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TOXINS OF MICROMYCETES IN GENERATIVE ORGANS OF PLANTS OF THE FAMILY *Fabaceae*

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Abstract

During the study of the role of associated microscopic fungi in the adaptation of plants to external influences, researchers focus mainly on such key aspects as i) the shift in the composition of the internal mycobiota during growth, ii) the direction of fungal colonization of vegetative and generative organs, and iii) concomitant changes in the metabolic status of the plant organism (J.A., Wearn et al., 2012; V. Arbona et al., 2013; J. Hong et al., 2016). The dynamics of DNA accumulation of *Alternaria*, *Cladosporium* and *Fusarium* fungi in different months of plant growth was revealed in meadow grasses of the Fabaceae family (O.P. Gavrilova et al., 2017; A.S. Orina et al., 2018) and seasonal fluctuations in the content of toxic metabolites characteristic of these groups of micromycetes were found (A.A. Burkin, G.P. Kononenko, 2018, 2019). The predominant localization of mycotoxins in leaves was established for meadow clover, white clover, Caucasian goat's rue, Washington lupine and melilot (G.P. Kononenko et al., 2019). In this study, we describe for the first time the complexes of toxic fungi metabolites in the generative organs of legumes. The aim of the work was to study the component composition and content of mycotoxins in the whole plants, flowers and beans of perennial legumes of 6 genera of the *Fabaceae* family. Meadow grasses of the genera *Trifolium* L. — meadow clover (*T. pratense* L.), alsike clover (*T. hybridum* L.), zigzag clover (*T. medium* L.), white clover (*T. repens* L.); of *Lathyrus* L. — meadow peavine (*L. pratensis* L.), spring peavine (*L. vernus* (L.) Bernh.); of *Vicia* L. — bush vetch (*V. sepium* L.), cow vetch (*V. cracca* L.); of *Lotus* L. — deer vetch (*L. corniculatus* L. s.l.); of *Lupinus* L. —Washington lupine (*L. polyphyllus* Lindl.), and of *Galega* L. — Caucasian goat's rue (*G. orientalis* Lam.) were collected from natural grass stands of the Moscow region in May—the first half of August 2019, wood vetch (*V. sylvatica* L.) and Japanese peavine (*L. japonicus* Willd. subsp. pubescens Korobkov) — in the second half of August of the same year on of the Kandalaksha Gulf of the White Sea (Republic of Karelia). The aboveground parts of plants, as well as flowers and beans, were kept at room temperature to an air-dry state and crushed in a laboratory mill. For extraction, a mixture of acetonitrile and water was used (84:16 v/v) at the ratio of 10 ml per 1 g of the sample. Extracts after 10-fold dilution with a buffer solution were assessed using indirect competitive enzyme immunoassay. The content of the mycotoxins — T-2 toxin (T-2), 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) was determined using commercial and research certified enzyme immunoassay systems (GOST 31653-2012). For the generative organs of most of the examined plants, both common features (preservation of the mycotoxin profile typical of the whole plant, with the absence or decrease in the content of a number of fungal metabolites) and peculiarities were revealed. In particular, in the flowers of three species of the genus *Trifolium* L., in general, the mycotoxin complex characteristic of the vegetative part was preserved, but the occurrence and accumulation of fusariotoxins were higher. The flowers of two species — alsike clover and zigzag clover were characterized by combined contamination of OA and CIT in comparable quantities, rare for plants. With a general low contamination, fusariotoxins T-2, DON and ZEN were present only in generative organs in the deer vetch. In all representatives of the genera *Vicia*, *Lathyrus*, *Lupinus*, and *Galega* the metabolic background in flowers as a whole was found weakened, in beans it turned out to be similar to the aboveground part without a sharp variation in the content of mycotoxins.

Keywords: legumes, flowers, beans, mycotoxins, ELISA

In recent years, when studying the mechanisms of adaptation to external influences, plants are increasingly considered as complex systems, which include micromycetes [1, 2]. Various connections can be established between fungi and a plant: from complete isolation to joint participation in biochemical processes [3-5]. The main plant responses to a change in habitat conditions include a shift in the composition of the internal mycobiota and the direction of colonization by fungi of vegetative and generative organs [6, 7], as well as the accompanying changes in the metabolic status of the organism [8-10].

In meadow grasses of the *Fabaceae* family, the dynamics of DNA accumulation of the fungi *Alternaria*, *Cladosporium*, and *Fusarium* was studied in different months of plant collection [11, 12], and seasonal fluctuations in the content of toxic metabolites characteristic of these groups of micromycetes were revealed [13-15]. With regard to the organotropism of endophytic fungi in these plants, preliminary data were obtained [16], but for meadow clover, white clover, Caucasian goat's rue, Washington lupine, and melilot, the predominant localization of mycotoxins in leaf blades was established; the same complex of metabolites with an increased content of individual components is retained in flowers of *Melilotus* sp. [17]. Mycotoxicological examination of generative organs in other members of this family was not carried out.

In this work, we were the first to describe complexes of toxic metabolites of imperfect fungi in the generative organs of leguminous plants.

Our purpose was to study the component composition and content of mycotoxins in flowers and beans in perennial legumes of the genera *Trifolium*, *Lathyrus*, *Vicia*, *Lotus*, *Lupinus,* and *Galega*.

Materials and methods. Meadow grasses of the *Fabaceae* family from natural grass stands of the Moscow Province were collected regularly, at weekly intervals in May—the first half of August 2019. According to the guides [18, 19], grasses were assigned to six genera: *Trifolium* L. — meadow clover *T. pratense* L., alsike clover *T. hybridum* L., zigzag clover *T. medium* L., white clover *T. repens* L.; *Lathyrus* L. — meadow peavine *L. pratensis* L., spring peavine *L. vernus* (L.) Bernh.; *Vicia* L. — bush vetch *V. sepium* L., cow vetch *V. cracca* L.; *Lotus* L. — deer vetch *L. corniculatus* L. s.l., *Lupinus* L. — Washington lupine (*L. polyphyllus* Lindl.); and *Galega* L. — Caucasian goat's rue *G. orientalis* Lam. Wood vetch (*V. sylvatica* L.) and Japanese peavine (*L. japonicus* Willd. subsp. *pubescens* Korobkov) were collected in the second half of August of the same year on the coast of the Kandalaksha Bay of the White Sea (Republic of Karelia).

The aboveground parts of plants, cut at a height of 3-5 cm from the soil surface, as well as flowers and beans separated from them, were immediately after collection placed in a ventilated room, kept at room temperature to an air-dry state, and ground in a laboratory mill. For extraction, a mixture of acetonitrile and water in a volume ratio of 84:16 was used at a flow rate of 10 ml per 1 g of the sample. Extracts after 10-fold dilution with phosphate buffered saline (pH 7.4) with Tween 20 were used for indirect competitive enzyme immunoassay.

The content of mycotoxins T-2 toxin (T-2), 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 analyzed using commercial and research certified enzyme-linked immunosorbent assay tests (GOST 31653-2012. Feedstuffs. Method of immunoenzyme mycotoxin determination. Moscow, 2012). The lower limits of quantitative measurements were 1 (EA, AB_1), 2 (T-2), 4 (OA, STE, ROA), 15 (AOL, ZEN, EMO), 20 (CIT, MPA), and 50 μg/kg (DON, FUM, CPA, PR) and corresponded to 85% of antibody binding.

The data were processed using descriptive statistics in Microsoft Excel 2013. We calculated the proportion of positive specimens (n^+) from the total number of those tested (n), the minimum, maximum content (μg/kg) of mycotoxins and the arithmetic mean value of the indicator (M) for positive samples.

Results. In the vegetative part of meadow clover, as noted earlier [9], the most common fusariotoxin was T-2, EA, AOL, CPA, EMO, OA, AB1, MPA, and PR were regularly detected; CIT, STE were rarely detected, and ROA was absent. In flowers, the component composition as a whole remained the same, while the frequency of occurrence and content increased for fusariotoxins (T-2, DON, ZEN, FUM), remained unchanged for AOL, OA, and AB1, and decreased for the rest of the components (Table 1).

1. The occurrence and concentration of mycotoxins in aboveground parts (1) **and flowers** (2) **of *Trifolium* L. plants** (Moscow Province, 2019)

Mycotoxin	*T. pratense* L.		*T. hybridum* L.		*T. medium* L.		*T. repens* L.	
	1 (n = 13)	2 (n = 13)	1 (n = 8)	2 (n = 8)	1 (n = 4)	2 (n = 3)	1 (n = 9)	2 (n = 9)
T-2	13	13	6	8	4	3	9	8
	2-8-55	2-69-795	3-4-7	2-10-16	2-6-9	8-65-180	2-5-15	2-83-470
DON	3	8	2	7	–	2	–	–
	68-115-160	63-110-160	95, 95	120-210-400		100, 170		
ZEN	3	6	2	7	4	3	3	3
	19-22-25	16-21-32	17, 17	20-45-84	15-19-25	25-45-61	19-20-21	19-22-26
FUM	1	1	–	5	–	2	–	–
	90	250		85–195–300		200, 355		
AOL	13	13	8	8	4	3	9	9
	64-130-180	63-125-315	30-70-105	44-300-630	120-155-210	240-290-370	21-38-54	40-76-160
OA	13	13	5	8	4	3	–	4
	6-20-31	6-17-54	5-7-10	10-55-110	8-9-10	9-31-56		5-7-8
CIT	3	2	5	7	–	2	–	2
	28-36-51	38, 40	21-39-59	30-79-180		32, 49		21, 25
STE	5	1	3	5	1	3	3	2
	12-17-21	30	12-17-22	12-41-75	16	24-29-37	12-13-13	21, 38
AB1	11	12	2	6	3	2	2	5
	1-2-4	1-3-8	1, 2	2-5-9	1-1-1	4, 8	2, 2	1-2-2
CPA	13	13	8	8	4	3	9	9
	39-230-590	31-95-245	50-135-240	63-160-315	62-86-105	47-77-135	40-72-105	32-76-115
MPA	12	8	8	7	4	3	9	9
	26-44-100	22-40-94	25-36-52	45-90-125	26-39-60	58-61-64	16-28-40	24-33-50
EA	10	6	6	7	4	3	7	5
	1-10-47	1-6-27	1-7-15	3-18-50	1-5-10	3-4-4	2-3-12	1-2-4
EMO	13	13	7	8	4	3	9	9
	315-960-2820	115-300-850	10-355-2240	33-130-240	32-82-125	50-195-405	50-150-250	35-185-645
PR	12	7	–	7	3	3	–	–
	335-530-795	305-405-575		400-585-795	425-530-670	670-790-945		
ROA	–	–	–	1	–	2	–	–
				18		10, 14		

N o t e. Mycotoxins are T-2 toxin (T-2), deoxynivalenol (DON), zearalenone (ZEN), fumonisins (FUM), ergot alkaloids (EA), alternariol (AOL), roridin A (ROA), aflatoxin B1 (AB1), sterigmatocystin (STE), cyclopiazonic acid (CPA), emodin (EMO), ochratoxin A (OA), citrinin (CIT), mycophenolic acid (MPA), and PR-toxin (PR); n is the number of studied specimens. The upper value in lines is the number of positive specimens (n^+) containing mycotoxins in an amount exceeding the lower range limit; concentration of relevant mycotoxin (μg/kg, minimal-mean-maximum) is indicated underneath. A dash means that the mycotoxin was not detected.

For white clover plants, we observed the ubiquitous or similar occurrence of the same mycotoxins, but in amounts lower than in meadow clover [13]. The flowers were characterized by an increased concentration of T-2, a tendency to an increase in the content of AOL, OA, CIT, EMO while maintaining the same amounts of CPA and MPA. In aslike clover and zigzag clover, which occupy an intermediate position in the content of mycotoxins [13], increased contamination with fusariotoxins and AOL was observed in flowers with an increase in indicators for other components. Consequently, in the flowers and vegetative part of plants of the genus *Trifolium*, both common features and differences in the composition and content of mycotoxins occurred (see Table 1).

All plants showed a tendency towards greater accumulation of fusariotoxins in flowers. Apparently, this phenomenon is associated with the similarity of their physiological reactions, leading to a general direction of shifts in the distribution of micromycetes. The detection of all analyzed fusariotoxins suggests a combined contamination of the flowers of meadow clover, aslike clover, and zigzag clover with a medium complex of *Fusarium* fungi, the identification of which has not yet been carried out. It is worth noting, that in recent years the presence of toxigenic species, in particular *F. graminearum* Schw., *F. sporotrichioides* Sherb., *F. culmorum* (W.C. Sm.) Sacc. was noted in endophytes in a phylogenetically diverse group of wild-growing cereal grasses, and the study of their distribution in leaves, inflorescences, and seeds has begun [20-23].

An increase in the accumulation of AOL in flowers is also among the interspecific features, which is especially pronounced in aslike clover, less clearly in zigzag clover and white clover, and is not characteristic of the meadow clover. It is important to note that in cereal grasses the active producer of this toxin, the small-spore species *Alternaria alternata*, is referred to as typical endophytes [7].

Besides, OA and CIT were detected in the flowers of aslike and zigzag clover in comparable amounts, which was quite unusual (see Table 1). This situation is extremely rare. It was observed only in one of the 22 examined legume species - licorice (*Glycyrrhiza glabra* L.) [15] and was not observed in crucifers [17]. The simultaneous occurrence of these toxins has been described in both higher plants and lichens [24], but, as a rule, the amount of CIT is an order of magnitude higher than that established for OA. The joint presence of these mycotoxins with a frequency of 38% was also detected in grain, but not in equal amounts [25]. There is still no clarity about the sources of OA and CIT in grasses; however, it is known that habitat conditions have a noticeable effect on biosynthetic processes in the micromycete *Penicillium verrucosum* [26-28], which is capable of producing both metabolites [29]. In flowers of meadow clover and white clover, CIT was found only in some of the samples in quantities close to the limit of its determination (see Table 1), and in the seeds of these plants only OA was detected and there was no CIT (unpublished data of the authors).

The mycotoxicological assessment of one of the representatives of the genus *Lotus*, the horned flower, performed in this work for the first time, showed that in the aboveground part of the plant, the complex of fungal metabolites included AOL, CPA, and EMO, as well as OA and EA in smaller amounts (Table 2). It was not possible to detect OA and EA in the same amounts in flowers, while retaining the three main contaminants, but IFC and PR were detected. The composition of the beans turned out to be similar to the vegetative mass and differed only in the increased content of EMO. Fusariotoxins were found only in flowers and beans: T-2 in all samples, ZEN and DON in some cases. This feature is of undoubted scientific interest and indicates the need for a more detailed examination of *L. corniculatus* on expanded samples of material.

In two *Vicia* species (*V. sepium* and *V. cracca*), known to be weakly contaminated with mycotoxins [15], there was a tendency to a decrease in their number in flowers as compared to the vegetative part, while the occurrence and concentration of AOL remained the same, and both indicators for EA decreased (see Table 2). In forest vetch (*V. sylvatica*), which we sampled in Karelia, in the terrestrial part and flowers, the content of AOL was also comparable (25 and 32 μg/kg), and the differences in EA (30 and 1 μg/kg) were quite significant.

In two species of the genus *Lathyrus*, characterized by different levels of contamination [15], there was a uniform decrease in the content of mycotoxins in flowers compared to the vegetative parts. Nevertheless, in *L. vernus*, the AOL and

CPA contamination remained unchanged (Table 3), as in the *L. japonicus* specimens from the geographically remote area (Karelia) with 23 and 43 μg/kg AOL and 50 and 42 μg/kg CPA in the aerial parts and flowers, respectively.

2. The occurrence and concentration of mycotoxins in aboveground parts (1), **flowers** (2) **and beans** (3) **of deer vetch and *Vicia* L. plants** (Moscow Province, 2019)

Mycotoxin	*V. sepium* L.		*V. cracca* L.		*Lotus corniculatus* L. s.l.		
	1 (n = 4)	2 (n = 4)	1 (n = 5)	2 (n = 5)	1 (n = 4)	2 (n = 3)	3 (n = 3)
T-2	1	2	2	2	–	3	3
	3	2, 3	2, 3	2, 2		2-3-3	3-6-10
DON	–	–	1	1	–	–	1
			100	95			71
ZEN	–	–	1	-	–	1	2
			16			16	16, 18
FUM	–	–	2	-	–	–	–
			380, 420				
AOL	4	4	4	5	4	3	3
	20-26-34	20-29-35	14-29-38	23-33-47	22-36-50	31-42-48	40-41-44
OA	1	–	1	1	4	-	3
	5		8	9	5-7-8		5-10-18
CIT	1	–	1	–	–	–	1
	47		30				23
STE	1	–	2	–	–	–	–
	14		12, 17				
AB_1	–	–	–	–	–	–	–
CPA	4	4	5	5	3	3	2
	160-200-275	79-140-195	50-130-200	48-100-160	39-65-97	52-70-105	61, 105
MPA	4	4	–	–	–	2	2
	16-23-38	14-16-18				13, 20	25, 32
EA	4	3	5	3	3	–	2
	10-19-26	2-3-5	3-14-35	2-9-24	2-2-3		3, 11
EMO	2	1	–	1	4	3	3
	13, 25	16		25	40-75-140	49-75-120	165-205-250
PR	–	–	–	–	–	1	–
						260	
ROA	–	–	–	–	–	–	–

N o t e. Mycotoxins are T-2 toxin (T-2), 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), and PR-toxin (PR); n is the number of studied specimens. The upper value in lines is the number of positive specimens (n^+) containing mycotoxins in an amount exceeding the lower range limit; concentration of relevant mycotoxin (μg/kg, minimal-mean-maximum) is indicated underneath. A dash means that the mycotoxin was not detected.

Specific features extended to beans. In the spring vetch, the composition of mycotoxins was replenished with T-2, DON and PR, the frequency of detecting MPA was reduced with the same regular detection and constant concentration of AOL and CPA, as in the aboveground part, and in the meadow, there were clear differences in practically no contamination of beans and vegetative plants was observed (see Table 3).

For plants of the genera *Lupinus* and *Galega*, despite the contrasting content of mycotoxins [14], the differences between the generative organs and vegetative ones had common features (Table 4). In many-leaved lupine of 14 components characteristic of the aerial part, all were found in flowers except FUM, CIT, and PR, and in beans 8 were found, excluding DON, STE, and AB_1. The occurrence and content of all other mycotoxins, except for AOL, in flowers and beans decreased. For the eastern goa's rue, a generally similar trend was observed: in flowers, of 12 mycotoxins 8 were detected, except for FUM, OA, CIT, STE, in beans 7, except for ZEN and AB_1, with one case of OA detection. The frequency of detection of T-2, CPA, MPA and EMO decreased while their average content remained unchanged (see Table 4).

Thus, despite the interspecific features, perennial leguminous plants as a whole are characterized by the preservation of mycotoxin complexes typical for the vegetative part in flowers, with a tendency to a decrease in their content. It is

possible that in plants with a long interrupted growth cycle, only a part of producers is involved in specific processes accompanying the transition to the generative phase of development. It should be noted that in two species of annual cruciferous plants (white mustard and field cabbage), the set of mycotoxins in flowers turned out to be much wider due to the group of fusariotoxins DON, DAS, ZEN, FUM, as well as MPA, EMO, and PR [17, 30]. In this regard, in the future, it is of interest to examine annual leguminous grasses that are rarely found in biocenoses of Central Russia, such as *Vicia angustifolia* Reichard, *V. hirsula* (L.) S.F. Gray, *V. tetraspermum* (L.) Schreb. and *V. pannonica* Crantz.

3. The occurrence and concentration of mycotoxins in aboveground parts (1), **flowers** (2) **and beans** (3) **of *Lathyrus* L. plants** (Moscow Province, 2019)

Mycotoxin	*L. vernus* (L.) Bernh.			*L. pratensis* L.		
	1 (n = 20)	2 (n = 4)	3 (n = 8)	1 (n = 9)	2 (n = 4)	3 (n = 6)
T-2	–	–	3 2-2-3	9 2-6-10	4 2-3-6	5 3-4-5
DON	–	–	4 64-74-83	7 105-220-455	1 97	5 125-175-240
ZEN	–	–	–	9 19-36-52	4 16-27-42	6 24-29-33
FUM	–	–	–	4 95-450-1095	–	4 89-205-355
AOL	20 21-59-100	4 39-54-63	8 33-54-86	9 60-350-960	4 10-160-580	6 98-150-190
OA	4 4-5-6	–	6 5-12-23	9 16-36-59	1 32	6 43-66-105
CIT	4 23-30-40	–	3 24-27-33	8 40-65-125	1 32	6 32-42-56
STE	6 11-15-19	–	2 12, 15	9 19-63-120	1 10	6 22-30-38
AB1	2 2, 2	–	3 2-3-4	9 3-10-19	1 2	6 2-3-4
CPA	18 72-160-255	4 79-130-180	8 74-115-225	9 63-145-315	2 66, 79	4 53-65-78
MPA	10 15-19-25	–	1 16	9 29-46-79	2 13, 22	5 26-41-60
EA	20 3-21-100	4 4-6-8	5 2-8-20	9 4-36-155	1 3	6 3-7-12
EMO	9 12-21-38	2 19, 19	6 12-24-60	9 17-71-130	1 18	6 17-25-37
PR	–	–	1 232	4 400-720-1585	–	4 425-610-815
ROA	–	–	–	4 14-185-255	–	6 3-44-110

N o t e. Mycotoxins are T-2 toxin (T-2), deoxynivalenol (DON), zearalenone (ZEN), fumonisins (FUM), ergot alkaloids (EA), alternariol (AOL), roridin A (ROA), aflatoxin B1 (AB1), sterigmatocystin (STE), cyclopiazonic acid (CPA), emodin (EMO), ochratoxin A (OA), citrinin (CIT), mycophenolic acid (MPA), and PR-toxin (PR); n is the number of studied specimens. The upper value in lines is the number of positive specimens (n^+) containing mycotoxins in an amount exceeding the lower range limit; concentration of relevant mycotoxin (μg/kg, minimal-mean-maximum) is indicated underneath. A dash means that the mycotoxin was not detected.

The taxonomic assignment of micromycetes responsible for the formation of a complex of mycotoxins in herbs is still unclear. Nevertheless, in the mycobiota of higher plants of 17 families, including *Fabaceae*, the dominance of representatives of the genera *Curvularia, Acremonium, Alternaria, Penicillium, Fusarium, Stemphylium*, and *Cladosporium* [16] has already been shown, among which potentially toxigenic species are known [31]. In addition, associated fungi of other systematic groups may also be present in it, since modern science receives more and more evidence of the transfer of genome sites in the process of evolution from one organism to another [32, 33]. Participation in the toxinogenesis of endophytic fungi proper, involved in mutually beneficial symbiosis and do not exist autonomously, cannot be ruled out.

The role of endophytic fungi in plant responses to biotic and abiotic influences remains the focus of attention of researchers [34-38]. However, information on the distribution of endophytes in the host organisms is still extremely limited and contradictory. On plants of 17 families, including *Fabaceae*, it was shown that the fungi associated with them are localized mainly in the stems and leaves, to a lesser extent populate flowers, fruits, ears and inflorescences, and the root system is the most favorable organs for their existence [16]. In cereals (cocksfoot, wheatgrass, and timothy grass), the organotropic confinement of fungi *Fusarium, Alternaria*, and *Cladosporium* was established by quantitative PCR: the amount of DNA in generative organs (ears/panicles) was significantly higher than in stems and leaves [39].

An analysis of the results obtained in this work shows that toxin-forming micro-mycetes can be involved in the formation of generative organs in leguminous plants and in the response to changes in the environment. It can be assumed that they are involved in metabolic shifts that serve to transmit transformed signals from receptors that perceive changes in external factors through the "disturbance" of the hormonal status, the system of secondary messengers, genome activity, or the behavior of the cytoskeleton [40]. Given this nature of the interaction with the plant, associated toxin-forming micromycetes should rather be attributed to symbions than to endocommensals, although the data obtained in this work do not give any grounds to judge the typification of micromycetes that provide the production of mycotoxins.

4. The occurrence and concentration of mycotoxins in aboveground parts (1), **flowers** (2) **and beans** (3) **of *Lupinus* L. and *Galega* L. plants** (Moscow Province, 2019)

Mycotoxin	*L. polyphyllus* Lindl.			*G. orientalis* Lam.		
	1 (n = 17)	2 (n = 7)	3 (n = 7)	1 (n = 15)	2 (n = 5)	3 (n = 8)
T-2	17	7	6	9	5	3
	3-5-11	4-6-9	2-3-4	2-3-4	3-3-4	2-2-3
DON	16	1	–	–	–	–
	63-89-125	63				
ZEN	17	7	4	7	5	–
	16-29-47	16-34-50	12-17-21	16-25-33	19-27-34	
FUM	1	–	–	1	–	–
	56			95		
AOL	17	7	7	15	5	8
	65-155-295	63-105-130	19-145-785	11-31-66	25-47-81	14-27-63
OA	14	4	2	1	–	1
	5-8-12	5-6-6	5, 6	5		5
CIT	5	–	–	3	–	–
	20-36-74			23-24-25		
STE	15	1	–	5	–	–
	12-24-33	15		11-18-38		
AB1	17	7	–	3	4	–
	3-5-8	2-3-4		2-2-2	2-2-2	
CPA	17	7	4	14	5	4
	130-475-870	150-260-355	68-73-79	41-115-255	82-110-140	74-105-125
MPA	16	7	2	8	5	1
	13-29-47	18-23-29	14, 16	15-22-37	16-20-22	20
EA	16	7	4	12	4	4
	8-70-160	2-4-10	3-9-16	3-12-50	2-3-4	2-3-3
EMO	17	7	6	8	4	2
	31-155-355	25-49-85	26-84-130	12-14-15	13-17-25	11, 26
PR	7	–	–	–	–	–
	205-290-400					
ROA	–	–	–	–	–	–

N o t e. Mycotoxins are T-2 toxin (T-2), deoxynivalenol (DON), zearalenone (ZEN), fumonisins (FUM), ergot alkaloids (EA), alternariol (AOL), roridin A (ROA), aflatoxin B1 (AB1), sterigmatocystin (STE), cyclopiazonic acid (CPA), emodin (EMO), ochratoxin A (OA), citrinin (CIT), mycophenolic acid (MPA), and PR-toxin (PR); n is the number of studied specimens. The upper value in lines is the number of positive specimens (n^+) containing mycotoxins in an amount exceeding the lower range limit; concentration of relevant mycotoxin (μg/kg, minimal-mean-maximum) is indicated underneath. A dash means that the mycotoxin was not detected.

Thus, in the generative organs of perennial legumes of the genera *Trifolium, Lathyrus, Vicia, Lotus, Lupinus*, and *Galega*, the mycotoxin complexes are generally similar to those found in the vegetative parts and in some species have differences in the content or ratio of individual components. A number of features have been established in the contamination of flowers of plants of the genus *Trifolium*: the combined occurrence of ochratoxin A and citrinin in comparable quantities (*T. hybridum* L., *T. medium* L.), a sharply increased content of alternariol (*T. hybridum*), increased accumulation of T -2 toxin, deoxynivalenol, zearalenone and fumonisins (*T. pratense*, *T. hybridum, T. medium*), and T-2 toxin (*T. repens*). In two species of the genus *Lathyrus*, a common feature was a tendency towards a decrease in the content of mycotoxins in flowers, while for beans there was a similarity with a whole plant (*L. pratensis*) and replenishment of the composition with T-2 toxin, deoxynivalenol, PR-toxin with a decrease in the detection rate of mycophenolic acid (*L. vernus*). In sheepfoot (*Lotus corniculatus*), fusariotoxins were found only in the generative organs. In the examined representatives of the genera *Vicia, Lupinus*, and *Galega*, the metabolic background in flowers was generally weakened, and in beans it did not have clear signs of a change in the component composition and a sharp variation in the content. Taxonomically related species and subspecies of the genera *Lotus, Vicia, Lupinus*, and *Galega* are promising for the successful continuation of work on assessing the contribution of toxigenic micromycetes to internal processes and plant responses to external factors. The comparative mycotoxicological analysis of the generative and vegetative organs, carried out by us, in the future it is advisable to extend to plants and other taxonomic groups, which will make it possible to form more definite ideas about the physiological role of associated microscopic fungi.

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