 2011. More evident differences are in the content of zearalenone. While in 2008, the content of zearalenone was 115.76 ppb, but it was only 6.15 ppb in 2009, and only 1.23 ppb in 2011. In 2010, the content of zearalenone was even below the limit of detection. 2010 was characterized by very low ($P < 0.05$) content of T2 toxin. Moisture, temperature and availability of nutrients and oxygen belong to the important factors influencing the growth of mould [18]. The combination of these factors can have a significant share on the annual fluctuations in the concentrations of mycotoxins. In 2008, when the highest occurrence of mycotoxins in green fodder was determined, the highest average annual temperature was measured and the precipitation was evenly distributed in each month. During the year there was enough precipitation for plant growth. In contrast, the following years had

lower mean annual temperature and especially autumn months were characterized by a lack of precipitation. Sometimes precipitation curve falls below the curve of temperatures, which indicates lack of moisture for plant growth. This may be reflected also on the growth of mould and subsequent mycotoxin production.

3.2. Silages

Grass specie had no influence on the content of mycotoxins in silages of the first cut (Table 2). Differences between species are minimal in produced silages. However, there is an interesting difference in the content of mycotoxins between green mass and silages. The increase in the content of deoxynivalenol, zearalenone and T2 toxin in silages compared with green mass shows Table 3. DON content in silages increased up to 405.2%. The highest content was found at mixture with *Poa pratensis* (167.70 ppb). Nevertheless, Charmley et al. reports the transition of DON to the milk from the value of 6 mg kg^{-1} [8]. European Commission (Commission Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding (2006/576/EC)) advisory guideline for DON is 5 mg kg^{-1} DM. Zearalenone content increased up to 868% in silage from *Festulolium pabulare*. The lowest content of zearalenone was determined in silage mixture with *Festuca rubra* (66.89 ppb). The guidance value for zearalenone in Europe is 500 mg kg^{-1}. According to D'Mello, zearalenone concentration ranging from 0.2 to 1.0 mg kg^{-1} is even toxic for rodents [19]. Forage with a zearalenone content higher than 0.5 mg kg^{-1} is not advised for feeding [20]. Except for fumonisin and aflatoxin, where no difference between the green mass and silage was found, the smallest changes in T2 toxin were recorded. T2 toxin content in silages increased by a maximum of 86.8 % at *Festulolium pabulare*. However, the increase of mycotoxins in silages is in some cases very significant. Silage is a process where lactic acid bacteria ferment simple sugars and produce acids, which reduce the pH and consequently there is a reduction of growth of undesirable microorganisms (Garon et al., 2006). The increase in mycotoxin produced silages was probably caused by the production of mycotoxins during withering and probably during the first phase of aerobic fermentation. Anaerobic environment reduces the growth of fungi and silage is from this perspective effective strategy to prevent the growth of mycotoxin [6]. Silage is contaminated with mycotoxins and has consequent reduced health feed safety already in the field, at least during the first hours after the start of ensiling. This finding supports the fact that DON and zearalenone, as well as other *Fusarium* mycotoxins, are produced in silages [18]. On the other hand, there are also studies showing the development of these mycotoxins in silages [21-24]. In any case, the results indicate that mycotoxins have been degraded silage process. However, there are studies demonstrating

strong reduction of mycotoxin promoting silage process [25,26]. However, we do not support this assumption. Cluster analysis (Fig. 2) shows on the one hand the similarity of intergeneric hybrids (*Festulolium pabulare* and *Festulolium braunii*) and on the other hand, a cluster of *Lolium perenne* and mixtures with both *Festuca rubra* or *Poa pratensis*.

Table 2. Influence of species, preservative and year on the content (ppb) of deoxynivalenol (DON), fumonisin (FUM), aflatoxins (AFL), zeatralenone (ZEA) and T2-toxin (T2) in silages from first cut.

Factor	DON	FUM	AFL	ZEA	T2
Species					
Lolium perenne	141.39	<LOQ	<LOQ	66.07	20.37
Festulolium pabulare	156.73	<LOQ	<LOQ	47.92	45.19
Festulolium braunii	143.60	<LOQ	<LOQ	46.34	43.04
Mixture with *Festuca rubra*	161.97	<LOQ	<LOQ	66.89	38.58
Mixture with *Poa pratensis*	167.70	6.07	0.21	54.46	19.96
p	0.5142	0.4207	0.2551	0.1577	0.8363
Preservative					
Untreated	139.19^{a}	<LOQ	<LOQ	60.28	21.81
Chemical	182.71^{b}	<LOQ	<LOQ	53.40	21.64
Biological-enzymatic	140.93^{a}	3.66	0.14	55.33	56.83
p	0.0042	0.3765	0.4899	0.6803	0.2137
Year					
2008	164.61	0	<LOQ	53.95^{ab}	12.67
2009	156.49	<LOQ	<LOQ	73.24^{a}	42.97
2010	141.73	3.72	0.15	41.81^{b}	44.65
p	0.2553	0.3590	0.4037	0.0016	0.2929

Mean values in the same column with different superscripts (^{a,b,c}) are significant at the P<0.05 level after Scheffe test analysis

Table 3. Difference (%) of content of deoxynivalenol (DON), zeatralenone (ZEA) and T2-toxin (T2) between green mass and silages. GM = green mass, S = silages

Factor	DON			ZEA			T2		
	GM	S	*Rel. %*	GM	S	*Rel. %*	GM	S	*Rel. %*
Lolium perenne	41.03	141.39	*344.6*	17.06	66.07	*387.3*	24.80	20.37	*82.1*
Festulolium pabulare	31.02	156.73	*505.2*	4.95	47.92	*968.0*	24.19	45.19	*186.8*
Festulolium braunii	36.98	143.60	*388.3*	36.45	46.34	*127.1*	24.94	43.04	*172.6*
Mixture with *Festuca rubra*	42.15	161.97	*384.3*	47.37	66.89	*141.2*	30.40	38.58	*126.9*
Mixture with *Poa pratensis*	40.19	167.70	*417.3*	48.15	54.46	*113.1*	29.98	19.96	*66.6*

The detected preservatives did not prevent mycotoxin production. In the case of deoxynivalenol even supplementation organic acids lead to an increase (P <0.05) the content. It is precisely the addition of organic acids, in particular propionic acid, which has antifungal effects [27]. However acids and inoculants have no effect on mycotoxins that have been already synthesized (Binder, 2007). Year affected (P <0.01) content of zearalenone in silages. The lowest content of zearalenone (P <0.05) was found in silages in 2010, similar to the green mass.

4. Conclusions

Mycotoxins belong to the secondary metabolites having harmful effects on mammals. It is clear that their contents are monitored and their effects are intensively studied. In this study, we investigated several factors influencing the content of these secondary metabolites in green mass and silages prepared from various grass species. It can be concluded based on the results obtained that low temperatures can be beneficial for not-production of mycotoxins, however, processing of green matter for silage can be also source for mycotoxins occurrence, which should be also taken into account.

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Revidovaná verze

How do grass species, season and ensiling influence mycotoxin content in forage?

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Abstract: Mycotoxins are secondary metabolites produced by fungi species and that have harmful effects on mammals. The aim of this study was to assess the content of mycotoxins in fresh-cut material of selected forage grass species both during and at the end of the growing season. We further assessed mycotoxin content in subsequently produced first-cutting silages with respect to the species used in this study: *Lolium perenne* (cv. Kentaur), *Festulolium pabulare* (cv. Felina), *Festulolium braunii* (cv. Perseus), and mixtures of these species with *Festuca rubra* (cv. Gondolin) or *Poa pratensis* (Slezanka). Mainly the mycotoxins deoxynivalenol, zearalenone and T-2 toxin were detected in the fresh-cut grass material while fumonisin and aflatoxin contents were below the detection limits. July and October were the most risky periods for mycotoxins to occur. During cold temperatures in November and December, the occurrence of mycotoxins in

fresh-cut material declined. Although June was a period with low incidence of mycotoxins in green silage, contents of deoxynivalenol and zearalenone in silages from the first cutting exceeded by several times those determined in their biomass collected directly from the field. Moreover, we observed that use of preservatives or inoculants did not prevent mycotoxin production.

Keywords: grass; silage; mycotoxin; environmental factor

1. Introduction

Clean and healthy phytomass is a prerequisite for producing high-quality forage. Potential plant contaminants include various epiphyte microflora as undesirable clostridia (*Clostrium spp.*) and fungi (*Fusarium spp.*, *Puccinia spp.*) [1,2]. Development of microscopic fungi may lead to the formation of mycotoxins [3], which are secondary metabolites produced especially by the fungi *Aspergillus*, *Penicillium* and *Fusarium* [4]. Mycotoxins are produced due to interactions and reactions of fungi to environmental conditions [5]. While such production is especially associated with stress caused by extreme weather conditions or damage from insects or animals, mycotoxin contamination of silages is nevertheless associated with failure in silage management practices [6].

Mycotoxins can cause serious health problems in the human population. The incidence of liver cancer caused by aflatoxins is believed to be increasing each year, for example, and up to 28.2% of liver cancer cases may be due to aflatoxins [7]. Mycotoxins naturally have negative impacts also upon livestock, causing alterations in hormonal functions, poor feed utilization, lower rates of body weight gain, and possibly death. Moreover, some mycotoxins may pass into milk, which could represent risk for the food chain [8-11].

Preventing the occurrence of mycotoxins in forage should begin in the field, and certain guidelines have been suggested and practices recommended to avoid that. These include to use varieties or hybrids that are well adapted to the given growing area and that are resistant to fungal diseases [12].

Various grasses are used for grazing and producing stored forages, and it exists considerable differences between these grass species. Among those species, *Lolium perenne* is susceptible to fungal infestation. By contrast, *Festulolium* ssp. are considered to be resistant [3]. Interspecific hybrids of *Festulolium* ssp. may combine the endurance of the *Festuca* spp. with the high quality of the *Lolium* spp. Poa pratensis and Festuca rubra are ones of the rhizomatic grasses, which are used to thicken the lower floor stand and contribute to the density of the stands [13].

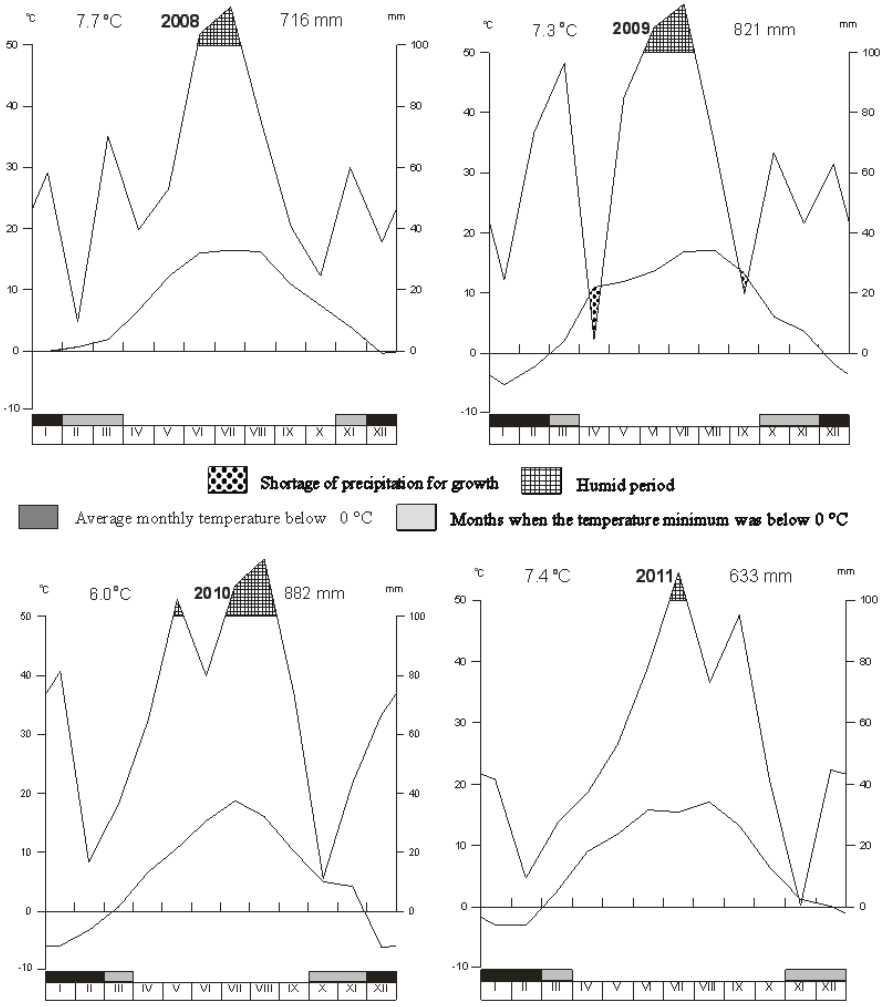
The aim of the present study was to assess mycotoxins content in feedstuffs under Central European environmental conditions [14,15] and the risk to health safety posed by mycotoxins in fresh-cut material of selected forage grass species both during and at the end of the growing season. Furthermore, mycotoxin content was assessed in subsequently produced first-cutting silages with respect to the various species used in this study: *Lolium perenne* (cv. Kentaur), *Festulolium pabulare* (cv. Felina), *Festulolium braunii* (cv. Perseus), and mixtures of these species with *Festuca rubra* (cv. Gondolin) or *Poa pratensis* (Slezanka). The choice of species considered the facts described above, and the various species were either potentially susceptible to diseases or potentially more resistant to disease. When producing silage, a chemical preservative or biological inoculant was applied.

2. Experimental Section

2.1. Plant Material and Cultivation

A small-plot experiment was established in 2007 at the Research Station of Fodder Crops in Vatín, Czech Republic (49°31'N, 15°58'E, 560 m a.s.l.). The climate at the station can be characterized by the 1970–2000 mean annual precipitation of 617 mm and mean annual temperature of 6.9 °C. Figure 1 reports precipitation and mean temperature during the observation years (2008–2011). These data were obtained from a meteorological station situated at the experimental location. The soil type used in our experiments was Cambisol as a sandy-loam on a diluvium of biotic orthogneiss. In the years of observation, the contents of soil nutrients were 89.1 mg kg^{-1} P, 231.6 mg kg^{-1} K, and 855 mg kg^{-1} Ca; pH was 4.76. The experimental plots were fertilized with 50 kg ha^{-1} N in spring (March). Times of cutting were the beginning of June, end of July, beginning of October, beginning of November and beginning of December. Biomass from the first cutting was ensiled. The experiment was carried out in triplicate. A split-plot design was used with plots of 1.5 × 10 m. The plots were harvested using a self-propelled mowing machine with an engagement width of 1.25 m. Harvested area was 12.5 m^2. Stubble height was 0.07 m. The grasses were harvested at the earing stage.

Figure 1. Precipitation and mean temperatures in years 2008–2011 at Research Station of Fodder Crops, Vatin, Czech Republic.



2.2. Fresh-Cut Material and Silages Preparation

Mycotoxin contents of fresh-cut material and in silages were evaluated. In evaluating fresh-cut material, species was the first factor examined (Table 1). Season was the second factor examined, and it was defined by time of cutting, as follows: beginning of June, end of July, beginning of October, beginning of November and beginning of December. The combined effects of the two factors were also observed. In evaluating silages, grass species was the first factor examined. The second factor was use of inoculant, the groups being untreated, treated with chemical ingredient (formic acid [43% *w*/*w*], propionic acid [10% *w*/*w*], ammonium formate [30% *w*/*w*], benzoic acid [2% *w*/*w*]), and treated with biological–enzymatic inoculant (containing *Enterococcus faecium*, *Lactobacillus plantarum*, *Pediococcus acidilactici*, *Lactobacillus salivarius*, cellulase, hemicellulase, and amylase, with 1 x 10^{11} CFU/g). The amount of chemical ingredient added was 4 l t^{-1} of ensiled material and that of biological additive was 10 g t^{-1}. Biological additive was diluted in water at the rate of

2 l t^{-1}. Chemical and biological additives were applied by spraying onto fresh-cut material. During the application, the material was mixed in order to spread the additives evenly. Material for ensiling was harvested from the first cutting in the first week of June. Grasses were allowed to wilt and dry for 20 to 30 h after mowing. The wilted biomass was ensiled in containers with diameter and height 0.15 m and 0.64 m, respectively. Silages were sampled 90 d after closing the containers. Silages were observed in the three years 2008 (1st harvest year), 2009 (2nd harvest year) and 2010 (3rd harvest year). In the fourth harvest year, silages were not produced due to low grass yields.

Silages sampled 90 d after ensiling were assessed for pH, acidity of water extract (AWE), as well as contents of lactic acid (LA), acetic acid (AA), butyric acid (BA) and NH_3. Values of pH were from 4.05 to 4.26. Content of lactic acid (LA) was from 10.39 to 16.63 %, content of acetic acid (AA) was from 1.23 to 3.25 %, content of NH_3 was from 0.1541 to 0.1752 % and content of ethanol was from 1.88 to 4.28 %.

2.3. Mycotoxin Determination

Green forage samples and silages were dried at 60 °C, ground to a particle size of < 1 mm, then analyzed for content of the mycotoxins deoxynivalenol (DON), zearalenone (ZEA), fumonisin (FUM), aflatoxin (AFL) and T-2 toxin (T-2) using enzyme-linked immunosorbent assay (ELISA) according to Skladanka *et al.* (2011) [16]. ELISA is a competitive, direct enzyme-linked assay for quantitative analysis. The toxin concentration is expressed in parts per billion (ppb). The data were processed statistically using STATISTICA.CZ Version 8.0 (Czech Republic). The results are expressed as means (x). The results obtained were then further analyzed using ANOVA and Scheffé's method. Cluster analysis was performed to create graphical representations.

3. Results and Discussion

3.1. Fresh-Cut Material

In our study, mainly the mycotoxins DON, ZEA and T-2 were detected. The contents of FUM and AFL were below the limits of detection in the majority of samples. The lowest DON content in fresh-cut material was found in *F. pabulare*, at 31.02 ppb (Table 1). The highest DON content in the fresh-cut material was determined for the mixture with *F. rubra*, at 42.15 ppb. Similarly, the lowest levels of ZEA were determined in the fresh-cut material of *F. pabulare*. Due to high variability among samples, no statistically significant influence of grass species on mycotoxin content was confirmed. There nevertheless was a clear lower tendency for mycotoxins to occur in *F. pabulare*. This is evidenced by the results of the

cluster analysis (Figure 2), where *F. pabulare* stands outside a cluster of the other species for June, October and December.

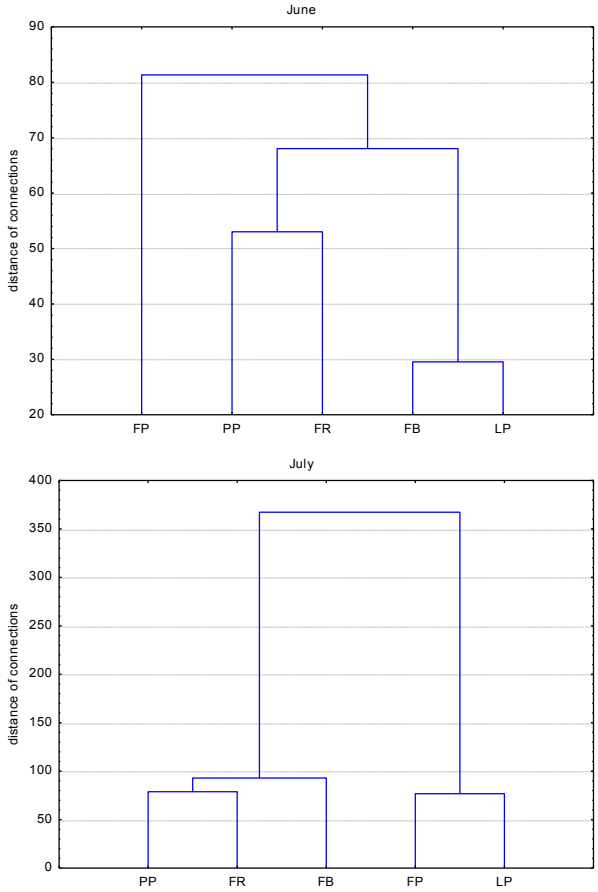
Table 1. Influence of species, season (time of cutting) and year on the content (ppb) of deoxynivalenol (DON), fumonisin (FUM), aflatoxins (AFL), zearalenone (ZEA) and T-2 toxin (T-2) in fresh-cut material of grasses.

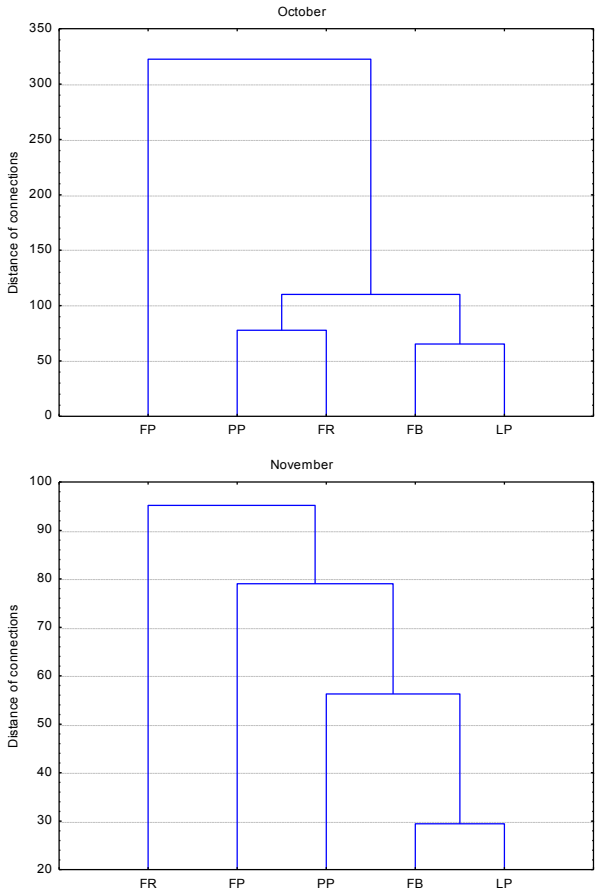
Factor	DON		FUM		AFL		ZEA		T-2	
	x	s.d.	x	s.d.	x	s.d.	x	s.d.	x	s.d.
Species										
Lolium perenne	41.03	6.12	<LOQ	-	<LOQ	0,01	17.06	15,52	24.80	5,76
Festulolium pabulare	31.02	6.27	<LOQ	-	0.07	0,05	4.95	2,70	24.19	6,27
Festulolium braunii	36.98	5.49	<LOQ	-	<LOQ	0,01	36.45	25,05	24.94	5,33
Mixture with *Festuca rubra*	42.15	6.72	<LOQ	-	<LOQ	0,01	47.37	31,50	30.40	6,45
Mixture with *Poa pratensis*	40.19	7.56	<LOQ	-	<LOQ	0,01	48.15	30,99	29.98	6,34
p	0.6347		-		0.5288		0.4581		0.7976	
Season (time of cutting)										
Beginning June	16.09[a]	3,76	<LOQ	-	<LOQ	0,01	1.46	1,43	24.70	6,35
End July	51.90[b]	6,55	<LOQ	-	0.09	0,05	61.18[a]	33,51	28.49	6,70
Beginning October	41.94[b]	5,90	<LOQ	-	<LOQ	0,01	86.55[a]	37,83	36.49	5,80
Beginning November	41.58[b]	6,99	<LOQ	-	<LOQ	0,01	1.88	1,80	18.25	5,72
Beginning December	39.86[ab]	5,97	<LOQ	-	<LOQ	0,01	2.91	1,97	26.39	4,90
p	0.0004		-		0.0176		0.0045		0.1112	
Year										

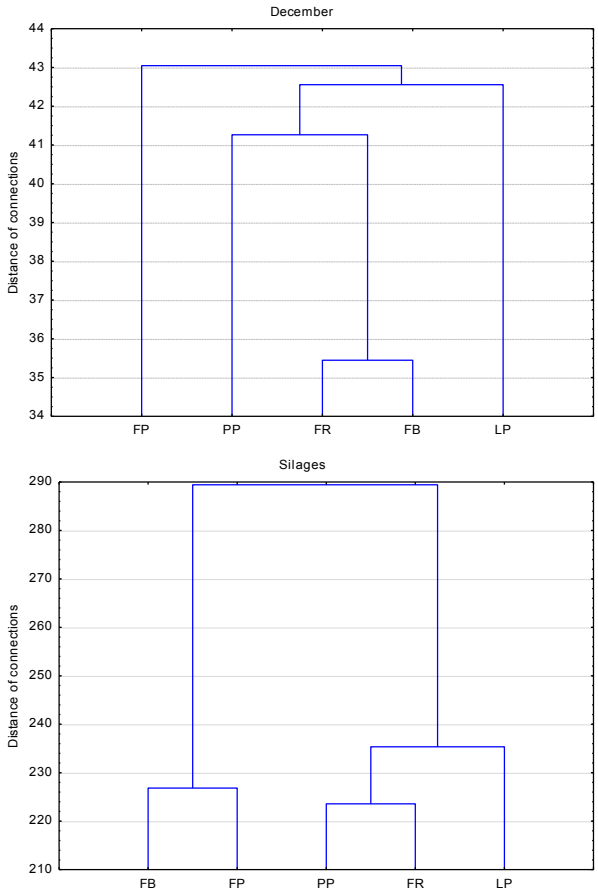
2008	37.63^{ab}	7,65	<LOQ	-	<LOQ	0.01	115.76^{a}	37,82	34.89^{ab}	5,26
2009	46.28^{a}	5,67	<LOQ	-	0.08	0.04	6.15^{b}	2,50	48.37^{b}	3,99
2010	47.13^{a}	3,64	<LOQ	-	<LOQ	0.01	<LOQ^{b}	0,01	5.34^{c}	3,03
2011	22.06^{b}	3,78	<LOQ	-	<LOQ	0.01	1.23^{b}	1,14	18.87^{ac}	4,51
p	0.0019		-		0.0138		0.0000		0.0000	
Species x Season	0.7797		-		0.6986		0.9950		0.9766	
Species x Year	0.9552		-		0.3676		0.6122		0.9906	
Season x Year	0.0000		-		0.0518		0.0000		0.0001	

Mean values in the same column with different superscripts (^{a,b,c}) are significant at the $p < 0.05$ level after Scheffé's method analysis. LOQ = limit of quantification. x = mean. s.d. = standard deviation.

Figure 2. Cluster analysis of evaluated species (Euclidean distance), 2008–2011.




October
Distance of connections
350
300
250
200
150
100
50
0
FP
PP
FR
FB
LP
November
Distance of connections
100
90
80
70
60
50
40
30
20
FR
FP
PP
FB
LP



LP = *Lolium perenne*, FP = *Festulolium pabulare*, FB = *Festulolium braunii*, FR = mixture with *Festuca rubra*, PP = mixture with *Poa pratensis*.

Time of cutting influenced ($p < 0.01$) especially the contents of DON and ZEA. Deoxynivalenol content was highest ($p < 0.05$) at the end of July (51.90 ppb). High DON content remained also in October (41.94 ppb) and November (41.58 ppb). In December, DON decreased to 39.86 ppb. Similarly, high ZEA content was found in late July (61.18 ppb), and this culminated in October at 86.55 ppb. The population density of filamentous fungi is known to be positively associated with the senescence process of plants [17], and yet forage from November and December had low levels of ZEA (1.88 and 2.91 ppb, respectively). Reduction of mycotoxins in forage during late autumn and early winter is also evidenced by the analysis for T-2. In as much as the onset of winter would be associated with the death of biomass and the senescent processes would themselves be associated with microscopic fungi capable of producing mycotoxins, one would expect rather greater increase of mycotoxins as autumn and winter drew nearer and nearer. In fact, however, the opposite was true. Low temperatures reduce the risk from mycotoxins. It is obvious that the higher humidity of the

growing season contributes to the development of mold, but low temperatures inhibit formation of mycotoxins. Fall of temperature under 5 °C in November and December can lead to reduction of enzymatic activity of mold and lower production of mycotoxins, which comprise stress reaction on the lower temperature [18-20]. Denijs *et al.*, Engels and Krämer, and Behrendt *et al.* had also observed the influence of not only biotic but also abiotic factors on the production of mycotoxins [17,21,22]. Moreover, higher levels of mycotoxins occurring during winter months were reported by Golinski *et al.* [23]. Forage from the beginning of June is generally characterized by low levels of mycotoxins, and this is especially evident ($p < 0.01$) for DON and ZEA.

The interannual variability of the average DON, ZEA and T-2 contents was significant ($p < 0.01$). In the case of DON, there was an obvious difference ($p < 0.05$) especially between 2010 and 2011. Even more evident differences occurred in ZEA content. While in 2008 ZEA content was 115.76 ppb, it was only 6.15 ppb in 2009 and just 1.23 ppb in 2011. In 2010, ZEA content was even below the limit of detection. Meanwhile, 2010 was characterized by very low T-2 content ($p < 0.05$). There were differences among the evaluated years in terms of total rainfall and its distribution as well as in average annual temperatures and temperature changes.

Moisture, temperature and availability of nutrients and oxygen are among the important factors influencing mold growth [24]. The combination of these factors can have a significant proportionate influence on annual fluctuation in mycotoxin concentrations. In 2008, when the greatest occurrence of mycotoxins in green forage was determined, the highest average annual temperature was measured and precipitation was well distributed within and between months. There was sufficient precipitation for plant growth through the year. By contrast, the following years had lower mean annual temperatures and especially the autumn months were characterized by a lack of precipitation. Sometimes, the precipitation curve falls below the curve of temperatures, which indicates lack of moisture for plant growth. This may be reflected also in the growth of mold and subsequent mycotoxin production. Temperature may affect the utilization of certain nutrients in the soil, and in particular phosphorus [25,26]. Reduced nutrients availability can cause plants to have lower resistance to disease and subsequently to be subject to mold development. The year 2008 was among the warmest, and there was a higher incidence of ZEA in the green plant material. In 2009, when there was an obvious drought and rainfall was insufficient for plant growth, higher levels of T-2 were found.

3.2. Silages

Grass species had no influence on the content of mycotoxins in silages from the first cutting (Table 2). Differences between species were minimal in the silages produced. There were, however, interesting differences in the contents of mycotoxins between fresh-cut material and silages. The increase in the contents of DON, ZEA and T-2 in silages compared with fresh-cut material is shown in Table 3. DON content in silages increased by as much as 400%. This rise could be due to a higher temperature after closing of the silo containers. Higher temperatures constitute a stress factor that can trigger production of mycotoxins. After closing the silo containers, the aerobic phase, during which aerobic microorganisms consume the remaining oxygen, produces heat. Mold growth then diminishes during the following anaerobic phase, but the mycotoxins already produced are nevertheless preserved in the silages.

The highest content of mycotoxin generally and of DON in particular (167 ppb) was found in the mixture with *P. pratensis*. Charmley *et al.* have reported that DON may be passed to milk when its content in feedstuffs reaches the level of 6 mg kg^{-1} [8]. The European Commission advisory guideline for DON is 5 mg kg^{-1} of dry matter (Commission Recommendation of 17 August 2006 on the presence of DON, ZEA, ochratoxin A, T-2 and HT-2 toxins, and fumonisins in products intended for animal feeding [2006/576/EC]). Zearalenone content increased by as much as 868% in silage from *F. pabulare*. The highest ZEA content was determined in the silage mixture with *F. rubra* (66.89 ppb). The guidance value for ZEA in Europe is 500 µg kg^{-1} of dry matter. According to D'Mello, ZEA in concentrations ranging from 0.2 to 1.0 mg kg^{-1} is even toxic to rodents [27]. It is advised not to use for feeding purposes forage with ZEA content higher than 0.5 mg kg^{-1} [28]. Aside from FUM and AFL, for which no differences between the fresh-cut material and silages were found, the smallest changes after ensiling were recorded for T-2. T-2 content in silages increased by a maximum of 86.8% in the case of *F. pabulare*.

The increase of mycotoxins in silages was in some cases very significant. Ensiling is a process whereby lactic acid bacteria ferment simple sugars and produce acids. This reduces the pH and consequently there is diminished growth of undesirable microorganisms (Garon *et al.*, 2006). The increase of mycotoxins within the silages was probably caused by the production of mycotoxins during wilting of the cut grass and the first phase of aerobic fermentation. Because an anaerobic environment reduces the growth of fungi, ensiling is from this perspective an effective strategy to prevent the production of mycotoxins [6]. Material for producing silage is contaminated with mycotoxin-producing fungi already in the field, and consequently the feeding safety continues to diminish at least through the first several hours after the start of ensiling. Our findings support earlier observations that DON, ZEA and other *Fusarium* mycotoxins are produced in silages [24]. In any case, our results indicate that

mycotoxins were generally not degraded by the ensiling process. Nevertheless, there are other studies demonstrating potential for strongly reducing mycotoxins production during the ensiling process by using, for example, inoculants [29,30].

Cluster analysis (Figure 2) in relation to the silages shows, on the one hand, a similarity between the intergeneric hybrids (*F. pabulare* and *F. braunii*) and, on the other hand, a cluster of *L. perenne* and both mixtures including *F. rubra* or *P. pratensis*.

Table 2. Influence of species, preservative or inoculant, and year on the content (ppb) of deoxynivalenol (DON), fumonisin (FUM), aflatoxins (AFL), zearalenone (ZEA) and T-2 toxin (T-2) in silages from the first cutting of grasses.

Factor	DON		FUM		AFL		ZEA		T-2	
	x	s.d.	x	s.d.	x	s.d.	x	s.d.	x	s.d.
Species										
Lolium perenne	141.39	6,29	<LOQ	0,02	<LOQ	0,02	66.07	10,80	20.37	11,29
Festulolium pabulare	156.73	19,04	<LOQ	0,02	<LOQ	0,02	47.92	7,99	45.19	25,39
Festulolium braunii	143.60	13,24	6.07	6,04	<LOQ	0,01	46.34	8,96	43.04	26,10
Mixture with *Festuca rubra*	161.97	13,86	<LOQ	0,02	<LOQ	0.01	66.89	6,89	38.58	23,88
Mixture with *Poa pratensis*	167.70	15,82	<LOQ	0,02	0.21	0,12	54.46	6,64	19.96	12,1
p	0.5142		0.4207		0.2551		0.1577		0.8363	

Preservative or inoculant										
Untreated	139.19[a]	8,91	<LOQ	0,01	<LOQ	0,01	60.28	6,35	21.81	13,64
Chemical	182.71[b]	12,41	<LOQ	3,62	<LOQ	0,01	53.40	8,23	21.64	11,67
Biological-enzymatic	140.93[a]	7,22	3.66	0,01	0.14	0,08	55.33	5,23	56.83	20,14
p	0.0042		0.3765		0.4899		0.6803		0.2137	
Year										
2008	164.61	8,70	<LOQ	0.01	<LOQ	0,01	53.95[ab]	5,59	12.67	5,22
2009	156.49	15,19	<LOQ	0.01	<LOQ	0,08	73.24[a]	6,94	42.97	11,82
2010	141.73	6,81	3.72	3.62	0.15	0,01	41.81[b]	4,68	44.65	23,94
p	0.2553		0.3590		0.4037		0.0016		0.2929	
Species x Preservative	0.9502		0.4596		0.3666		0.5255		0.3641	
Species x Year	0.4784		0.4560		0.3753		0.9177		0.8801	
Preservative x Year	0.0004		0.4212		0.3174		0.0362		0.2586	

Mean values in the same column with different superscripts ([a,b,c]) are significant at the $p < 0.05$ level after Scheffé's method analysis. x = mean. s.d. = standard deviation.

Table 3. Differences (%) in content (ppb) of deoxynivalenol (DON), zearalenone (ZEA) and T-2 toxin (T-2) between fresh-cut material and grass silages. FCM = fresh-cut material, S = silages.

Factor	DON			ZEA			T-2		
	FCM	S	*Rel. %*	FCM	S	*Rel. %*	FCM	S	*Rel. %*
Lolium perenne	41.03	141.39	*344.6*	17.06	66.07	*387.3*	24.80	20.37	*82.1*
Festulolium pabulare	31.02	156.73	*505.2*	4.95	47.92	*968.0*	24.19	45.19	*186.8*

Festulolium braunii	36.98	143.60	*388.3*	36.45	46.34	*127.1*	24.94	43.04	*172.6*
Mixture with *Festuca rubra*	42.15	161.97	*384.3*	47.37	66.89	*141.2*	30.40	38.58	*126.9*
Mixture with *Poa pratensis*	40.19	167.70	*417.3*	48.15	54.46	*113.1*	29.98	19.96	*66.6*

The preservatives used in our study did not prevent mycotoxin production although, these materials are commonly used by farmers with the aim to improve the ensiling process. In the case of DON, the addition of organic acids even led to an increase ($p < 0.05$) in the content. It is precisely the addition of organic acids, and in particular propionic acid, which has antifungal effects [31]. Nevertheless, acids and inoculants have no effect on mycotoxins that already have been synthesized.

We observed an effect of year on ZEA content in silages ($p < 0.01$). The lowest ZEA content ($p < 0.05$) was found in silages during 2010, in which year ZEA concentrations were similar to those in fresh-cut material.

4. Conclusions

Mycotoxins are secondary metabolites having harmful effects on mammals. Their concentrations are therefore monitored and their effects intensely studied in fresh material. In this study, we investigated several factors influencing the content of these secondary metabolites in fresh-cut material and silages prepared from various grass species. It can be concluded that low temperatures can be beneficial for inhibiting the production of mycotoxins. This is well documented by the above results, however, these conditions can be taken into the account in some part of Europe, mainly in the middle and northern. On the other hand, these places are beneficial for the growing of the mentioned specie, because they are also resistant to that environment together with the lower content of mycotoxins. It should also be taken into account that the processing of green material for silage can itself contribute to increasing mycotoxin concentrations.

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