AGRICULTURAL BIOLOGY,
2017, V. 52, № 2, pp. 409-417
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

Fodder and feed additives

UDC 636.085.19:636.086.2/.3:632.4(470)

doi: 10.15389/agrobiology.2017.2.409rus
doi: 10.15389/agrobiology.2017.2.409eng

MYCOTOXINS IN THE LEGUMES OF NATURAL FODDER OF THE EUROPEAN RUSSIA

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The authors declare no conflict of interests
Acknowledgements:
Supported in part by Russian Science Foundation (project № 14-16-00114)
Received August 26, 2016

Abstract

Evaluation of the negative effects of mycotoxins on ruminants and horses is a complex scientific problem and has important economic significance. Mycotoxicoses of animals caused by feeding leguminous grasses have been known for a long time, but their causes remain largely unclear. The purpose of this work was a comparative study of mycotoxin contamination of legumes of the genera *Lathyrus*, *Trifolium*, *Vicia*, *Melilotus*, *Medicago*, *Galega* and *Lupinus* from the natural fodder lands of the European part of Russia. The collection of ground parts of plants was carried out in May-September 2015 in the Moscow, Tver, Leningrad, Pskov, Novgorod, Smolensk, Astrakhan regions, Perm Krai, and the Republic of Karelia. The mycotoxins determined by enzyme immunoassay were T-2 toxin (T-2), diacetoxycirpenol (DAS), deoxynivalenol (DON), zearalenone (ZEN), fumonisins (FUM), alternariol (AOL), roridin A (ROA), aflatoxin B_1 (AB_1), sterigmatocystin (STE), cyclopiazonic acid (CPA), emodin (EMO), ochratoxin A (OA), citrinin (CIT), mycophenolic acid (MPA), PR toxin (PR) and ergot alkaloids (EA). In grasses of the genus *Lathyrus* (peavines), all the analyzed mycotoxins (with the exception of FUM and ROA) were found in more than 80 % of the samples; for clovers (*Trifolium*), the same frequency was found in T-2, OA, MPA, EA, AOL, CPA and EMO, and in species of the genus *Vicia* (vetchs), sweetclovers (*Melilotus*) and alfalfa (*Medicago*) there were only EA, AOL, CPA and EMO. A common feature for the genera *Lathyrus*, *Trifolium* and *Vicia* plants were high, > 1000 μg/kg, accumulation of DAS, AOL and CPA, and for peavines and clovers, PR also. Among the peculiarities of meadow clover (*T. pratense* L.), we found the ultra-high content of EMO, up to 30 000 μg/kg and more. White clover (*T. repens* L.) was characterized by moderate contamination as compared to other species of the genus. Representatives of *Vicia*, bush vetch (*V. sepium* L.) and cow vetch (*V. cracca* L.), showed similarities in the frequency of the majority of mycotoxins, but in cow vetch with a smaller occurrence in comparison to bush vetch, especially visible for FUM, the upper limits of the contents of EA, AOL, ROA, STE, MPA were higher. Meadow sweetclovers and alfalfa were found to be contaminated less than other grasses, while sweetclovers were close to *Vicia* in terms of detection frequency and amounts of OA and PR, and alfalfa was low in MPA and relatively high in EMO accumulation. Among other crops less common in meadows, *Lupinus polyphyllus* Lindl. had the highest mycotoxicological load — of 16 metabolites analyzed, all but FUM and ROA were more than 80 % in frequency. At Caucasian goat's rue (*Galega orientalis* Lam.) and narrow-leaved vetch (*Vicia sativa* L.) mycotoxins were detected less often and in smaller quantities. In this study previously published data on mycotoxicological analysis for meadow clover and white clover (A.A. Burkin and G.P. Kononenko, 2015) have been confirmed, but for other legumes the findings are presented for the first time.

Keywords: meadow grasses, legumes, *Lathyrus*, *Trifolium*, *Vicia*, *Melilotus*, *Medicago*, *Galega*, *Lupinus*, mycotoxins, T-2 toxin (T-2), diacetoxycirpenol (DAS), deoxynivalenol (DON), zearalenone (ZEN), fumonisins (FUM), alternariol (AOL), roridin A (ROA), aflatoxin B_1 (AB_1), sterigmatocystin (STE), cyclopiazonic acid (CPA), emodin (EMO), ochratoxin A (OA), citrinin (CIT), mycophenolic acid (MPA), PR toxin (PR), ergot alkaloids (EA)

Mycotoxicosis of ruminants and horses, in rations of which grain and grass feed complement each other, have been receiving increasing attention in recent years [1-4]. It is known that fusariose grains and its derivative products are sources of T-2 toxin, zearalenone, deoxynivalenol [5, 6], fumonisins [7]. Besides, there is a threat of contamination of barley and maize with ochratoxin A [8], and the products form sunflower seeds (seedcake, oilcake) are often contaminated ин both ochratoxin A and citrinin. Risks from the use of grass fodders that predominate in the diet or serve as the only feed of ruminants are yet to be evaluated. Recently, there has been confirmation of the extensive combined contamination of mycotoxins for mowing grasses on long-term use fields [10-12], as well as for dry, haylage and ensilage feeds [13-16].

The specific internal mycobiota in mixed spontaneously arising plant communities, the susceptibility of plant stand to fungal diseases, including those caused by toxin-forming fungi, are formed as a result of complex direct and indirect biocenotic relations, the action of ontogenetic and environmental factors. That is why the evaluation of the negative effect of mycotoxins in free grazing is among the extremely complex problems. One of the solutions is a survey of wide samples of a botanically representative homogeneous material with regard to the areas of growth and the phases of vegetation. Recently, in meadow grasses this approach allowed to obtain the first information on the main carriers of toxicological load among wild cereals and to characterize the mycotoxin complex in some leguminous plants [17].

In the present study, for meadow clover and white clover, the results have been confirmed of which we have already reported [17]. Data on the prevalence of mycotoxins in vetches, peas, sweetclovers, alfalfa, lupine and goat's rue were obtained for the first time.

The aim of the work is a comparative study of mycotoxin contamination of leguminous grasses (*Lathyrus*, *Trifolium*, *Vicia*, *Melilotus*, *Medicago*, *Galega* и *Lupinus*) on natural forage lands in the European Russia.

Technique. The study was performed on 842 specimens of *Leguminaceae* family plants of genera *Lathyrus* (n = 111), *Trifolium* (n = 310), *Vicia* (n = 227), and sweetclovers (*Melilotus* spp., n = 76), alfalfa (*Medicago* spp., n = 88), *Galega orientalis* Lam. (n = 18) and Washington lupine (*Lupinus polyphyllus* Lindl., n = 12). For attribution of systematic groups we used identification keys [18, 19]. Species of genera *Lathyrus*, *Trifolium* and *Vicia* are described in the section "Results", among sweetclovers there were *M. albus* Medik. and *M. officinalis* (L.) Pall., though these species could not be identified before the beginning of flowering. To the plants of the genus *Medicago* were referred *M. sativa* L. with significant polymorphism and *M. lupulina* L. Samples was collected from May to September 2015 in Moscow (Balashikha, Dmitrov, Noginsk, Odintsovo, Podolsk regions and forest park in the floodplain of the Setun River, Moscow), Leningrad (Gatchina, Luga, Pushkin, Slantsy, Tosnensky regions), Pskov (Novorzhevsk region), Novgorod (Starorussky region), Smolensk (Gagarinsk region), Tver (Vyshnevolotsk region), Astrakhan (Enotayevsk region), in the Republic of Karelia (Loukhsk region) and Perm (Chaikovsky region), on meadows of different types, cattle stations and pastures, open slopes, on the banks of rivers, streams, lakes (reservoirs), clearing of the woods and outskirts, swaths, openings, willow stands and bushes, roadsides and head lands, near houses, on waste lands, field boundaries and roadside meadows. The aerial parts of plants were cut 3-5 cm from the soil surface, were air-dried in a room and milled.

For extraction, a mixture of acetonitrile and water was used in a volume ratio of 84:16 (10 ml per 1 g of sample). Extracts after 10-fold dilution with phosphate-buffered saline solution (pH 7.4) with Tween 20 were used for indirect competitive enzyme-linked immunosorbent assay. The content of T-2 toxin

(T-2), diacetoxyscirpenol (DAS), deoxynivalenol (DON), zearalenone (ZEN), fumonisins (FUM), ergot alkaloids (EA), alternariol (AOL), roridin A (ROA), aflatoxin B_1 AB_1), sterigmatocystin (STE), cyclopiazonic acid (CPA), emodin (EMO), ochratoxin A (OA), citrinin (CIT), mycophenolic acid (MPA), PR- toxin (PR) were determined using certified immunoenzyme test systems [20]. The lower limits of measurements corresponded to 85 % of antibody binding.

1. Species of genera *Lathyrus*, *Trifolium* and *Vicia* collected in different territories of the European Russia (May-September 2015)

Species	Number of samples
Lathyrus	
L. pratensis L.	93
L. vernus (L.) Bernh.	12
L. palustris L.	3
L. sylvestris L.	3
Trifolium	
T. hybridum L.	56
T. montanum L.	20
T. pratense L.	115
T. repens L.	69
T. medium L.	50
Vicia	
V. sepium L.	87
V. cracca L.	117
V. sylvatica L.	9
V. sativa L.	14

Results. Species of genera *Lathyrus*, *Trifolium* and *Vicia* in the samples are given in Table 1.

Mycotoxicological analysis for five genera of legumes represented by several species reflected an equally high frequency of detection of EA, AOL, CPA and EMO (in 80 % and more samples) (Table 2). All other mycotoxins were found less often in peas (*Vicia*), sweetclovers (*Melilotus*) and alfalfa species (*Medicago*), though T-2, OA and MPA were also common in clovers (*Trifolium*), and all tested mycotoxins, except of FUM and ROA, was frequent in vetches (genus *Lathyrus*) (see Table 2). Moreover, peas, sweetcloves and alfalfa were noticeably inferior to vetches and clovers in the frequency of contamination with fusariotoxins T-2, DON, ZEN, FUM, and also AB_1, OA, PR.

2. Occurrence (%) **and accumulation of mycotoxins** (μg/kg) **in legumes of different genera** (European Russia, May-September 2015)

Mycotoxin	*Lathyrus* spp. (*n* = 111)	*Trifolium* spp. (*n* = 310)	*Vicia* spp. (*n* = 227)	*Melilotus* spp. (*n* = 76)	*Medicago* spp. (*n* = 88)
T-2	89 (2-10-41)	86 (2-10-205)	45 (2-17-445)	63 (2-6-110)	59 (2-6-41)
DAS	86 (170-995-3020)	55 (50-325-4680)	20 (79-365-1035)	13 (100-260-425)	28 (79-265-630)
DON	81 (76-340-2065)	41 (55-190-520)	22 (74-150-375)	11 (74-125-180)	20 (79-140-250)
ZEN	88 (24-59-200)	55 (20-45-380)	22 (21-43-100)	30 (15-32-56)	35 (20-37-67)
FUM	61 (52-307-775)	22 (66-170-1780)	12 (76-185-420)	8 (74-95-120)	16 (87-190-265)
EA	98 (2-48-795)	93 (2-16-280)	85 (2-16-600)	97 (2-13-89)	89 (1-7-26)
AOL	98 (28-830-6310)	99 (30-300-1905)	96 (14-98-1515)	100 (20-105-1070)	100 (21-110-795)
ROA	77 (3-63-675)	30 (3-25-240)	22 (4-24-105)	11 (4-16-38)	20 (4-14-26)
AB_1	87 (2-15-89)	56 (2-5-79)	27 (2-5-19)	22 (2-3-7)	25 (2-4-6)
STE	92 (15-82-500)	65 (6-29-315)	48 (8-68-1320)	61 (8-20-45)	32 (10-24-56)
CPA	99 (79-885-5130)	99 (52-675-3550)	98 (63-425-2040)	99 (62-435-1335)	98 (76-330-935)
EMO	99 (21-205-775)	99 (20-2300-35500)	82 (10-49-775)	78 (16-51-335)	86 (13-170-2950)
OA	95 (6-73-200)	87 (3-25-245)	48 (4-9-28)	32 (5-8-13)	32 (4-11-76)
CIT	91 (31-145-500)	69 (8-63-820)	34 (25-76-315)	53 (19-49-185)	30 (20-52-200)
MPA	86 (14-88-830)	88 (12-45-530)	41 (11-40-280)	68 (13-39-250)	32 (10-25-47)
PR	85 (190-835-2755)	71 (23-510-1700)	26 (30-355-685)	28 (104-235-515)	31 (105-325-765)

N o t e. T-2 — T-2 toxin, DAS — diacetoxyscirpenol, DON — deoxynivalenol, ZEN — zearalenone, FUM — fumonisins, EA — ergot alkaloids, AOL — alternariol, ROA — roridin A, AB_1 — aflatoxin B_1, STE — sterigmatocystin, CPA — cyclopiazonic acid, EMO — emodin, OA — ochratoxin A, CIT — citrinin, MPA — mycophenolic acid, PR — PR-toxin; *n* — number of samples examined. The minimum-average-maximum content of mycotoxin is given in parentheses.

Common to the genera *Lathyrus*, *Trifolium* and *Vicia* plants was a high accumulation of DAS (> 1000 μg/kg) and ZEN, EA, ROA, STE (> 100 μg/kg), while *Vicia* had amounts of STE > 1000 μg/kg. In addition, vetches and clovers were distinguished by a significant PR (> 1000 μg/kg) and OA content (> 100 μg/kg), the highest levels of occurrence of ROA, STE and accumulation of DON, EA, AOL, CPA were noted in vetches, and the ultrahigh contamination of EMO (> 30,000 μg/kg) was characteristic of clovers. Meadow sweetclovers and alfalfa were found to be less contaminated with mycotoxins with the maximum accumulation of DAS, ZEN, FUM, EA, PR, AB_1, STE was an order of magnitude lower, and there was a similarity to peas according to the frequency of detection and the amounts of OA and PR. In alfalfa, a low level MPA and a relatively high EMO (up to 2950 μg/kg) were noted.

Within each of the genera, groups with a narrow range (by an order of magnitude or within the order of magnitude) and a wide (two to three orders of magnitude) range of variation in the accumulation of mycotoxins were distinctly distinguished. The variability in the amounts of toxins could be due to both specific plant characteristics and seasonal fluctuations. Most often wide variation was noted in clovers (for 12 toxins), vetches and peas (for 7-8 toxins). These legumes were the most diverse in species composition (5 species of *Trifolium* and 4 species of *Lathyrus* and *Vicia*) (see Table 1). On the contrary, only a few metabolites (T-2, AOL, CPA in sweetclover, and EMO in alfalfa) varied considerably in quantity among the white sweetclover and yellow melilot, as well as wild medic with an admixture of clover (18 samples out of 88 ones). Perhaps, these species differed little in mycotoxin contamination due to the close composition of micromycetes and their similar response to external factors.

The interspecies differences in *Lathyrus* and *Trifolium* genera plants can be seen from Table 3.

3. Occurrence (%) **and accumulation of mycotoxins** (μg/kg) **in legumes of genera** ***Lathyrus*** **and** ***Trifolium*** (European Russia, May-September 2015)

Mycotoxin	Meadow vetch (*n* = 93)	Spring vetch (*n* = 12)	Meadow clover (*n* = 115)	Zigzag clover (*n* = 50)	White clover (*n* = 69)
T-2	96 (2-10-41)	75 (4-5-7)	98 (3-11-205)	86 (3-13-160)	78 (2-6-41)
DAS	97 (240-1035-3020)	33 (170-240-315)	71 (50-370-4675)	56 (63-225-500)	19 (50-170-315)
DON	89 (76-360-2065)	58 (105-125-195)	59 (55-190-520)	48 (120-225-390)	9 (84-135-200)
ZEN	99 (24-60-200)	42 (26-34-42)	62 (20-50-380)	72 (28-46-79)	59 (20-32-47)
FUM	72 (52-230-775)	8 (125	38 (77-195-1780)	20 (84-110-165)	7 (84-98-125)
EA	98 (2-56-795)	100 (2-13-48)	97 (2-22-280)	94 (2-20-115)	88 (2-9-60)
AOL	100 (28-940-6310)	100 (48-165-255)	100 (100-395-1905)	100 (33-440-1585)	99 (30-100-315)
ROA	87 (3-66-675)	33 (3-11-32)	43 (3-26-215)	36 (5-33-240)	19 (4-12-54)
AB_1	98 (3-16-89)	42 (2-6-12)	86 (2-6-79)	62 (2-5-14)	22 (2-3-4)
STE	99 (15-87-500)	75 (16-45-100)	84 (10-30-195)	76 (8-30-91)	41 (8-21-37)
CPA	100 (115-975-5130)	100 (205-445-1150)	100 (150-910-2500)	100 (140-1025-3550)	99 (74-350-935)
EMO	99 (25-235-775)	100 (39-66-125)	100 (125-5535-35500)	94 (39-330-4265)	100 (20-345-3090)
OA	100 (9-82-200)	75 (6-8-12)	100 (7-36-245)	90 (6-18-39)	58 (3-8-16)
CIT	96 (31-150-500)	92 (31-130-325)	78 (23-75-820)	82 (25-68-255)	61 (22-46-125)
MPA	95 (14-93-830)	67 (16-30-52)	97 (15-57-530)	82 (12-39-130)	86 (15-36-85)
PR	96 (190-860-2755)	33 (300-455-590)	99 (145-570-1700)	84 (190-560-1215)	14 (23-290-595)ё

N o t e. T-2 — T-2 toxin, DAS — diacetoxyscirpenol, DON — deoxynivalenol, ZEN — zearalenone, FUM — fumonisins, EA — ergot alkaloids, AOL — alternariol, ROA — roridin A, AB_1 — aflatoxin B_1, STE — sterigmatocystin, CPA — cyclopiazonic acid, EMO — emodin, OA — ochratoxin A, CIT — citrinin, MPA — mycophenolic acid, PR — PR-toxin; *n* — number of samples examined. The minimum-average-maximum content of mycotoxin is given in parentheses.

Meadow vetch predominating among the samples (93 of 111 ones) showed the specialties of contamination, characteristic of the genus (see Table 2). All mycotoxins, other than FUM, were present in more than 80 % of the samples, and there was a significant accumulation (> 1000 μg/kg) of DAS, DON, AOL, CPA, PR and > 100 μg/kg for the rest toxins, except for AB_1 and T-2. Given such a richness of mycotoxins, grass mixtures with meadow vetch should be used with caution. In addition, this species is characterized by a wide quantitative variability in DAS, DON, EA, AOL, ROA, which may be a consequence of hypersensitivity of mycobiota to growth conditions and plant growth phases. Spring vetch which was rare among the samples (12 out of 111 ones) was noticeably inferior to the meadow vetch in contamination (Table 3). Only EA, AOL, CPA, EMO were found regularly, the ranges of none of the mycotoxins went beyond the limits found in the meadow vetch, the maximum accumulation > 1000 μg/kg was retained only in the CPA, and the amounts of ZEN, EA, ROA, OA and MPA were an order of magnitude lower, not reaching 100 μg/kg. When comparing these species, one can not ignore the fact that they have different areas, i.e. the meadow vetch usually grows in meadows, and is rarely found in the outskirts, clearings of the woods or in the clarified areas of the forest, whereas the spring vetch is common in forests and openings.

For meadow clover, the results coincided with those described earlier [17]. In the preliminary study in 2014, conducted on a collection of 35 samples in the Moscow, Tver regions and the Republic of Karelia, mycotoxins were represented by 12-15 components in all habitats and in different periods of vegetation (July, August and September), and the EMO content was kept ultrahigh (up to 27,540 μg/kg). In this large-scale study, accumulation of > 1000 μg/kg was found for a greater number of toxins, i.e. for DAS, FUM, AOL and PR in addition of CPA. In meadow clover in comparison to the meadow vetch, T-2, DAS, FUM and EMO also were among the mycotoxins with a wide varying accumulation. Apparently, a greater number of toxin-forming micromycetes affecting this culture are sensitive to habitat. Both species with the greatest variety of putative sources of toxic metabolites deserve special attention of researchers as objects for the assignment and identification of epiphytic fungi.

Two species of the genus *Trifolium*, the zigzag clover and the white clover, were represented in a smaller number of samples, but with comparable sample sizes. In the zigzag clover, the same mycotoxins and in the same amounts as in meadow clover were regularly detected, but, as a rule, with smaller range of variation. The differences concerned only the indicators of the greatest accumulation of DAS, ZEN, FUM and OA, as well as EMO, for which the range amonts shifted by an order of magnitude toward lower values (see Table 3). For the white clover, a lesser degree of contamination was revealed compared to two other species of this genus. The upper limits of DAS, ZEN FUM, EMO, OA levels coincided with the zigzag clover (unlike meadow), but with a much more rare detection of DAS, DON, FUM, ROA, AB_1, STE, PR and reduced accumulation of EA, ROA, as well as AB_1, STE, CPA. Moreover, the MPA level did not exceed 100 μg/kg. Thus, a large-scale mycotoxicological study confirmed the earlier conclusion [17] about clear advantages of white clover, the main component of pastures throughout the European Russia and in Siberia, over meadow clover.

4. Occurrence (%) **and accumulation of mycotoxins** (μg/kg) **in lupine, goat's rue and three species of *Vicia*** (European Russia, May-September 2015)

Mycotoxin	Bush vetch (*n* = 87)	Tufted vetch (*n* = 117)	Common vetch (*n* = 14)	Washington lupine (*n* = 12)	Goat's rue (*n* = 18)
T-2	51 (2-17-445)	40 (2-9-62)	71 (2-56-280)	100 (3-8-12)	56 (2-3-5)
DAS	26 (160-340-850)	15 (79-375-955)	14 (130-135-140)	83 (195-255-405)	11 (215-275-330)
DON	36 (78-145-375)	12 (87-175-315)	36 (74-130-190)	92 (105-205-365)	11 (110-115-120)
ZEN	29 (21-44-78)	17 (25-44-100)	7 (30)	100 (31-51-83)	22 (31-48-63)
FUM	23 (76-200-420)	3 (83-105-130)	Не выявлен	33 (79-130-230)	17 (110-175-275)
EA	89 (2-10-37)	80 (2-17-600)	100 (3-44-345)	100 (5-33-75)	89 (2-20-79)
AOL	100 (14-115-860)	94 (17-85-1515)	100 (50-95-245)	100 (140-425-795)	100 (30-195-1410)
ROA	26 (4-22-76)	21 (4-20-105)	Не выявлен	50 (6-16-27)	6 (3)
AB_1	32 (2-5-19)	19 (2-4-12)	50 (2-3-5)	100 (3-8-23)	6 (2)
STE	52 (9-45-325)	47 (8-92-1320)	29 (12-20-35)	100 (16-38-71)	28 (12-16-23)
CPA	100 (77-445-1585)	96 (63-420-2040)	100 (91-485-1660)	100 (315-630-1260)	89 (81-355-610)
EMO	83 (10-57-775)	85 (16-44-250)	79 (17-34-74)	100 (165-415-740)	72 (18-49-125)
OA	71 (4-9-28)	32 (4-8-24)	71 (8-10-13)	92 (8-15-24)	56 (5-9-13)
CIT	39 (25-100-315)	29 (25-61-165)	43 (27-40-68)	83 (33-82-210)	22 (32-37-42)
MPA	54 (13-35-82)	33 (11-50-280)	29 (17-20-25)	67 (16-33-66)	44 (12-23-32)
PR	31 (31-350-685)	24 (30-350-655)	7 (250)	100 (220-355-595)	17 (135-215-315)

N o t e. T-2 — T-2 toxin, DAS — diacetoxyscirpenol, DON — deoxynivalenol, ZEN — zearalenone, FUM — fumonisins, EA — ergot alkaloids, AOL — alternariol, ROA — roridin A, AB_1 — aflatoxin B_1, STE — sterigmatocystin, CPA — cyclopiazonic acid, EMO — emodin, OA — ochratoxin A, CIT — citrinin, MPA — mycophenolic acid, PR — PR-toxin; *n* — number of samples examined. The minimum-average-maximum content of mycotoxin is given in parentheses.

Members of genus *Vicia*, the bush vetch and tufted vetch, had similarities in the frequency of detection of the majority of mycotoxins, but in tufted vetch there was a tendency to a decrease in this parameter, especially noticeable for FUM (Table 4). However, in this species the upper limits of the EA, AOL, ROA, STE, MPA contents were higher. The possibility of intensive accumulation of STE (up to 2320 g/kg), noted earlier for *Vicia* spp. [17], was also characteristic of tufted vetch (see Table 4). By the levels of fusariotoxins DAS, DON, ZEN and a number of other toxins, the species did not differ, the maximum content of DAS (1035 μg/kg) in the *Vicia* spp. (see Table 2) was found for wood vetch. In tufted vetch, a wide fluctuations in the amounts of mycotoxins were much more frequent than in bush vetch. Here, too, we can assume the effect of higher environmental plasticity of micromycetes, but one can not exclude the consequences of the fact that clear identification of closely related species and varieties is often hampered by morphological differences which are hard to detect.

Cultivated leguminous plants are often found in grass stands of natural forage lands. In the areas of sample collection, a single growth or clumps of Washington lupine, goat's rue and common vetch were observed. Among them, Washington lupine was the most abundant in mycotoxicological load. Of the 16 mycotoxins tested, 13 (all but FUM, ROA and MPA) were detected at a frequency of more than 80 %. Fusariotoxins T-2, DAS, DON and ZEN were almost common, although in small amounts. Significant susceptibility to different species of *Fusarium* was previously established for annual fodder lupins, *L. luteus* L., *L. albus* L. and *L. angustifolius* L. [21], but the formation of metabolites is uncharacteristic of the main pathogens *F. avenaceum* (Fr.) Sacc. and *F. oxysporum* (Schlecht) Snyd et Hans. Perhaps, their appearance in the plant is provided by accompanying species. Frequent contamination of Washington lupine with a wide range of mycotoxins should be considered when growing this crop for sideration and especially for subsequent silage.

Goat's rue is considered promising because of its high yield, it is attractive for grazing, harvesting hay, haylage, silage. We have shown that it was characterized by a moderate contamination with mycotoxins by prevalence and content (see Table 4). In goat's rue, as well as in peas, sweetcloves and alfalfa, only EA, AOL, CPA and EMO were regularly identified, but the goat's rue plants differed from peas by a smaller accumulation of DON, CIT, ROA, and STE, and was close to sweetclovers in EMO levels, and to alfalfa in low level of MPA (see Table 2).

Common vetch on the whole was characterized by weak contamination, it combined certain features of other species of this genus, tufted vetch (practical absence of FUM) and bush vetch (occurrence of OA), but there were distinctive signs (absence of ROA and smaller contents of EMO, STE and CIT).

Comparison of susceptibility to contamination with mycotoxins for species within the genus (see Table 3, 4) and between genera of leguminous plants (see Table 2) showed that the differences were comparable and could be very significant. We have previously marked this for lichens [20, 22], which, forming the basis of vegetation cover for deer pastures, serve as a food for many other animals in the wild.

Sources and mechanisms for the formation of the mycotoxicological status of plants are still unclear, but lately an increasing role in this has been attributed to endophytic fungi [23]. Thus, the cause of widely known intoxication of ruminants and horses (salivation, locoism, "pea disease") accompanying grazing in legumes, the red clover (*Trifolium pratense* L.), *Astragalus* spp., *Oxytropis* spp. in the USA and *Swainsona canencens* (Benth.) F. Muell. in Australia, are indolizidine alkaloids of fungi inhabiting tissues of these plants [24]. The presence of EA in pasture cereals is associated with the endophytes of the genus *Ne-*

otyphodium [25], the hypericin-emodin metabolism in Saint-John's wort (*Hypericum perforatum* L. common for the meadow is provided by endophytic fungus *Thielavia subthermophila* Mouch. [26], and search for fungi responsible for EMO biosynthesis in plants is expanding [27]. Therefore, a reasonable approach to the formation of grass stands with productive longevity, is based on diversity of the cenotic interactions and environmental factors [28, 29].

Thus, the predisposition to the accumulation of fungal metabolites toxic to animals should be taken into account in the economic use of leguminous crops along with their yield, nutritional value, and resistance to drought, salinity, temperature changes, pest damage and diseases.

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