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BIOLOGICAL FEATURES OF THE RESPONSE OF FODDER GRASSES TO THE USE OF IODINE ON AGROSOD-PODZOLIC SOILS OF VARIOUS CULTIVATION LEVELS

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Abstract

A geochemical anomaly of iodine deficiency the North-Western region of the Russian Federation negatively affects the yield and quality of marketable products of regional agriculture and feed, the viability and productivity of farm animals, and the health of the population. In this study, for the first time in the conditions of the region, the optimal concentration levels of the KI solution for foliar fertilization and the time period of treatment on the annual and perennial grasses dominating in the structure of the acreage of the Non-Chernozem region were established. Our goal was to study the biological characteristics and evaluate the parameters of responsiveness of forage grasses to changes in the concentration of the KI solution and the period of time of iodine foliar treatments. The research was carried out in 2019-2021 in the Menkovo branch of the Agrophysical Institute (Gatchinsky District, the Leningrad Province). Two micro-field experiments were laid down in the system of a long-term fundamental field agrophysical experiment in the field crop rotation link: potatoes—annual grasses + perennial grasses—perennial grasses of the 1st year of use—perennial grasses of the 2nd year of use. The object of the study was mixed crops. Annual grasses were presented by the oat (*Avena sativa* L.) variety Skakun and the garden vetch (*Vicia sativa* L.) variety Vera, perennial grasses were presented by the red clover (*Trifolium pratense* L.) variety Orpheus and the timothy (*Phleum pratense* L.) variety Leningradskaya 204. Both experiments had a two-factor scheme. Factor A is the degree of cultivation of sandy loam agrosod-podzolic soil (medium-cultivated, well-cultivated and highly cultivated). The scheme of the first experiment on factor B included nine variants of the concentration of the KI solution: 0, 0.005, 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, and 0.64 %. Foliar treatments of annual grasses were carried out in the booting stage of oat, perennial grasses were in the tillering stage. In the second experiment, four variants of the time period of foliar treatment with 0.02 % KI solution were studied by factor B: KI-0 — control without treatment; KI-1 — early treatment in the tillering stage of oats, red clover and timothy; KI-2 — late treatment in the booting stage of oat and in the stage of branching of red clover; KI-3 — two-fold treatments in terms corresponding to variants KI-1 and KI-2. The yield of the aboveground biomass of grasses used for the preparation of feed was counted by a continuous weight method from a 1 m^2 plot. The placement of plots by repetitions and variants was systematic. The repetition in the first experiment was threefold, in the second — sixfold. A chemical-analytical analysis of selected soil and plant samples was carried out. As a result of short-term field experiments, it was found that the responsiveness of forage grasses to the iodine foliar treatment under a geochemical anomaly of iodine deficiency is determined by a combination of weather-climatic and agrochemical soil conditions with biological characteristics of crops and depends on the period of time of treatment and the concentration of the KI solution. For annual grasses, the treatment was more effective in the booting stage of oat (yield increased by an average of 2.49 t/ha, or 29 %; $p \leq 0.05$), whereas for perennial grasses in the tillering stage of red clover and timothy (an increase of 3.39 t/ha, or 18 %; $p \leq 0.05$). The optimal C_{KI} for the treatment of annual grasses was 0.16 %, regardless of the degree of cultivation of the soil, and of perennial grasses on soils of medium, good and high cultivation was 0.04, 0.08 and 0.16 %, respectively. The increase ($p \leq 0.05$) in productivity reached 3.69-9.38 t/ha, or 67-80 %, for annual grasses and 3.91-8.03 t/ha, or 22-30%, for perennial grasses. The positive effect of iodine increased with the optimization of soil and agrochemical conditions to good and high cultivation by 68 and 128 %. Due to high tolerance to the concentration of the KI solution, toxic effect was detected only at C_{KI} 0.32-0.64 %, when crop losses reached 19 %. Legume types of herbs were more sensitive to the excess of iodine. The reduction of iodine toxicity in the experiments was facilitated by an increase in soil cultivation and a change in the botanical composition of crops with an increase in the proportion of cereals. Perennial grasses accumulated 9 % less iodine than annual ones. In the variants with optimal C_{KI}, the iodine content in the aboveground biomass of annual and perennial grasses increased on average from 119 and 88 to 766 and 628 μg/kg, that is, 6.4-fold and 7.1-fold. The accumulation of nitrates, on the contrary, decreased ($p \leq 0.05$) by 13 % in annual and 11 % in perennial grasses. The maximum level of iodine accumulation in the green mass of annual grasses were about 600 on medium cultivated soil, 900 on well-cultivated soil, and 1500 μg/kg on highly cultivated soil. In perennial grasses less sensitive to soil cultivation, this value practically did not depend on soil and agrochemical conditions and amounted to 900 μg/kg. One of the signs of iodine toxicity was a 23-33 % ($p \leq 0.05$) increase in the content of nitrates in products.

Keywords: fodder grasses, annual grasses, perennial grasses, iodine, nitrates, iodine fertilizers, agrosod-podzolic soil, cultivation, productivity

Improving the quality of agricultural products is one of the main challenge [1, 2]. The global problem is still the lack of iodine, due to the geochemical features of its distribution and behavior in the environment. The implementation of long-term state programs for the prevention of iodine deficiency made it possible to get rid of the most severe human pathologies caused by chronic iodine deficiency in food, but did not fully solve the problem either in Russia [3, 4] or in the European Union [4, 5]. Spatial heterogeneity of soils for iodine, local soil contamination with a radioactive isotope in Belarus and Russia [6-8], and limited availability of iodine-fortified foods for a part of the population, mainly rural, along with the low content of the element in soils and waters, complicate iodine deficiency [9].

Animal husbandry, which is the main commercial sector of agriculture in the Non-Chernozem region [2], also faces the negative consequences of iodine deficiency in feed [10, 11]. Despite the fact that the world has accumulated extensive scientific data on the effective use of iodine microfertilizers [12-15], including together with selenium [15-19], in the North-West region of Russia, various aspects of their use have been systematically studied only in the Kaliningrad region [20]. It was shown that even the coastal position of the region does not allow compensating for the deficiency of iodine in arable soils caused by soil genesis.

Multi-scale field experiments revealed the advantage of foliar treatment with a iodine microfertilizer over its application to the soil [21-23], as well as increased toxicity and some superiority of the iodide (I^-) over the iodate (IO^{3-}) [24-27]. It has been found out that iodine in optimal concentrations, interacting with amino acids, proteins and enzymes, stimulates the synthesis of sugars and proteins, enzymatic (peroxidase and oxidoreductase) and antioxidant activity of plant cells [28, 29]. By enhancing the biosynthesis of tryptophan and its transamination into indole auxins which promote meristem cell elongation, iodine also promotes nutrient transport in plants [30].

Thereof, in optimal concentrations, iodine activate the production processes, increase tolerance of agrocenoses to negative biotic and abiotic factors, and improve the quality of commercial products in terms of accumulation of iodine, proteins, vitamins, and sometimes sugars [18, 19, 28]. On the contrary, an excess of iodine inhibits part of the nitrogen cycle enzymes (nitrate reductase, glutamate dehydrogenase) and protein biosynthesis, including through increased productucion of the phytohormone ethylene, and promotes the accumulation of nitrates [31, 32]. The optimal dosage, accumulation in plant biomass, and the toxicity of iodine depend on the biological properties of crops, varieties, as well as soil and agrochemical conditions [26, 27, 33, 34].

Despite the quite obvious theoretical prerequisites, the problem of improving the iodine status of fodder crops has been ignored by the regional scientific community for many years. In fact, there are no recommendations on the types, dosages, terms and modes of treatment of forage grasses with iodine microfertilizers. Note, biofortification with iodine increases the yield of grasses and improves their nutritional value, which, in turn, increases the productivity of livestock and the quality of dairy products [11, 30], as iodine, covalently binding to milk casein, forms a complex which is physiologically most suitable and valuable for humans.

This paper is the first to indicate the concentrations of KI solutions for foliar application and timing of threaamaents optimal under the conditions of the Leningrad Province for the annual and perennial grasses that dominate in the sown areas of the Non-Chernozem Region.

Our goal was to evaluate the biological response of forage grasses to various KI concentrations and timing of foliar treatment with potassium iodide.

Materials and methods. The studies were carried out in 2018-2021 in the Menkovsky branch of the Agrophysical Research Institute (API, Gatchinsky District, Leningrad Province). Two microfield tests were incorporated in a long-term fundamental experiment (agrophysical station) on the field crop rotation, the potatoes—annual grasses + perennial grasses—perennial grasses of the 1st year of use—perennial grasses of the 2nd year of use.

Of grasses for mixed crops, annual herbs (oats *Avena sativ*a L. cv. Skakun of the FRC Nemchinovka, Russia; vetch *Vicia sativa* L. cv. Vera of Federal Williams Research Center of Forage Production & Agroecology, Russia) and perennial herbs (red clover *Trifolium pratens*e L. cv. Orfey of Rudnitsky FARC of the North-East, Russia; meadow timothy grass *Phleum pratense* L. cv. Leningradskaya 204 of Belogorka Leningrad Research Institute of Agriculture — a branch of the Lorkh Federal Research Center for Potatoes, Russia) were used.

Both experiments had a two-factor scheme. Factor A was the degree of cultivation of sandy loamy agro-podzolic soil (medium-cultivated, well-cultivated and highly cultivated) due to the long-term use of organic fertilizers and lime. The humus content in the topsoil is 2.51, 3.48 and 4.46%, respectively;mobile phosphorus compounds amounted to 199, 325 and 364 mg/kg, mobile potassium compounds to 49, 162 and 274 mg/kg, total iodine to 0.94, 1.22 and 1.48 mg/kg, with pH_{KCl} 5.12, 5.99 and 6.25.

The first experiment included nine concentrations of the KI working solution (C_{KI}) as factor B (0, 0.005, 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, and 0.64%). Foliar treatment of annual grasses was carried out at the time of oats stem extention, of perennial grasses at tillering. In the second experiment, the timing of foliar treatment uding 0.02% KI solution was as follows: KI-0 for control without treatment, KI-1 for early treatment at tillering of oats, red clover and timothy grass, KI-2 for late treatment at stem extention of oats and branching of red clover, KI-3 for double treatment, the timing is as for KI-1 and KI-2.

Spraying was carried out in the evening in calm weather using a backpack sprayer STIHL SG51 (Andreas Stihl AG & Co. KG, Germany) with a working fluid flow rate of 30 ml/m^2. The working solution was prepared using crystalline KI, chemically pure (Troitsky Iodine Plant, Russia).

The yield of aboveground grass biomass used for fodder preparation was taken into account by a continuous weight method from a plot of 1 m^2. Placement of plots according to repetitions and variants is systematic. The repetition in the first experiment was 3-fold, in the second 6-fold.

Chemical assays of 1-1.2 kg bulk green mass samples of harvested grasses composed of 10 individual samples, was carried out in 3 replications using standardized methods (the study was carried out by accredited laboratories of GSAS

Pskovskaya and API. The content of iodine in the dry green mass of annual and perennial grasses was determined according to GOST 31660-2012 (Moscow, 2012) by the stripping voltammetric method after dry ashing and dissolution of the precipitate in sulfuric acid using Ecotest-VA-iodine (OOO Ekoniks-Expert, Russia). The concentration of nitrates was estimated according to GOST 13496.19-2015 (Moscow, 2016) by the ionometric method after extraction with a 1% solution of potassium alum (an HI98191, Hanna Instruments, Germany).

Statistical processing was carried out by the dispersion method after checking the compliance of the sample with the normal distribution law in the Statistica 7.0 software package (StatSoft, Inc., USA). The significance of differences in deviations was assessed at a 5% significance level using Fisher's *F*-test expressed via LSD_{05} for each factor and their interaction. The tables and figures show the average values (*M*) and a confidence interval with a standard error of the mean (±SEM).

Results. A significant impact on the production process of unfavorable weather and climatic conditions at the beginning of the growing season was shown in all years of research. The early summer drought characteristic of the region [2] reduced the hydrothermal coefficient in June, during which both crops have the most intensive growth period, to 0.2-0.7 units. Annual grasses and perennial grasses of the 1st year of use were especially hard hit. From the critical consequences of the June drought in 2021, the sowing of perennial grasses of the 2nd year of use was saved by waterlogging of the soil in April-May.

A direct consequence of this was not only the unusually low productivity of annual grasses, but also their very high responsiveness to increasing the effective fertility of agro-soddy-podzolic soil (Table 1). Due to the optimization of the water and potassium regimes of the soil, which control the watering of the cell cytoplasm, as the cultivation of the soil increased to good and high, the productivity of annual grasses increased by 59 and 195%, respectively ($p \leq 0.05$). For perennial grasses, similar parameters for 2 years averaged 52 and 72% ($p \leq 0.05$).

Under such a critical weather and climatic parameters, the responsiveness of annual grasses to foliar feeding with a solution of potassium iodide turned out to be unexpectedly high, 27% vs. 13% ($p \leq 0.05$) for perennial grasses. The optimal C_{KI} for annual grasses was 0.16%, regardless of the degree of soil cultivation, and for perennial grasses on soils of medium, good, and high cultivation, it was 0.04, 0.08 and 0.16%, respectively. The optimal concentration of the working solution of potassium iodide in this experiment turned out to be significantly higher than previously on potato crops [34], oats [36] and winter rapeseed [37]. The increase in productivity from foliar treatment with iodine reached 3.69-9.38 t/ha, or 67-80% ($p \leq 0.05$), for annual grasses and 3.91-8.03 t/ha, or 22-30% ($p \leq 0.05$), for perennial grasses. The probable reason for such a significant effect of this technique, along with compensation for the lack of iodine in the soil, was the presence of potassium in the composition of the microfertilizer, which plays one of the key roles in plant drought resistance. The protective physiological function of iodine itself is largely associated with an increase in the antioxidant activity of the cell cytoplasm by stimulating the synthesis of glutathione, ascorbic acid, and phenolic compounds and preventing the oxidative degradation of proteins, nucleic acids, and carbohydrates [25, 38, 39]. A.V. Sindireva et al. [36] in pot trials proved a threefold increase in catalase activity in the aboveground biomass of oats.

The positive effect of iodine on both crops increased markedly as the soil and agrochemical conditions were optimized. The increase in the yield of grass green mass from foliar feeding with iodine at the optimal concentration during the transition from medium to good and high degree of cultivation increased by 33-102 and 105-154%, respectively ($p \leq 0.05$).

1. Yields of annual and perennial grasses depending on cultivation level of agro-podzolic soil and the KI concentration (C_{KI}) (n = 3, M±SEM, Menkovsky branch of the Agrophysical Research Institute, Gatchinsky District, Leningrad Province, 2019-2020)

Variant		Annual grasses			Perennial grasses		
soil cultivation (factor A)	C_{KI}, % (factor B)	yield, t/ha	Δ due to KI		yield, t/ha	Δ due to KI	
			t/ha	%		t/ha	%
Medium cultivated	0	4.62±0.32			17.50±0.79		
	0.005	5.10±0.27	0.49	11	17.79±0.51	0,29	2
	0.01	5.19±0.28	0.58	12	18.64±0.77	1,14	7
	0.02	5.98±0.19	1.36	30	20.39±0.84	2,88	16
	0.04	6.49±0.23	1.87	41	21.42±0.46	3,91	22
	0.08	6.61±0.09	1.99	43	22.28±0.64	4,78	27
	0.16	8.30±0.22	3.69	80	22.38±0.39	4,88	28
	0.32	7.45±0.17	2.84	61	20.07±0.58	2,57	15
	0.64	5.76±0.17	1.15	25	16.94±0.51	–0,57	–3
Well cultivated	0	7.36±0.10			26.54±1.10		
	0.005	7.59±0.07	0.23	3	26.86±0.97	0,32	1
	0.01	8.38±0.26	1.02	14	27.79±0.57	1,25	5
	0.02	9.44±0.29	2.08	28	29.51±0.32	2,97	11
	0.04	10.65±0.25	3.29	32	32.65±0.39	6,11	23
	0.08	11.45±0.27	4.09	56	34.43±0.34	7,89	30
	0.16	12.26±0.34	4.90	67	34.80±0.56	8,26	31
	0.32	11.61±0.29	4.25	69	30.74±0.34	4,20	16
	0.64	10.41±0.39	3.05	37	28.26±0.68	1,72	6
Highly cultivated	0	13.65±0.31			30.03±0.71		
	0.005	14.12±0.32	0.47	3	30.29±0.68	0,25	1
	0.01	15.38±0.27	1.73	13	31.38±0.45	1,35	4
	0.02	17.07±0.34	3.42	25	33.40±0.71	3,37	11
	0.04	19.25±0.34	5.60	41	33.77±0.33	3,73	12
	0.08	20.94±0.44	7.31	54	36.62±0.41	6,59	22
	0.16	23.03±0.55	9.38	69	38.06±0.68	8,03	27
	0.32	22.12±0.56	8.47	62	34.55±0.90	4,52	15
	0.64	18.95±0.29	5.30	39	30.72±0.93	0,69	2
LSD_{05}							
factor A		0.31			1.39		
factor Б		0.53			1.48		
A×Б		0.92			$F_{факт.} < F_{05}$		

N o t e. Annual grasses — oat (*Avena sativa* L.) cv. Skakun and common vetch (*Vicia sativa* L.) cv. Vera, perennial grasses — red clover (*Trifolium pratense* L.) cv. Orfey and meadow timothy grass (*Phleum pratense* L.) cv. Leningradskaya 204.

In contrast to potatoes which showed acute sensitivity to excess iodine already at C_{KI} 0.06-0.08% [34], the response of herbs was more plastic. Legume species (common vetch and red clover) responded more sharply to the increase in C_{KI}, i.e., at the 0.64% concentration, marginal leaf necrosis was detected, similar to that described by P.G. Lawson et al. [22]. Yield losses from excess iodine vs. optimal concentrations averaged 19% ($p \le 0.05$) and were comparable to a 20% decrease in lettuce and kohlrabi yields reported by P.G. Lawson et al. [22].

The decrease in the sensitivity of crops to an excess of KI was facilitated by the improved soil cultivation which was associated with botanical composition of crops. If on medium cultivated soil the common vetch in crop biomass reached 71-78%, on highly cultivated soil 43-47%, for red clover, the distribution was more even, 76-88 and 69-73%, respectively. Therefore, annual grasses tolerated excess iodine better on cultivated soils than perennial herbs. In addition, according to C.L. Mackowiak et al. [40), due to the enrichment with humic acids, cultivated soils have a significantly higher potential for iodine detoxification than less humus soil types.

Due to the biological specificity of development, crops responed differently to the timing of foliar feeding with 0.02% KI solution (Table 2).

For annual grasses, the late spraying at booting stage was more favorable (an increase in yield averaged 2.49 t/ha, or 29%), for perennial grasses, it was earlier foliar reatmenr at tillering (with an increase by 3.39 t/ha, or by 18%)

(p ≤ 0.05). An obvious reason for this was the poor development of oat and vetch plants in the tillering phase (the projective soil cover is less than 5%), as a result of which only an insignificant part of the fertilizer got to the plants. In the same phase, in red clover and timothy grass, the projective soil leaf cover exceeded 60%, which, in combination with a favorable water regime, ensured the advantage of this variant.

2. Yields of annual and perennial grasses depending on cultivation level of agro-podzolic soil and the timing of KI application (*n* = 6, *M*±SEM, Menkovsky branch of the Agrophysical Research Institute, Gatchinsky District, Leningrad Province, 2019-2020)

Variant		Yield, t/ha	ΔYiled					
soil cultivation (factor A)	timing of treatment (factor B)		total		due to soil cultivation		due to KI	
			t/ha	%	t/ha	%	t/ha	%
		Annual grasses						
Medium cultivated	KI-0	4.53±0.18						
	KI-1	5.94±0.08	1,41	31			1,41	31
	KI-2	6.27±0.12	1,74	38			1,74	38
	KI-3	5.29±0.14	0,76	17			0,76	17
Well cultivated	KI-0	7.44±0.12	2,91	64	2,91	64		
	KI-1	9.39±0.16	4,86	107	3,45	58	1,95	26
	KI-2	9.65±0.20	5,12	113	3,38	54	2,21	30
	KI-3	8.64±0.18	4,11	91	3,35	63	1,20	16
Highly cultivated	KI-0	13.88±0.39	9,35	206	9,35	206		
	KI-1	14.82±0.11	10,29	227	8,88	149	0,94	7
	KI-2	17.37±0.19	12,84	283	11,10	177	3,49	25
	KI-3	12.96±0.15	8,43	186	7,67	145	–0,92	–7
LSD_{05}			2,95		0,92		1,97	
		Perennial grasses						
Medium cultivated	KI-0	15.47±0.35						
	KI-1	19.97±0.59	4,50	29			4,50	29
	KI-2	18.90±0.50	3,43	22			3,43	22
	KI-3	20.50±0.19	5,03	33			5,03	33
Well cultivated	KI-0	19.69±0.37	4,22	27	4,22	27		
	KI-1	22.78±0.26	7,31	47	2,81	14	3,09	16
	KI-2	21.48±0.46	6,01	39	2,58	14	1,79	9
	KI-3	23.28±0.33	7,81	50	2,78	14	3,59	18
Highly cultivated	KI-0	20.52±0.44	5,05	33	5,05	33		
	KI-1	23.10±0.16	7,63	49	3,13	16	2,58	13
	KI-2	21.67±0.65	6,20	40	2,77	15	1,15	6
	KI-3	23.14±0.39	7,67	50	2,64	13	2,62	13
LSD_{05}			1,03		0,52		0,60	

N o t e. Annual grasses — oat (*Avena sativa* L.) cv. Skakun and common vetch (*Vicia sativa* L.) cv. Vera, perennial grasses — red clover (*Trifolium pratense* L.) cv. Orfei and meadow timothy grass (*Phleum pratense* L.) cv. Leningradskaya 204.

The response to repeated spraying also differed drastically. While perennial grasses showed a positive trend towards higher yields after treatment, annuals showed very noticeable toxicity. It is not yet possible to find an unambiguous explanation for the latter, given the high efficiency of iodine in the first experiment.

Possessing a high physiological activity, iodine significantly influenced the quality of the green mass of grasses used for fodder production. Of the nine parameters studied (dry matter, nitrogen, phosphorus, potassium, crude protein, crude fiber, crude ash, iodine and nitrates), two turned out to be the most sensitive, the content of iodine and nitrates (Fig. 1). As in the work of V.I. Panasina et al. [37], in winter rapeseed, iodine in most of the studied dosfges was absorbed by the surface of grass leaves due to a barrier-free mechanism. In the first experiment, an almost linear functional dependence of the iodine content in the green mass was established up to C_{KI} 0.16% for perennial grasse, up to C_{KI} 0.04% for annual grasses on medium cultivated soil and up to C_{KI} 0.32% for annual grasses on well and highly cultivated sois. As a result, the maximum parameters of iodine accumulation in the green mass of annual grasses amounted to approx. 600 μg/kg on medium cultivated soil, 900 μg/kg on well-cultivated soil, and 1200 μg/kg on highly cultivated soil. For perennial grasses less sensitive to soil cultivation, this value was practically independent of soil and agrochemical conditions and amounted to 900 μg/kg. These parameters can be taken as the limit at which irreversible toxic reactions are found in herbs [22]. These values significantly exceeded those previously reported by A.V. Sindireva et al. [36] ofor oat, which is probably due to the specific conditions of the pot trials. However, R. Li et al. [12] succeeded in biofortifying horticulture products to an iodine content of 1330-4000 μg/kg, and an absolute maximum of 10000 μg/kg was achieved on tomatos [41].

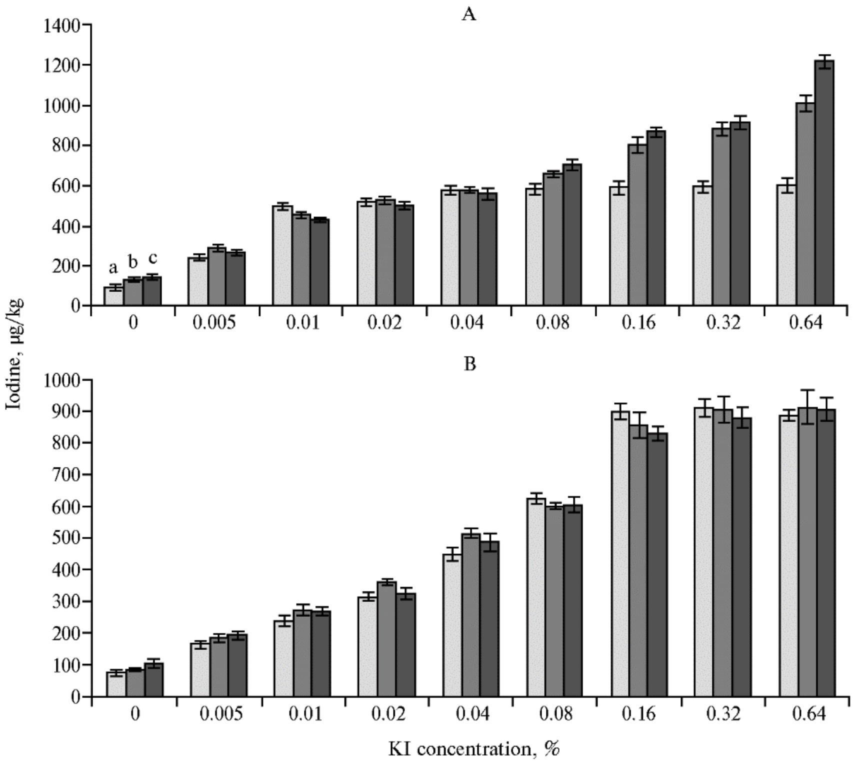

Fig. 1. Accumulation of iodine in the green mass of annuals (oat *Avena sativa* L. cv. Skakun, common vetch *Vicia sativa* L. cv. Vera) (A) **and perennial grasses (red clover *Trifolium pratense* L. cv. Orfei, meadow timothy *Phleum pratense* L. cv. Leningradskaya 204)** (B) **depending on the concentration of the KI solution and soil cultivation level:** a — medium cultivated, b — well cultivated, c — higly cultivated (n = 3, M±SEM, Menkovsky branch of the Agrophysical Research Institute, Gatchinsky District, Leningrad Province, 2019-2020).

On average, the accumulation of iodine in the aboveground plant biomass in the fertilized variants was 619 ґg/kg for annual grasses and 557 μg/kg for perennial grasses. The lag of perennial grasses in this indicator by 9% was most likely due to the effect of "biological" dilution in the significantly superior yield of the latter. In the optimal options for the impact on crop productivity, the iodine content in the aboveground biomass of annual and perennial grasses was increased on average from 119 and 88 to 766 and 628 μg/kg, that is, by 6.4 and 7.1 times ($p \leq 0.05$).

The accumulation of nitrates in annual grasses largely depended on the soil cultivation level which determined soil nitrogen status. The content of N-NO_3^- in the arable layer of medium, well- and highly cultivated soil in the first decade of June was 19, 32 and 44 mg/kg, respectively, and increased in well- and highly cultivated soil by 34 and 122% ($p \leq 0.05$) (Fig. 2). Among perennial grasses, red clover which is little dependent on soil nitrogen, prevailed, and these herbs weakly respond to soil cultivation level.

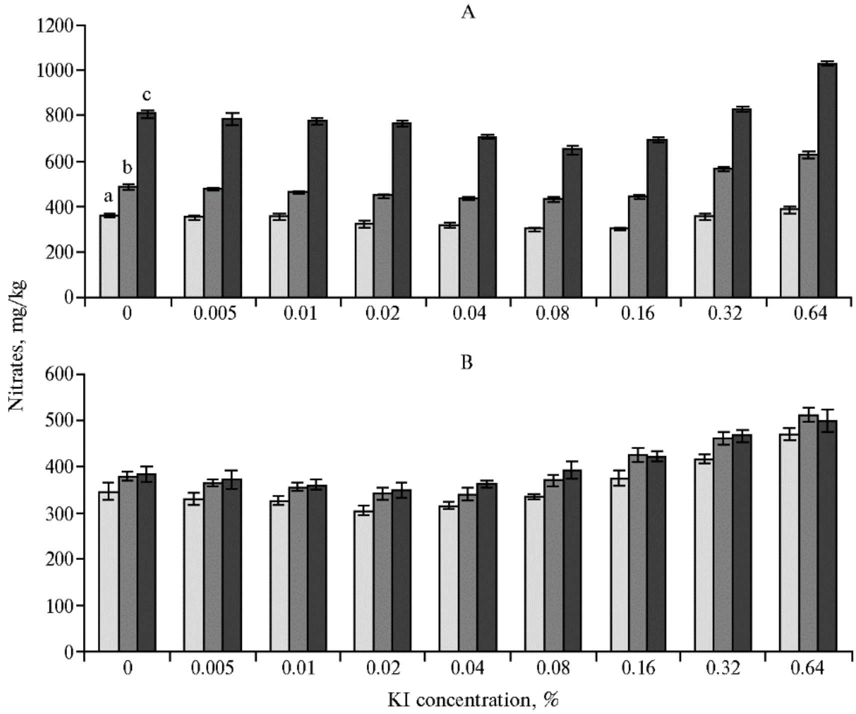

Fig. 2. Accumulation of nitrates in the green mass of annuals (oat *Avena sativa* L. cv. Skakun, common vetch *Vicia sativa* L. cv. Vera) (A) **and perennial grasses (red clover *Trifolium pratense* L. cv. Orfei, meadow timothy *Phleum pratense* L. cv. Leningradskaya 204)** (B) **depending on the concentration of the KI solution and soil cultivation level:** a — medium cultivated, b — well cultivated, c — higly cultivated (*n* = 3, *M*±SEM, Menkovsky branch of the Agrophysical Research Institute, Gatchinsky District, Leningrad Province, 2019-2020).

The effect of iodine on the accumulation of nitrates in grass biomass was much more complex. The important factors were the different availability of soil with nitrate nitrogen, which stimulated the biosynthetic effect in the area of low concentrations of the working solution and inhibited the reduction of nitrates in the cell in variants with high C_{KI} values [31, 32]. The maximum effect of reducing the accumulation of nitrates in the grass green mass occurred at C_{KI} 0.08% for annual grasses and C_{KI} 0.02% for perennial grasses. On average, for these treatments and crops, it reached 63 mg/kg (from 461 to 398 mg/kg), or 14%. In the test options that were optimal in terms of productivity, the average decrease in the content of nitrates reached 13% for annual grasses and 11% for perennial grasses ($p \leq 0.05$). Probably, largely for this reason, in the experiments of V.I. Panasin et al. [37] at KI concentrations up to 0.1%, there was a significant increase in the content of crude protein in the green mass of winter rapeseed.

An increase in the concentration of the working solution to the maximum values caused an increase in the accumulation of nitrates in the biomass of annuals by 128 mg/kg (from 552 to 680 mg/kg), or by 23%. In perennials, accumulation of nitrates increased by 123 mg/kg (from 370 to 493 mg/kg), or by 33% ($p \leq 0.05$), due to inhibition of reducing enzymes. Data comparable in terms of the relative increase in the content of nitrates under the influence of excess iodine (by 10-30%) were obtained for lettuce, wild carrot, and garden spinach [31, 32, 42].

In the second experiment, the nature of the influence of soil-agrochemical conditions and iodine foliar application on the qualitative parameters was largely similar (Fig. 3). Thus, the foliar use of 0.02% KI solution at the optimal time increased the iodine content in the green mass of annual grasses 5.1-fold (from 109 to 561 ґg/kg), of perennials 3.4-fold (from 99 to 333 ґg /kg). Unlike the perennials, annual grasses which are sensitive to the optimization of soil properties, reduced the iodine accumulation from medium level of cultivation to well- and highly cultivated soils due to “biological” dilution.

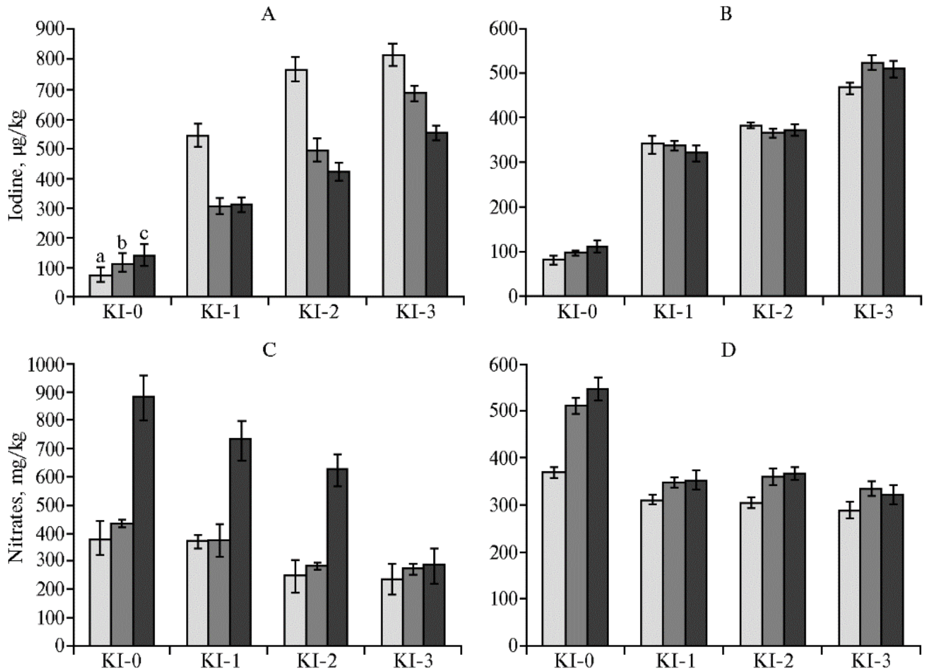

Fig. 3. Accumulation of iodine (A, B) **and nitrates** (C, D) **in the green mass of annuals (oat *Avena sativa* L. cv. Skakun, common vetch *Vicia sativa* L. cv. Vera)** (A, C) **and perennial grasses (red clover *Trifolium pratense* L. cv. Orfei, meadow timothy *Phleum pratense* L. cv. Leningradskaya 204)** (B, D) **depending on timing of KI application and soil cultivation level:** a — medium cultivated, b — well cultivated, c — higly cultivated (n = 6, M±SEM, Menkovsky branch of the Agrophysical Research Institute, Gatchinsky District, Leningrad Province, 2019-2020).

From the standpoint of providing better conditions for fixing iodine in the biomass, the later dates of foliar feeding were more effective. Justified in this regard was the two-time foliar spraying of grasses with a 0.02% KI solution, the positive effect of which on well- and highly cultivated soils was significantly higher. This option of using iodine microfertilizers also provided the best results for reducing the content of nitrates in grass biomass, especially on highly cultivated agro-soddy-podzolic soils. Their content in the green mass was reduced by 67% in annuals and by 41% in perennial grasses ($p \leq 0.05$), which is significantly higher than the values (18%) previously achieved both in our experiment on potatoes [34] and and on other crops [31, 32, 42].

Thus, the responsiveness of fodder grasses to iodine foliar apploction under geochemical iodine deficiency is determined by a combination of weather, climatic and soil agrochemical conditions with the biological properties of crops and depends on the timing of treatmens and the concentration of the KI working solution. For annual grasses, the optimal time for foliar application was the booting stage of oats, for perennial grasses, it was tillering of red clover and timothy grass. The yield of the annuals and perennials increased by 29% and 18%, respectively ($p \leq 0.05$). The optimal concentration of the working solution KI for annual grasses did not depend on the soil and agrochemical conditions and reached

0.16%. In perennial grasses, it was 0.04% on medium cultivated soil, 0.08% on well-cultivated soil, and 0.16% on highly cultivated soil. Due to the activation of the bioproduction due to the treatments, the yield of green mass increased ($p \le 0.05$) in annual grasses by 3.69-9.38 t/ha (67-80%), in perennial grasses by 3.91-8.03 t/ha (22-30%). The content of iodine in the aboveground biomass of annuals and perennials increased ($p \le 0.05$) on average from 119 and 88 to 766 and 628 mg/kg, that is, by 544 and 614%, while nitrates, on the contrary, decreased ($p \le 0.05$) by 11-13%. The toxic effect of excess iodine, expressed in a decrease in crop productivity by 19% and an increase in the content of nitrates in the green mass by 23-33%, occurres at C_{KI} 0.32-0.64%. Legume components of grass mixtures, the common vetch and red clove are more sensitive to excess iodine.

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