

## Changes in weed community composition in a long-term trial with different crop rotations and herbicide treatments

Langzeitstudie über Veränderung der Unkrautflora unter dem Einfluss von Fruchtfolgen und Herbizidbehandlungen

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### Abstract

The impact of herbicide application on weed community changes was studied in a long-term field experiment conducted since 1972 at two sites in the Czech Republic; Pernolec and Hněvčeves. The ongoing trial comprises multi-crop and simple crop rotations with 50 and 75% cereals, respectively. Three herbicide treatments were used: (1) untreated; (2) synthetic auxins (MCPA; 2,4-D; only in simple crop rotation) and (3) targeted herbicide combinations, including especially sulfonylureas, triazines, ureas and synthetic auxins. Weed species composition and weed density were assessed at the trial beginning, during the trial and in the present (2013-2016). Changes in weed flora composition were found out. In Hněvčeves, abundance of some species such as *Galium aparine*, *Stellaria media*, and *Vicia* spp. increased on untreated plots; the abundance of *Apera spica-venti*, *Fumaria officinalis*, and *Tripleurospermum inodorum* increased on treated plots. In Pernolec, some species receded in all variants, e.g. *Myosotis arvensis*, *Raphanus raphanistrum*, and *Scleranthus annuus*; the abundance of *Centaurea cyanus*, *Tripleurospermum inodorum*, *Veronica* spp., and *Spergula arvensis* increased in untreated plots. No significant differences affected by time and treatment were detected in population densities of *Apera spica-venti*, *Arabidopsis thaliana*, and *Stellaria media*. The species composition of the weed community was affected by explanatory variables in the following order: treatment < time < type of cultivated crop. The long-term study confirmed weed population shifts over time were caused by interaction between the management factors and environmental conditions.

**Keywords:** Crops, field trial, herbicides, long-term, weed changes

### Zusammenfassung

Der Einfluss von Herbizidapplikationen auf die Unkrautartenverteilung wurde seit 1972 in einer Langzeitfeldstudie auf zwei Standorten in Pernolec und Hněvčeves, Tschechische Republik, untersucht. Der Versuch beinhaltet eine blattfruchtbetonte und eine getreidebetonte Fruchtfolge mit unterschiedlich hohen Getreideanteilen von 50 % beziehungsweise 75 %. Drei Herbizidbehandlungen wurden untersucht: (1) unbehandelt, (2) synthetische Auxine (MCPA, 2,4-D, nur in der getreidebetonten Fruchtfolge), (3) gezielte Herbizidapplikationen insbesondere Sulfonylharnstoffe, Triazine, Harnstoffe und synthetische Auxine. Sowohl die Zusammensetzung der Unkrautspezies als auch die Unkrautdichte wurden zu Beginn, während des Versuchs und detaillierter in den Jahren 2013-2016 ermittelt. Es wurden Veränderungen in der Zusammensetzung der Unkrautflora festgestellt. In Hněvčeves erhöhte sich die Häufigkeit einiger Arten wie *Galium aparine*, *Stellaria media* und *Vicia* spp. in den unbehandelten Parzellen. Wohingegen sich hier die Häufigkeit von *Apera spica-venti*, *Fumaria officinalis* und *Tripleurospermum inodorum* in den behandelten Parzellen erhöhte. In Pernolec nahm die Pflanzendichte einiger Arten (z. B. *Myosotis arvensis*, *Raphanus raphanistrum*, *Scleranthus annuus*) über alle Varianten hinweg ab. Die Häufigkeit der Arten *Centaurea cyanus*, *Tripleurospermum inodorum*, *Veronica* spp. und *Spergula arvensis* erhöhte sich in den unbehandelten Parzellen in Pernolec. Es wurden keine signifikanten Effekte durch Zeit und Behandlung auf die Populationsdichte von *Apera spica-venti*, *Arabidopsis thaliana* und *Stellaria media* festgestellt. Die Zusammensetzung der Unkrautspezies wurde zunehmend durch die Variablen Behandlung, Zeit und Typ der Kulturpflanze beeinflusst. Diese Langzeitstudie bestätigt Veränderungen in der Unkrautpopulation über die Zeit durch Interaktion zwischen Managementfaktoren und den Umweltbedingungen.

**Stichwörter:** Feldfrüchte, Feldversuch, Herbizide, Langzeitstudie, Veränderung der Unkrautflora

### Introduction

Weed communities on arable land have been influenced by environmental factors (soil and climatic conditions) and farming practices (crop type, soil cultivation, weed control methods)

(LÉGERE et al., 2005; POTTS et al., 2010). The weed flora has changed in response to agricultural intensification accompanied with simplification of crop rotations (STOATE et al., 2001; MEYER, 2013) and excessive herbicide and fertilizer uses (STORKEY et al., 2010; SALONEN et al., 2012). Many surveys in various countries analyzed the relative importance of particular management factors on weed species composition (HYVÖNEN et al., 2003; STREIT et al., 2003; KELLER et al., 2004; ANDREASEN and STREIBIG, 2010). Some works suggested crop type as the major factor determining species composition, which is related to different farming practices in various types of crop (FRIED et al., 2008; LOSOSOVÁ and CIMALOVÁ, 2009; PINKE et al., 2012; NOWAK et al., 2015). Widespread use of herbicides has had the significant impact on weeds in the last 50 years. In general, repeated use of a single mode of action has resulted in weed communities shifting to an occurrence of herbicide-tolerant species (MARSHALL et al., 2003; MURPHY and LEMERLE, 2006). Available herbicides considerably changed over time and these changes had become a key factor in altering the abundance of weed population. Changes in chemical weed control contributed to less diversity of weed community and proliferation of tolerant species (MIKULKA et al., 2009; GRUNDY et al., 2010; MEYER, 2013). However some studies reported inconclusive effect of herbicide treatment on weed species diversity (DERKSEN et al., 1995; MAYOR and DESSAINT, 1998).

Although many works assessed the effects of herbicides on weed communities (DERKSEN et al., 1995; GRUNDY et al., 2009), weed shifts are rarely studied in the long-term. That is because of problems with determining history of changes in management practices and wide spectrum of active ingredients of herbicides (FRIED et al., 2008; PINKE et al., 2012; DE MOL et al., 2015). The objective of this study was to assess the long-term changes of weed community in the long-term experiment as a response to herbicide treatment and cultivated crops. We compared integrated approach of weed management (multi-crop rotation, targeted herbicide use) with less diversified approach: a) low input system with simple crop rotation and use of low-cost, mostly auxinic herbicides and b) simple crop rotation but with targeted herbicide use. The aim of this study was to determine long-term shifts of weed populations caused by herbicide application and cultivated crops.

## Materials and Methods

### Experimental sites

The long-term field trial has been conducted since 1972 at two experimental stations of the Crop Research Institute in the Czech Republic: Hněvčeves (50.31487 N, 15.71612 E) and Pernolec (49.77053 N, 12.68099 E). The site characteristics are listed in Table 1.

**Tab. 1** Experimental site characteristics.

**Tab. 1** Beschreibung der Forschungsstandorte.

Experimental site	Growing region	FAO Classification	Altitude (m)	Average annual temperature (°C)	Average annual rainfall (mm)
Hněvčeves	sugar beet	Haplic Luvisol on loess	265	8.2	573
Pernolec	potato	Cambisol on orthogneiss	530	7.1	559

The trial was established in two different crop rotation systems (CR) with specific cereal percentages: (1) multi-crop CR (MCR) with 50% cereals and 50% broadleaved crops and (2) simple CR (SCR) with 75% cereals and 25% legumes. Three herbicide treatments were used: (1) untreated control; (2) treatment by synthetic auxins in simple crop rotation (MCPA; 2,4-D in cereals and bentazone in peas) and (3) treatment by targeted herbicide combinations according to observed weed infestation. These combinations included different sulfonylureas, triazines, ureas and synthetic auxins during the trial period. The experiment was carried out as a split-plot design with 5 combinations of crop rotations (main plots) and treatments (sub-plots): MCR\*untreated,

MCR\*targeted, SCR\*untreated, SCR\*auxins and SCR\*targeted; with 4 replications of each combination. The 100m<sup>2</sup> (10m by 10m) plots were established 10m from field boundaries and separated from each other by 1 to 2m on all sides to eliminate interaction between treatments. Uniform practices of soil tillage, fertilization and fungicide treatment accompanied weed management on each plot.

#### Assessments and statistical analysis

Weed species composition and weed density were assessed at the beginning of the trial, during the trial and in the present (2013-2016). Weed data were recorded at 2 to 6-leaf stage of the crop before herbicide application. Individual plants in each plot were counted in four 0.25 m<sup>2</sup> random sampling squares (0.5m by 0.5m). Densities in the four squares were aggregated to provide 1m<sup>2</sup> samples. Headlands and plot edges were excluded from sampling. Weed species were identified at the species level when possible; some species were identified at the level of the genus (e.g. *Veronica* spp.). Botanical nomenclature was adapted according to KUBAT et al. (2002).

Multivariate analyses in the CANOCO 5 software (TER BRAAK and ŠMILAUER, 2012) were performed for data exploration. Prior to the analysis logarithmic transformation of data was made. Due to gradient length on the first canonical axis in the compositional turnover in Detrended Correspondence Analysis (DCA), optimal ordination method was selected. If values were more than 4.0 SD units, unimodal Canonical Correspondence Analysis (CCA) was used. In case of values below 3.0 SD units, linear Redundancy Analysis (RDA) was used. Both methods could be used for values 3-4.0 SD units. Three explanatory variables were compiled: time (year), crop type (winter cereals, spring cereals, legumes, and root crops), treatment (untreated, treatment by auxins, targeted treatment by combination of herbicides). Gross and net effects of explanatory variables on weed species composition were tested using Monte-Carlo permutation tests for 999 permutations at P=0.05 significance level, following the methodology of LOSOSOVÁ et al. (2004). The gross effect was tested using separate CCAs or RDAs with single explanatory variables. The net effect was tested using partial CCAs or RDAs with a single explanatory variable and other variables as covariates. The ratio of particular canonical eigenvalues to the sum of all eigenvalues was used to measure the proportion of explained variation. Rare species were downweighted in case of CCA. Complete analysis was carried out only for Pernolec. At Hněvčeves, the comparison of initial and present situation was performed due to lack of some continuous data from the trial.

## Results and discussion

### Weed density

At trial commencement in 1972, the average weed density and number of different weed species before herbicide treatment in winter wheat were 198 plants m<sup>-2</sup> of 21 species at Pernolec and 86 plants m<sup>-2</sup> of 11 species at Hněvčeves. In total, 53 different species at Pernolec and 29 at Hněvčeves were identified over the experimental period.

The application of herbicides decreased the weed density (Tab. 2). The average weed density was significantly higher in untreated plots than in treated plots at both localities and crop rotations. Simultaneously simple crop rotation evinced higher weediness than multi-crop rotation in both, untreated plots and targeted treatment plots. It is in agreement with KOOCHEKI et al. (2009) who found out that rotation of sugar beet-winter wheat caused 28% reduction in the weed seed bank compared to continuous wheat. Treatment by synthetic auxins affected a limited weed spectrum; therefore infestation was higher than at targeted treated plots.

**Tab. 2** Current weed density before herbicide treatment in winter wheat; the last assessment in 2016 (Hněvčeves), in 2014 (Pernolec, MCR) and 2015 (Pernolec, SCR). Average number of plants m<sup>-2</sup> (Avg.) ± standard deviation (SD). SCR - simple crop system, MCR - multi-crop system.

**Tab. 2** Unkrautdichte vor Herbizidbehandlungen in Winterweizen; letzte Bonitur 2016 (Hněvčeves), 2014 (Pernolec, MCR) und 2015 (Pernolec, SCR). Pflanzen je m<sup>2</sup> (Avg.) ± Standardabweichung (SD). MCR – blattfruchtbetonte Fruchtfolge, SCR - getreidebetonte Fruchtfolge.

Variant/locality	Hněvčeves		Pernolec	
	Avg.	SD	Avg.	SD
untreated SCR	221	34.9	186	37.5
auxins SCR	201	9.3	152	21.6
targeted SCR	163	17.9	104	10.4
untreated MCR	209	16.5	152	39.1
targeted MCR	155	22.4	16	7.8

### Explanatory variables

All variables (crop type, time, treatment) together explained 24.9% and 32.2% of the total variation in weed species data at simple and multi-crop system, respectively. Unexplained variation was associated with other factors, probably the environmental conditions.

**Tab. 3** Gross and net effects of explanatory variables and their interactions (\*) on the weed species composition in locality Pernolec. (Partial) CCAs were used for SCR; (partial) RDAs were used for MCR. Eigenvalue = sum of all canonical eigenvalues (total inertia for SCR = 2.53; for MCR=1.0); % = percentage of explained variance; F = ratio for the test of significance of all canonical axes; P-value = corresponding probability value obtained using the Monte-Carlo permutation test. SCR = simple crop rotation, MCR = multi crop rotation.

**Tab. 3** Brutto- und Nettoeffekte erklärender Variablen und deren Interaktionen (\*) auf die Unkrautartenzusammensetzung am Standort Pernolec. (Partielle) kanonische Korrespondenzanalyse wurde für SCR, (partielle) lineare Redundanzanalyse wurde für MCR benutzt. Eigenvalue= Summe aller kanonischen Eigenwerte (Gesamtvariabilität für SCR = 2.53; für MCR = 1.0); % = prozentualer Anteil an der Gesamtvariabilität; F = Verhältnis des Signifikanztests der kanonischen Achsen; P = durch Monte-Carlo-Permutations-test ermittelter zugehöriger Wahrscheinlichkeitswert; MCR = blattfruchtbetonte Fruchtfolge, SCR = getreide-betonte Fruchtfolge.

Explanatory variables	SCR				MCR			
	Eigenvalue	%	F	P	Eigenvalue	%	F	P
All factors	0.63	24.9	10.71	0.001	0.32	32.2	9.3	0.001
<b>Gross effects</b>								
crop type	0.34	13.4	12.8	0.001	0.18	17.7	7.2	0.001
time	0.26	10.4	19.24	0.001	0.15	14.9	16.5	0.001
treatment	0.03	1.1	0.91	n.s.	0.03	3.0	3.2	0.004
<b>Net effects</b>								
crop type	0.34	13.3	14.37	0.02	0.14	14.25	7.2	0.001
time	0.26	10.3	22.31	0.001	0.11	11.4	16.5	0.001
treatment	0.03	1.1	1.19	n.s.	0.03	3.0	5.1	0.001
treatment*time	0.02	1.0	0.79	n.s.	0.01	1.8	1.8	0.034
treatment*crop type	0.03	1.2	0.58	n.s.	0.02	2.4	1.2	n.s.
time*crop type	0.19	7.5	8.9	n.s.	0.11	11.7	10.1	0.001

Study of PINKE et al. (2012) suggested the environmental variables accounted for two times more variance than management variables. DE MOL et al. (2015) showed variation in species composition was more related to environmental factors (9.1% explained variance) than management factors (4.7% explained variance). On the contrary CIMALOVÁ and LOSOSOVÁ (2009) revealed that on regional scale, the relative importance of different crop types and their associated management was higher than climatic variables. The amount of variation in weed species data explained by net effects of particular variables, as detected by partial CCAs (for SCR) and RDAs (for MCR), was the highest for crop type (Tab. 3). Many studies confirmed a strong impact of crop type on species composition (FRIED et al., 2008; PINKE et al., 2012; NOWAK et al., 2015). Unlike our findings, MOL et al. (2015) found

out the small contribution of the factor year to the variance of species composition. Treatment evinced the smallest or insignificant effect. It is agreement with DE MOL et al. (2012) who did not find a correlation between weed species composition and the applied herbicides. Unlike that, STREIT et al. (2003) confirmed the significant effect of herbicide application on weed population. Effect of herbicide treatment on weed species in long-term experiments may be buffered thanks to persistent soil seedbank (HYVÖNEN and SALONEN, 2001).

### Species composition

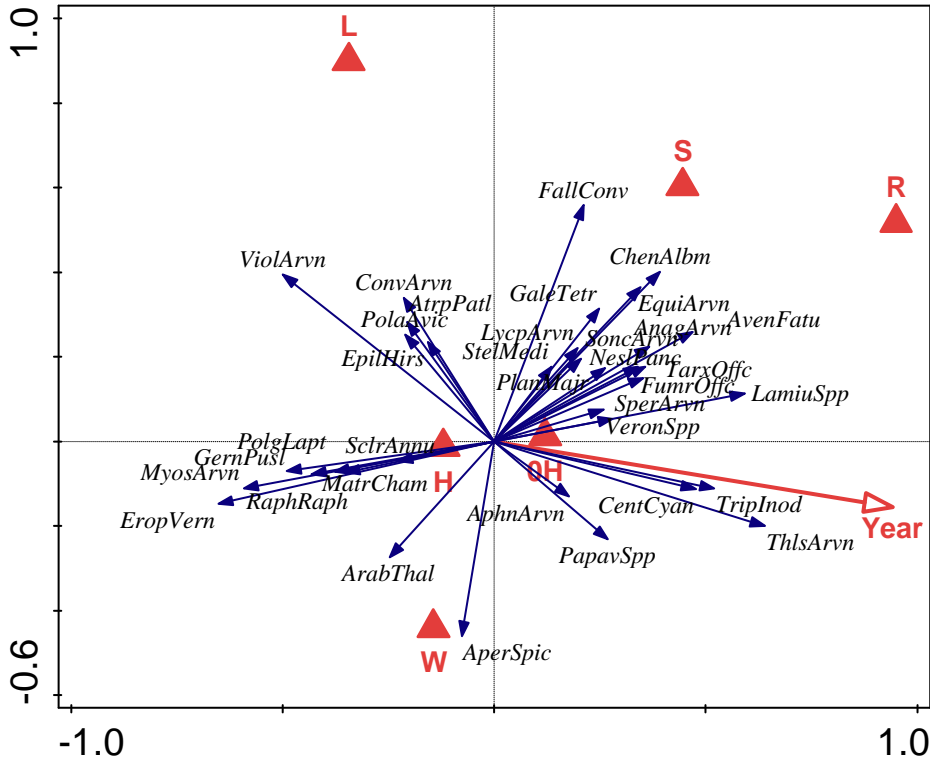
At the beginning of the trial, the following species dominated at Hněvčeves: *Thlaspi arvense*, *Papaver rhoeas*, *Lamium* spp., *Stellaria media*, *Veronica* spp., and *Fallopia convolvulus*. Abundance of some species such as *Lamium* spp., *Veronica* spp., *Stellaria media*, *Papaver* spp., *Galium aparine* increased on untreated plots. Abundance of other species such as *Apera spica-venti*, *Fumaria officinalis*, *Tripleurospermum inodorum*, and *Cirsium arvense* increased on treated plots (Tab. 4).

**Tab. 4** Weed species composition at Hněvčeves in winter wheat at trial commencement and the last assessment in 2016 (both crop rotations). Average no. of plants m<sup>-2</sup> (Avg.) and standard deviation (SD) for most frequent species; "-" = species not recorded.

**Tab. 4** Zusammensetzung der Unkrautarten in Hněvčeves in Winterweizen zu Versuchsbeginn und der letzten Erhebung 2016 (beide Fruchtfolgen). Pflanzen je m<sup>2</sup> (Avg.) und Standardabweichung (SD) für die am häufigsten auftretenden Unkrautarten; "-" = Art nicht vorhanden.

Weed species	Trial beginning		Simple crop rotation						Multi-crop rotation			
			Untreated		Auxins		Targeted		Untreated		Targeted	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
<i>Thlaspi arvense</i>	37	2.2	8	2.9	4	2.6	4	2.1	8	2.7	8	2.2
<i>Papaver</i> spp.	9	3.4	23	6.6	9	5.1	13	8.6	23	4.2	20	6.2
<i>Lamium</i> spp.	7	0.7	57	18.2	48	5.2	25	3.7	38	10.3	13	2.3
<i>Stellaria media</i>	6	4.9	26	3.9	20	4.7	7	1.9	30	5.6	10	5.2
<i>Veronica</i> spp.	5	0.9	57	13.6	58	6.4	72	14.7	26	9.2	42	9.4
<i>Fallopia convolvulus</i>	4	0.8	-	-	-	-	-	-	1	1.7	9	6.2
<i>Matricaria chamomilla</i>	4	1.5	-	-	-	-	-	-	-	-	-	-
<i>Galium aparine</i>	2	0.8	14	5.1	4	1.8	2	1.6	26	5.9	4	2.8
<i>Viola arvensis</i>	-	-	1	0.4	1	1.0	1	0.4	1	1.0	4	2.3
<i>Cirsium arvense</i>	-	-	3	2.5	5	2.9	4	1.9	7	3.3	1	0.9
<i>Vicia</i> spp.	-	-	1	0.8	-	-	< 1	0.4	5	3.2	-	-
<i>Sinapis alba</i>	-	-	25	7.7	31	5.5	23	5.1	37	2.7	32	6.8
<i>Fumaria officinalis</i>	-	-	3	1.1	16	3.4	6	1.7	4	1.1	6	3.5
<i>Tripleurospermum inodorum</i>	-	-	-	-	1	0.5	-	-	2	1.1	5	4.0
<i>Apera spica-venti</i>	-	-	3	3.3	4	3.5	8	3.9	1	1.7	4	4.5

The ordination diagrams in Figures 1 and 2 show changes of weed composition over years in Pernolec influenced by crop type and treatment. Some species receded in both crop rotations, regardless of treatment and crop type: *Myosotis arvensis*, *Raphanus raphanistrum*, *Scleranthus annuus*, and *Erophila verna*. No significant differences affected by time were detected in population densities of *Apera spica-venti*, *Arabidopsis thaliana*, *Stellaria media*, and *Fallopia convolvulus*.



**Fig. 1** Ordination diagram of Redundancy Analysis (RDA) - multi-crop rotation in Pernolec. Species with low weight are not shown. W = winter cereals, S = spring cereals, R = root crops, L = legumes; 0H = untreated, H = targeted treatment by combination of herbicides.

**Abb. 1** Ordinationsdiagramm der Redundanzanalyse (RDA) der blattfruchtbetonten Fruchtfolge in Pernolec. Spezies mit geringer Gewichtung werden nicht dargestellt. W = Wintergetreide; S = Sommergetreide; R = Hackfrucht; L = Leguminosen; 0H = unbehandelt; H = Kombination verschiedener Herbizide.

Many weed species responded to crop type. Those with the best fit were *Fallopia convolvulus*, *Chenopodium album*, *Anagallis arvensis*, *Galeopsis tetrahit* or *Lycopsis arvensis* (SCR) for spring cereals; *Avena fatua*, *Equisetum arvense*, *Sonchus arvensis*, *Taraxacum officinale*, and *Neslia paniculata* for root crops; *Apera spica-venti*, *Arabidopsis thaliana*, *Tripleurospermum inodorum*, *Poa annua* L. (SCR), and *Centaurea cyanus* for winter cereals; *Convolvulus arvensis*, *Polygonum aviculare*, and *Atriplex patula* for legumes. Most of species did not respond to treatment, but certain differences could be observed. The abundance of *Centaurea cyanus*, *Tripleurospermum inodorum*, *Veronica* spp., *Spergula arvensis*, and *Lycopsis arvensis* increased more on untreated plots.



## Conclusions

This long-term study confirmed changes in weed communities over years. We did not prove the relationship of these changes and specific herbicide treatment, however density of some weed species (e.g. *Raphanus raphanistrum* and *Erophila verna*) decreased faster on plots treated by herbicides compared to untreated plots and density of other weed species (e.g. *Centaurea cyanus*, *Tripleurospermum inodorum*) increased more on untreated plots. Crop was the major factor determining species composition and its effect was greater at multi-crop rotation, therefore it can be recommended to grow various crops in crop rotation to conserve weed species diversity.

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