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THE WEED SEED BANK ASSESSMENT IN TWO SOIL DEPTHS UNDER VARIOUS MINERAL FERTILISING

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Abstract

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The field trial at the experimental station of Slovak Agricultural University in Nitra - Kolíňany (Slovak Republic, maize growing region, Haplic Luvisol and Stagni-Haplic Luvisol) in 1997 year was established. Experiments were based on 14 ha area (424 x 432.2 m) by long strips method. The impact of different mineral fertilisers on six model crops was observed: winter wheat, spring barley, sunflower, winter oilseed rape, maize and sugar beet. Weed infestation of winter wheat, spring barley, maize and sugar beet as well as weed seed bank composition since 2000 year till 2002 year were detected. Three variants of mineral fertilisation were applied: variant 1 – without fertilisers, variant 2 – N-P-K fertilisation, steady state soil nutrients balance, variant 3 – high doses of N-P-K fertilisers (positive soil nutrients balance). Soil weed seed bank was analysed once per year before crop germination (on February) from depths 0–0.05 m and 0.20–0.25 m in five replicates. From the depth 0–0.05 m 26 weed species were found, from the depth 0.20–0.25 m 23 weed species, from late spring group mainly. *Chenopodium album, Stellaria media* and *Amaranthus* spp. (77.57 % from intact seeds in total) were the most occurred weeds in both depths. The year, depth of soil sampling and fertilisation did not have statistically significant impact on weed seeds number in the soil.

weeds, mineral fertilising, weed seed bank

Weedy plants overcome spatial and time distances in the fruits and seeds form; thereby, they determinate the state and weed infestation changes on arable land. The increase of weed population growth is conditioned by weed seeds and fruits input inside of arable land. It is accomplished by weed seeds dropping on concrete site, by seeds from organic fertilisers, by seeds for sowing and by uncontrollable inputs (wind, undomesticated animals). The reduction of weed infestation is given by decrease of dropping weed seeds on concrete site, by weed suppression and control, by increase of soil's self-cleaning ability and by weed seeds minimizing in materials that transport seeds (organic fertilisers, seeds for sowing). Weed infestation decreases when the reduction of seeds exceeds their entry into the soil, only. Viable seeds in the soil's reserve (i.e. weed seed bank) are the first assumption of actual weed infestation (Dvořák, Smutný, 2003).

Weed fruits and seeds are a viable reservoir in the upper part of the soil profile, which determinates the composition of weed flora in the concrete region (Caetano *et al.,* 2001). Management of agricultural systems has immediate and long-lasting effects for elements of weed coverage, population and diversity. Development of programs for integrated weed control requires a clear understanding of factors and mechanisms determinative the weed community dynamics in agroecosystems (Menalled *et al.,* 2001).

The goal of this work was to discover the impact of mineral fertilisation, years of research, and the depth of weed seed offtakes on weed seed soil bank in Kolíňany locality.

MATERIALS AND METHODS

A field experiment using a method of long belts was established in 1997 on experimental basis at Slovak Agricultural University (SUA) in Nitra – Kolíňany (university agricultural farm). The experiment was conducted on the base of a contract between the Duslo Šaľa, P.L.C. (mineral fertilisers producer) and the Department of Agrochemistry of the SUA in Nitra. The main goal of the experiment was to observe the effect of mineral fertilisers (N-P-K, without organic fertilising) on six model crops yield (winter wheat, spring barley, silage maize, sugar beet, winter oilseed rape and sunflower). The pilot experiment was established on Haplic Luvisol and Stagni-Haplic Luvisol with the various humus horizon thickness (from 0.23 m to 0.45 m) and with different depths of ground water (2.0 m at the top of the slope and 0.7 m–1.0 m in the valley below the slope) in the maize growing region, 12 km east of Nitra on 14 ha area (324 × 432.2 m). Each nutrition option was assigned on 0.54 ha area (18 m × 300 m); the area for one crop was 2 ha. Weed infestation of winter wheat, spring barley, maize and sugar beet as well as and weed seed bank since 2000 year till 2002 year were investigated. There were three mineral fertilising options: variant 1 – not fertilised (control = a negative balance of nutrients in the soil, e.g. 0 kg N, P, K); variant 2 – N-P-K fertilising according to the plant's requirements (steady balance of nutrients in the soil = fertilising as if for 5 t yield of cereal), variant 3 – high doses of N-P-K fertilisers (positive balance of nutrients in the soil = fertilising as if for 8 t of cereal). Concrete doses of mineral nutrients on variants 2 and 3 were applied as follows (per 1 ha in pure nutrients altogether): Variant 2 – winter wheat: 77.6 kg N, 25 kg P, 100 kg K; spring barley: 32.6 kg N, 25 kg P, 100 kg K; maize: 123.6 kg N, 31.2 kg P, 138 kg K; sugar beet: 54.5 kg N, 28 kg P, 120 kg K. Variant 3 – winter wheat: 120.1 kg N, 40 kg P, 160 kg K; spring barley: 55.1 kg N, 40 kg P, 160 kg K; maize: 199 kg N, 52 kg P, 230 kg K; sugar beet: 103.8 kg N, 45.5 kg P, 195 kg K.

The weed seed soil bank was analysed during 2000–2002 years once annually before crops germination (in February) from the same plot (plot No. 2, the top of the slope). Crops rotation on this plot was following: winter wheat (1999), maize for silage (2000), spring barley (2001), sugar beet (2002). Soil samples were taken by Kopecký cylinders, from the depth 0–0.05 m and 0.20–0.25 m for each fertilising variant in five repetitions and later air-dried. Before each one's washing, the samples were first covered with water for 24 hours in order to thoroughly disaggregate the soil. Next, they were washed through mesh screens with sieve meshes with 0.25 mm and 1 mm of a diameter. The portions washed out from both sieves were flushed into glass funnels lined with paper filters inside by distilled water. Individual particles from paper filters were dried and then placed under a binocular magnifying glass with magnification of 15–25 times. All undamaged weed seeds (or fruits) were selected by tweezers. The following literature based on plant seed and fruit designation was used: Lhotská, 1957; Deyl, Ušák, 1964; Lhotská *et al.*, 1984. Botanical terminology according to Marhold, Hindák (1998) and weed species classification into biological groups and their agricultural impact by Hron, Vodák (1959) were used (table I). Symbol "+" = low agricultural impact (less important weeds), "++" = middle agricultural impact (less dangerous weeds), "+++" = high agricultural impact (very dangerous weeds, Hron, Vodák, 1959).

Controversial weed species were determined with the aid of comparative plant seeds collection deposited at the Slovak Academy of Sciences in Archeological Institute in Nitra (Slovakia). The amounts of these seeds were recalculated for 1 m^2 area to the depth of 0.05 m (i.e. 0.05 m^3).

For the statistical evaluation of weed seed bank data, the analysis of variance (ANOVA, LSD test) was used, which is part of the STATGRAPHIC, program package version 5.0. Three parameters were statistically evaluated by using the ANOVA – LSD test: the year, fertilising, and depth. The third parameter – depth (Fig. 1) – was also examined further by the CANOCO version 4.5 multidimensional analysis of ecological data, also called the Monte-Carlo permutation test.

RESULTS AND DISCUSSION

During the three-year investigation period (2000–2002) 90 soil samples were taken from the field experiment (plot No. 2) in total from two depths and from each fertilising treatment. 29 weed species in weed seed soil bank were found belonging to eight biological weed groups. 25 species of them corresponded with weeds identified in above-ground flora (59 weed species were recorded here wholly). Tab. I shows their summary, amount and ranking into biological groups.

984 unharmed pieces of weed seeds (or fruits) were found at the depth 0–0.05 m by using the binocular magnifying glass. These pieces belonged to 26 species from six biological weed groups. At a depth 0.20–0.25 m, 1242 undamaged seeds (or fruits) were found appertain to 23 species together from five biological groups (Tab. I). In both depths late spring species of weeds prevailed – the dominating species was *Chenopodium album*. The second most common biological group was winter weeds, with the most frequent weed *Stellaria media*. *Amaranthus* spp., as a late spring weeds, were the third most common genus. *Amaranthus retroflexus*, *Amaranthus powelii* and their crossbreeds with *Amaranthus* spp. as arable crop, also occurred at the locality of this experiment. Therefore, their seeds were very difficult to identify. By reason of that we classified them as the *Amaranthus* spp. – genus – only, so that we avoided any possible confusion.

The recomputation of weed seeds (fruits) pieces per 1 m^2, differences between individual observed years, options of fertilising, and depths of extraction are displayed in Tab. II and in Fig. 1. Within the number of seeds evaluation, none of the parameters – year of observation, fertilising, depth – was statistically significant. Also, differences between individual levels of these three parameters were not significant (limit difference test: $Hd_{0.05}$, Tab. III, Fig. 1). *Chenopodium album*, *Stellaria media* and *Amaranthus* spp. created 77.57% of the weed seed soil bank together.

Hron, Kohout (1988), Slawinski, Golabek (2010) point out that *Chenopodium album* especially is the most widespread weed in the soil bank of weed seeds, where it commonly makes up more than 50% of this supply. This fact was fully confirmed at the site in our experiment. One single plant can give above 100 000 seeds with various lengths of dormancy and irregular germinability. They germinate and emerge the best from the topsoil or from a depth of 0.01 to 0.02 m and remain active and alive in the soil more than 40 years (Líška *et al*., 1995). Deyl, Ušák (1964) found that the ability to germinate is at least 10 years when the seeds are stored in a dry place; if they are stored in the soil, it is more than 10 years. The winter frost significantly increases germinability, which is given by the bursting of the hard seed coat. Because of a short vegetative period, *Chenopodium album* easy emerges from soil after the weakening of herbicidal effects.

Stellaria media has a very short vegetative period and year-round blooms, even during mild winters. Several thousand seeds ripen on one single plant

I: *List of weed species from weed seed soil bank (experimental station Kolíňany, Duslo Šaľa, P.L.C.) during 2000–2002 years*

English name	Scientific name	Agricult. impact	EWRS Codes	Number of seeds (pcs) (0–0.05m)	Number of seeds (pcs) (0,2–0.25m)	% share from total number	Biolog. group
Ivy-leaved Speedwell	*Veronica hederifolia* L.	+	VERHE	10	15	1.12	E
Common Field-Speedwell	*Veronica persica* Poir.	+	VERPE	1	0	0.04	E
Scarlet Pimpernel	*Anagallis arvensis* L.	++	ANGAR	4	3	0.31	ES
Black-bindweed, Wild Buckwheat	*Fallopia convolvulus* (L.) Á. Löve	+++	POLCO	2	3	0.22	ES
Knotgrass, Knotweed prostrate	*Polygonum aviculare* L.	++	POLAV	1	2	0.13	ES
Yellow Charlock	*Sinapis arvensis* L.	+++	SINAR	1	1	0.09	ES
Pigweed	*Amaranthus* spp.	+++	AMAXX	77	66	6.42	LS
Fat-hen	*Chenopodium album* L.	+++	CHEAL	540	704	55.88	LS
Sowbane	*Chenopodium hybridum* L.	+++	CHEHY	4	6	0.45	LS
Many-seeded Goosefoot	*Chenopodium polyspermum* L.	+++	CHEPO	33	27	2.70	LS
Barnyard grass, Cockspur grass	*Echinochloa crus-galli* (L.) P. Beauv.	+++	ECHCG	0	1	0.04	LS
Soft needle-leaf	*Polycnemum arvense* L.	+	PCNAR	37	40	3.46	LS
Pale Persicaria, Smartweed, Pale	*Persicaria lapathifolia* (L.) Gray	++	POLLA	21	10	1.39	LS
Smooth Sow-thistle	*Sonchus oleraceus* L.	++	SONOL	1	3	0.18	LS
Madwoman's Milk, Sun Spurge	*Tithymalus helioscopia* (L.) Scop. L. A.	++	EPHHE	2	0	0.09	LS
Common Chickweed	*Stellaria media* (L.) Vill..	++	STEME	134	206	15.27	W
Silky-bent grass, Loose Silky-bent	*Apera spica-venti* (L.) P. Beauv.	+++	APESV	32	43	3.37	W
Thymeleaf sandwort	*Arenaria serpyllifolia* L.	+	ARESE	1	1	0.09	W
Royal knight's-spur, Branching Larkspur	*Consolida regalis* S. F. Gray	++	CONRE	1	0	0.04	W
Henbit Dead-nettle	*Lamium amplexicaule* L.	++	LAMAM	11	26	1.66	W
Common Poppy, Corn Poppy	*Papaver rhoeas* L.	+++	PAPRH	2	2	0.18	W
Field Penny-cress	*Thlaspi arvense* L.	++	THLAR	2	0	0.09	W
Scentless Mayweed	*Tripleurospermum perforatum* Mérat (M.) Laínz	+++	MATIN	8	32	1.80	W
Field Pansy, Violet	*Viola arvensis* Murray	++	VIOAR	10	10	0.90	W
Curled Dock	*Rumex crispus* L.	+++	RUMCR	1	0	0.04	BP
Wild mint, Field mint, Corn mint	*Mentha arvensis* L.	++	MENAR	1	0	0.04	PSR
Codlins And Cream	*Epilobium hirsutum* L.	++	EPIHI	0	1	0.04	PSR
Canada Thistle, Creeping Thistle	*Cirsium arvense* (L.) Scop.	+++	CIRAR	47	39	3.86	PDR
White Dutch Clover	*Trifolium repens* L.	++	TRFRE	0	1	0.04	VC
Key: E – ephemeral weeds, ES – early spring weeds, LS – late spring weeds, W – winter weeds, BP – biennial and perennial weeds, PSR – perennial weeds with shallow roots, PDR – perennial weeds with deep roots, VC – volunteer crops			Total	984	1242	100	8 groups

II: *Weed seeds (pieces) at the depths 0–0.05 m and 0.2–0.25 m and at three variants of fertilising during 2000–2002 years (recalculation to 1 m² to the depth 0.05 m)*

Year	2000	2001	2002	2000	2001	2002	Total	Total	Total
Depth (m)	**0–0.05**	**0–0.05**	**0–0.05**	**0.2–0.25**	**0.2–0.25**	**0.2–0.25**	**0–0.05**	**0.2–0.25**	**(both depths)**
Variant 1	98	78	181	118	86	99	357	303	660
Average	19.6	15.6	36.2	23.6	17.2	19.8	71.4	60.6	132
Average per 1 m²	9 800	7 800	18 100	11 800	8 600	9 900	35 700	30 300	66 000
Variant 2	96	131	74	104	354	90	301	548	849
Average	19.2	26.2	14.8	20.8	70.8	18	60.2	109.6	169.8
Average per 1 m²	9 600	13 100	7 400	10 400	35 400	9 000	30 100	54 800	84 900
Variant 3	125	94	107	135	137	119	326	391	717
Average	25	18.8	21.4	27	27.4	23.8	65.2	78.2	143.4
Average per 1 m²	12 500	9 400	10 700	13 500	13 700	11 900	32 600	39 100	71 700

III: *Statistical evaluation of the number of weed seeds in soil bank (by year, fertilising and depth) in 2000–2002 years (test H_d 0.05)*

Source of variability	Sum of squares	Degrees of freedom	Mean of squares	F- ratio	Level of signifi-cance	Signifi-cance	Levels of factors	Number of seeds	
								$Hd_{0.05}$	Homogeneous groups
Year (A)	2.3820×10^8	2	1.1910×10^8	1.116	0.3330	-	Year 2000	5314.32	x
Fertilising (B)	1.5665×10^8	2	7.8325×10^7	0.734	0.4835	-	Year 2001	5314.32	x
Depth (C)	1.8490×10^8	1	1.8490×10^8	1.732	0.1921	-	Year 2002	5314.32	x
Interaction AxB	1.4938×10^9	4	3.7345×10^8	3.498	0.0112	*	Fertilising 1	5314.32	x
Interaction AxC	4.7707×10^8	2	2.3853×10^8	2.235	0.1140	-	Fertilising 2	5314.32	x
Interaction BxC	3.8302×10^8	2	1.9151×10^8	1.794	0.1733	-	Fertilising 3	5314.32	x
Uncontrollable factor	8.1128×10^9	76	1.0675×10^8				Depth 1	4339.13	x
Total (edited)	1.1046×10^{10}	89					Depth 2	4339.13	x

* means significant (P=0,95)

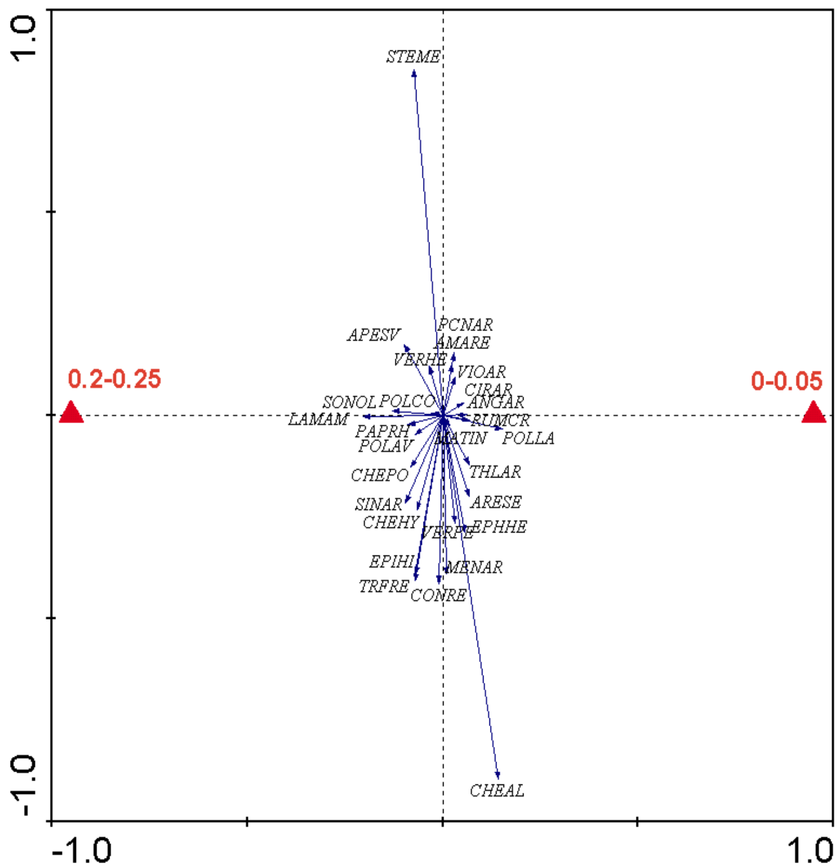
and then drop out gradually. The seeds have irregular germinability and their emergence from the topsoil and upper layers of the soil is basically continuous during the entire year. Seeds retain their viability in the soil for several years; Líška *et al.* (1995) report to 2–4 years. Deyl, Ušák (1964) state that seeds retain their germinability for up to 8 years in a dry place storage and 5 years in the soil storage. They spread easily and accidentally after the reduction of herbicidal effect (Hron, Kohout, 1988).

Amaranthus retroflexus is known for its extraordinary seed creation, where the number of seeds on one plant can reach several hundred thousand; according to Líška *et al.* (1995), the seeds come to between 0.5 – 1 million pieces. These seeds germinate the best at a depth of approximately 0.01 m to 0.02 m, even from the direct surface of the soil. Right after their ripening, they have lower germinability. The seeds retain viability of 1 to 10 years, even longer. A substantial share of seeds germinate or lose viability in the nearest three years.

Four weed species were found in the weed seed soil bank not recorded in the above-ground flora - *Arenaria serpyllifolia*, *Mentha arvensis*, *Trifolium repens* and *Polycnemum arvense*. While the findings of the first three mentioned weeds were sporadic, the finding of *Polycnemum arvense* was interesting (the fifth most common species). It represents a residue of an older weed seed soil supply obviously. It is a thermophilic species, relatively rare, appears in sparse or incomplete stands (Deyl, Ušák, 1964).

The vegetation composition develops on site depends on the related species ratio in the soil seed supply and of their germination characteristics (Roberts, 1986, in Šeffer, Jarolímek, 1988). If the soil is regularly cultivated to a specific depth, weed seeds have a tendency to spread uniformly (Roberts, 1981, in Šeffer, 1990). After the soil ploughing they can be concentrated in one layer (Šeffer, 1990), where they do not germinate and become dormant (Winkler, Zelená, 2001).

De Cauwer *et al.* (2010) state that weed seed bank density and composition is related to mineral fertilisation, by mineral nitrogen mainly. It was not confirmed in a case of Kolíňany locality. In a field experiment in England with weed seeds sowing into various soil depths, larger and heavier seeds germinated, e.g. *Veronica hederifolia* emerged from deeper soil layers (0.08 m). On the other hand, field germination of smaller and slighter seeds



1: *Ordination diagram of spatial analyses of weed seeds (or fruits) in the soil bank in two depths (0–0.05 m and 0.2–0.25 m) during the years 2000–2002*
Comment: To identify individual weed species according to EWRS codes, please use Tab. I.

(e.g. *Matricaria maritima* subsp. *inodora* and *Veronica arvensis*) decreased rapidly after exceeding a sowing depth of 0.01 m. Mutual relationships between size, shape, and seed germination are complex and specific to each weed (Grundy *et al.*, 2003). Regarding to the viability of weed seeds taken from the soil, it is radically shorter than that one of seeds left in the ground (Demo, 1999). Similarly, Sahoo (1998) found that weed seeds placed in the top layer of soil lost their viability in germination much faster than seeds placed into deeper soil. Swanton *et al.* (2000) discovered that the vertical distribution of the seed soil bank was affected by the soil tillage system, ploughing depth, and the soil type (sandy soil). Caetano *et al.* (2001) confirmed that the qualitative and quantitative distribution of the weed seed soil supply is dependent on cultivation practices in specific region, mainly. Ortega *et al.* (2003) recorded a large variability of the weed seed soil bank at a depth of up to 0.025 m in the time of germination, in viability and dormancy based on weed species, too. Its spatial variability was connected with the soil characteristics. Furthermore, Tyšer (2002) proved a statistically significant fluctuation of the weed seed soil supply within one vegetative period. During the autumn sampling of soil, a larger amount of whole weed seeds (alive and dead) in the soil supply was documented compared to the summer samplings. It was about 46% higher in the area with root crops growing and about 21% higher in the area with clover crops growing.

CONCLUSIONS

The results from investigation weed seed soil bank after collecting data for three years can not be evaluated exactly. 25 weed species were found in the soil bank from 59 detected weed species in the above-ground flora, only. The reasons could be various – the higher soil self-cleaning ability due to its tillage, by insects, birds, or rodents seeds consuming, or by the ability of some weed seeds to germinate soon after semination. Dvořák, Krejčíř (1976) support the opinion that there is no equal relationship between the weed seed soil bank and the actual weed infestation. A high soil weed seed supply of a certain species does not have to manifest itself across strong occurrence on crop stand and vice versa. The percentual representation of a certain species of seeds in the soil bank at the site is usually not correlated with the representation of the same species in actual weed infestation. The authors (Dvořák, Krejčíř, 1976; Dvořák, Smutný, 2003) confirmed a large variability in the horizontal and vertical stratification of weed seeds in the soil. Similarly, species diversity in the soil seed bank agrees with the species spectrum of the above-ground flora partly, only.

SUMMARY

The field trials at the experimental station of Slovak Agricultural University (SUA) in Nitra – Kolíňany (Slovak Republic) with cooperation of mineral fertilisers producer (DUSLO Šaľa, P.L.C.) in autumn 1997 were established by long strips method. The influences of various doses of mineral fertilisers only (N-P-K, without organic fertilisers) on six model crops: winter wheat, spring barley, sunflower, oil seed rape, maize and sugar beet were observed. Weed infestation of winter wheat, spring barley, maize and sugar beet and weed seed bank since 2000 till 2002 years were investigated. The whole area of experimental station was 14 ha (324 × 432.2 m), situated on maize growing region 12 km east of Nitra, based on Haplic Luvisol and Stagni–Haplic Luvisol with different thickness of humus horizon (from 0.23 m to 0.45 m) and different level of underground water (2.0 m on slope and 0.7–1.0 m in valley). The area for each crop was 2 ha, each treatment of fertilising had 0.54 ha (300 × 18 m). Three treatments of mineral fertilising were observed: treatment 1 – zero doses of mineral N-P-K fertilisation (negative nutrient balance in soil), treatment 2 – mineral N-P-K fertilisation in accordance of crop needs (equable nutrient balance in soil, e. g. = equivalent of 5 $t.ha^{-1}$ grain yields), treatment 3 – high doses of mineral N-P-K fertilisation (positive nutrient balance in soil, e. g. = equivalent of 8 $t.ha^{-1}$ grain yields). Weed seed soil bank was determined once a year before crop germination (February) from the depths 0–0.05 m and 0.20–0.25 m in each fertilisation treatment, with five replications. In the depth 0–0.05 m 26 weed species from 6 weed biological groups were found, in 0.20–0.25 m 23 weed species from 5 biological groups, from „the late-spring weeds“ group mainly. The dominant weed species from all 2226 pieces of intact seeds in both depths were *Chenopodium album, Stellaria media* and *Amaranthus* spp. (77,57% of total weed seed amount). Three parameters were observed with ANOVA – LSD-test: year, fertilisation and depth. The third parameter – depth was also assessed by multivariate analysis of ecological data – CANOCO, 4.5 version, by Monte-Carlo permutation test. None of these parameters was statistically significant.

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