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Weed infestation and its biodiversity under the influence of different herbicide variants application in maize

Zachwaszczenie i różnorodność biologiczna chwastów w warunkach stosowania różnych wariantów herbicydów w kukurydzy

Summary. The main objective of Integrated Pest Management is to minimize the negative impact of pesticide use on the environment. For this purpose, technologies are developed that involve reducing doses of herbicides whose efficacy can be enhanced by adding adjuvants. This field study aimed to determine the weed control efficacy of herbicide applied at reduced doses in relation to full dose at different growth stages of maize. In the experiment, pre-emergence herbicide was applied at stage BBCH 00, while a post-emergence herbicide was at the 3, 6, or 8 leaves of maize. The herbicides were applied at doses reduced to 60% or 80% with adjuvants or at a full dose. The study has shown that pre-emergence or early post-emergence (in BBCH 13) application of the herbicide had the most beneficial effect on reducing the number and biomass of weeds in maize. The use of the doses reduced by 20% with the addition of adjuvant gave the same weed-killing effect as a 100% dose.

Key words: integrated pest management, maize, herbicide doses, adjuvants, weed infestation

INTRODUCTION

The use of herbicides in maize crops is one of the elements of integrated weed management of this crop [Simić et al. 2020]. Nonetheless, indirect and mechanical methods are the basic weed management methods, while herbicides only complement them [Chojnacka et

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al. 2018, Simić et al. 2020]. In agricultural practice, plant protection products are the most cost-effective, and besides, they are the most efficient [Haliniarz et al. 2018].

Many factors influence herbicide efficacy [Schortgen and Patton 2020, Sobiech et al. 2020]. The main aspect is the selection of the active ingredient and its dose as well as application time and method [Chojnacka et al. 2018]. Moreover, to reduce the negative impact of herbicides on the natural environment, integrated weed management also involves a decrease in the number of weed control treatments, a reduction in herbicide dose [Niemczak et al. 2020], and application of herbicides in combination with adjuvants, i.e., improving the adhesion and penetration of the active ingredient into plant cell [Haliniarz et al. 2020].

The weed control efficacy of herbicide particularly applied at a reduced dose, is also affected by the physiology and morphology of a specific weed and its growth stage [Kieloch and Domaradzki 2011]. The earlier the treatment is performed, the better the effect can be achieved. With the growth of a plant, its sensitivity to herbicides diminishes, and higher doses are required to control it [Chojnacka et al. 2018]. In the case of foliar-applied agents, leaf blade number, size, distribution, and cover are important since they affect the uptake of the active ingredient [Wang and Liu 2007]. Maize is a poor competitor to weeds; hence, it is sensitive to weeds occurring in the crop [Cerrudo et al. 2012, Lehoczky et al. 2013, Głowacka and Flis-Olszewska 2022]. The sensitivity of maize to the competitive effects is due to its slow initial growth and wide interrows [Iqbal et al. 2020]. The critical period for the occurrence of weeds in maize crops depends on their numbers, species composition, competitiveness, and the condition of the crop plant [Knezevic et al. 2002, Evans et al. 2003, Woźnica and Idziak 2015]. According to Woźnica and Idziak [2015], reduction of weed infestation in maize cultivation should be conducted until the 8-leaf stage at the latest. Likewise, Ferrero et al. [1996] reason that maize exhibits the highest sensitivity to competition from weeds at the 1 to 7 visible leaf stages. Based on the results obtained by Woźnica and Idziak [2015] and Ferrero et al. [1996] in their studies, weeds were eliminated from the maize crop until the 8-leaf stage.

This three-year field study aimed to determine the weed control efficacy of herbicide applied at doses reduced to 80% and 60% together with adjuvants – oil adjuvant and surfactant – and at a full dose (100%), before maize emergence at stage BBCH 00, after the emergence of the crop plant, at stages BBCH 13, BBCH 16, and BBCH 18, as well as at split doses of 50% at stages BBCH 13 and BBCH 18. This study assessed the rationale for using reduced herbicide doses and performing weed control treatment at the latest possible time or using the split dose system by determining the effects on the maize crop.

MATERIAL AND METHODS

A field experiment was conducted over the period 2017–2019 at the Czesławice Experimental Farm (51°18'23"N, 22°16'2"E, Lubelskie voivodeship, Poland), belonging to the University of Life Sciences in Lublin, in a split-plot design in three replicates. The experiment was set up on loess-derived humic podzolic soil (PPh) [Kabała et al. 2019a], soil class II in terms of agricultural land suitability [Kabała et al. 2019b]. According to the soil classification following the Polish standard PN R 04033:1998 and the agronomic categories, the soil was classified in the silt group, subgroup: silt loam. The soil arable layer had a high content of phosphorus (76.3–77.7 $mg \cdot kg^{-1}$) and potassium (117.8–132.3 $mg \cdot kg^{-1}$) as well as a medium content of magnesium (79–85 $mg \cdot kg^{-1}$). The humus content was 1.59–1.63%, while the soil pH ranged 6.1–6.4.

Table 1. The types of herbicide treatments

I. Herbicide application date	Herbicide active ingredient	BBCH phase of maize	II. Herbicide application variants	Surfactant/oil adjuvant	Herbicide and adjuvant dose ($dm^3 \cdot ha^{-1}$)
T0	terbuthylazine – 187.5 $g \cdot L^{-1}$ mesotrione – 37.5 $g \cdot L^{-1}$ S-metolachlor – 312.5 $g \cdot L^{-1}$	BBCH 00	100%	–	4.0
			80% + A1	Trend 90 EC (ethoxylated isodecyl alcohol – 90%)	3.2 + 0.2
			80% + A2	Olejan 85 EC (rapeseed oil – 85%)	3.2 + 1.5
			60% + A1	Trend 90 EC (ethoxylated isodecyl alcohol – 90%)	2.4 + 0.2
			60% + A2	Olejan 85 EC (rapeseed oil – 85%)	2.4 + 1.5
T1 T2 T3	nicosulfuron – 40 $g \cdot L^{-1}$	BBCH 13 BBCH 16 BBCH 18	100%	–	1.0
			80% + A1	Trend 90 EC (ethoxylated isodecyl alcohol – 90%)	0.8 + 0.2
			80% + A2	Olejan 85 EC (rapeseed oil – 85%)	0.8 + 1.5
			60% + A1	Trend 90 EC (ethoxylated isodecyl alcohol – 90%)	0.6 + 0.2
			60% + A2	Olejan 85 EC (rapeseed oil – 85%)	0.6 + 1.5
T1 + T3	nicosulfuron – 40 $g \cdot L^{-1}$	BBCH 13 + BBCH 18	50%/50%	–	0.5 + 0.5
			40% + A1/ 40% + A1	Trend 90 EC (ethoxylated isodecyl alcohol – 90%)	0.4 + 0.2 0.4 + 0.2
			40% + A2/ 40% + A2	Olejan 85 EC (rapeseed oil – 85%)	0.4 + 1.5 0.4 + 1.5
			30% + A1/ 30% + A1	Trend 90 EC (ethoxylated isodecyl alcohol – 90%)	0.3 + 0.2 0.3 + 0.2
			30% + A2/ 30% + A2	Olejan 85 EC (rapeseed oil – 85%)	0.3 + 1.5 0.3 + 1.5

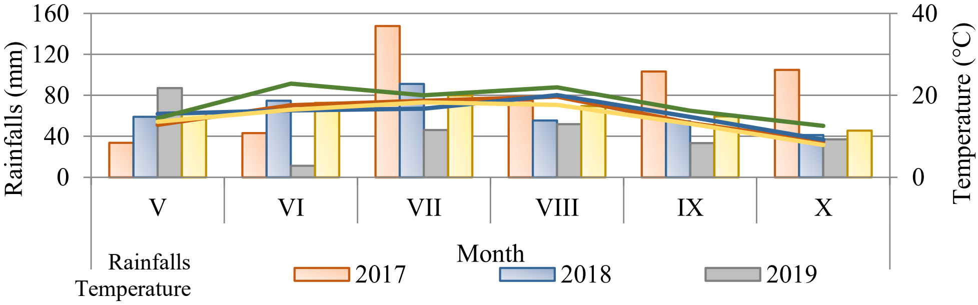


Fig. 1. Air temperature (°C) and rainfalls (mm) in maize growing season 2017–2019 and 1963–2010 according to the Meteorological Station in Czesławice

The field study was conducted in a medium early cultivar of maize – 'Tonacja FAO 220–230'. It is a three-line cultivar (TC) with semi-flint grain.

The soil was prepared for sowing by using typical tillage practices. Maize was sown in the first 10 days of May at a depth of 6 cm, a row spacing of 75 cm, and a plant spacing of 16.5 cm. In each year of the experiment, maize was the previous crop – a short-time monoculture. The plot size was 4.0 m × 4.5 m (18 m^2). The following fertilization was applied before maize sowing: ammonium phosphate (27 kg $N \cdot ha^{-1}$, 69 kg $P \cdot ha^{-1}$), urea (92 kg $N \cdot ha^{-1}$), and potassium salt (100 kg $K \cdot ha^{-1}$).

The experimental factors in the experiment (I. herbicide application time and II. herbicide application treatment) and their levels are shown in Table 1. The herbicides were applied with a field sprayer at a pressure of 0.25 MPa, whose spray liquid application dose was 250 $L \cdot ha^{-1}$.

Weed infestation and its parameters were evaluated twice. The first assessment term was at the 2–3 leaf stage of maize in the case of the soil herbicide (T0) and after 3–4 weeks from the application of the foliar herbicide (T1, T2, T3, T1+T3) [EPPO 2021a]. A 1 m × 0.5 m frame in two randomly selected plots was used twice. The dry-weight-rank method determined the following parameters: number of weeds per 1 m^2, number of species, floristic composition, and air-dry weight of all weed species (g). The second term of weed infestation assessment was before maize harvest at stages BBCH 85–87 [EPPO 2021a]. The weed species nomenclature followed Mirek et al. [2002], and EPPO codes were used [EPPO 2021b, EPPO 2022]. The obtained results were the basis for calculating the Shannon-Wiener species diversity index (H) [Shannon and Wiener 1948], the Simpson dominance index (D) [Simpson 1949], and the Sörensen qualitative and quantitative similarity index [Sörensen 1948].

Maize was harvested in the second 10-days period of October. Ten plants and ten cobs were collected from each plot. Grain yield was determined and expressed on a 15% moisture content ($dt \cdot ha^{-1}$). The number and dry weight of weeds and grain yield were also used to calculate relationships between the weed infestation indicators and maize yield. To this end, Pearson's linear correlation coefficient was determined, a linear regression analysis was performed, and the coefficient of determination was established (R2). Tukey's test estimated the significance of differences at a significance level of $p = 0.05$. Statistica 13.3 (StatSoft Polska) statistical software was used for calculations. The distribution conformity with normal distributions was verified with the Shapiro-Wilk test, while the homogeneity of the variance was tested with the Bartlett test. When necessary to homogenize the variance, the data were subjected to angular transformation before the variance analysis. Before statistical calculations, the percentage values were transformed using the following equation: $y = arcsinx\sqrt{x}$. In Figures 2 and 3, significant differences are marked by a bar above the graph bar and in tables by the letters. In Figures 4 and 5, the standard deviation is marked by a bar above the graph bar.

The meteorological data from the period the experiment was conducted (2017–2019) come from the weather station in Czesławice (Fig. 1).

Among all study years, 2017 was characterized by the highest total rainfall during the maize growing season (507.9 mm), with the largest rainfall recorded in July, while the lowest was in May. The 2018 growing season was similar to the long-term mean rainfall for 1963–2010 in terms of rainfall levels. The period from May to October 2019 was the poorest in rainfall compared to the same period in the other years of the study.

The growing season 2017 was the coldest compared with the other years of the study (<20°C), and the average temperature over this time was closest to the long-term mean. The 2019 growing season, on the other hand, proved to be the warmest one since in June – August, the average temperature exceeded 20°C, while the average for the entire season was 18.0°C.

RESULTS

The first term of weed infestation assessment – in the BBCH 12–13 of maize or 3–4 weeks after herbicide application

In BBCH 12–13 of maize in the case of application of the pre-emergence herbicide or after 3–4 weeks from the herbicide application with the post-emergence herbicide, the significantly lowest number of weeds per 1 m^2 was recorded in 2018 – 42.7 pcs. In contrast,

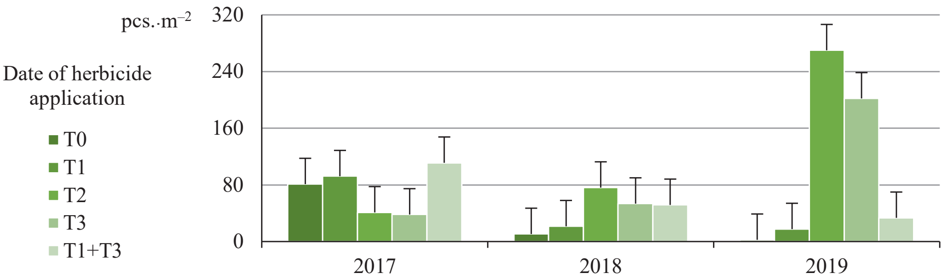
Table 2. Number of weeds in maize in the 2–3 leaves phase of maize (BBCH 12–13) or 3–4 weeks after herbicide application (I term) – mean for research years (pcs.·m^{-2})

Herbicide application variant	Date of herbicide application					Mean
	T0	T1	T2	T3	T1 + T3	
100%	30.1^a	38.2^a	127.1^a	109.1^a	52.2^a	71.4^a
80% + A1	27.1^a	37.7^a	120.1^a	84.3^a	70.8^a	68.0^a
80% + A2	35.7^a	44.2^a	120.3^a	100.2^a	58.0^a	71.7^a
60% + A1	24.8^a	49.8^a	153.5^a	94.9^a	60.7^a	76.7^a
60% + A2	39.0^a	48.8^a	124.2^a	100.8^a	85.3^a	79.6^a
Mean	31.3^d	43.7^d	129.0^a	97.9^b	65.4^c	–

Research year	mean
2017	72.7^b
2018	42.7^c
2019	105.1^a

The significant diffrences are marked by letters in the table.

it was highest in the experiment's third year (2019) – 105.1 pcs. (Tab. 2). Pre-emergence application of the herbicide (T0) and post-emergence at stage BBCH 13 of maize (T1) significantly reduced the occurrence of weeds in comparison to treatment T2 (BBCH 16), in which the significantly highest number of weeds was recorded.



The significant differences in the figures are marked by a bar above the graph bar

Fig. 2. Number of weeds per 1 m^2 in maize in the crop's 2–3 true leaves phase (BBCH 12–13) or 3–4 weeks after herbicide application (I term) – the interaction of research years and herbicide application date

Table 3. Species composition and number of weeds species in maize in the 2–3 leaves phase of maize (BBCH 12–13) or 3–4 weeks after herbicide application (I term) depending on herbicide application date – mean for research years (pcs.·m^{-2})

Specification		Date of herbicide application				
		T0	T1	T2	T3	T1+T3
Short-lived						
Species	*Chenopodium album*	5.8^{c}	25.4^{b}	42.3^{a}	31.8^{ab}	27.5^{b}
	Echinochloa crus-galli	5.4^{b}	2.5^{b}	4.5^{b}	10.2^{a}	5.0^{b}
	Polygonum lapathifolium subsp. *lapathifolium*	3.1^{b}	1.8^{b}	12.3^{a}	6.0^{b}	3.1^{b}
	Galinsoga parviflora	2.9^{b}	8.1^{b}	46.3^{a}	31.5^{a}	13.8^{b}
	Amaranthus retroflexus	2.7^{a}	1.0^{b}	0.2^{c}	1.2^{b}	0.3^{c}
	others	6.9^{b}	2.9^{b}	16.6^{a}	15.4^{a}	11.0^{a}
Number of short-lived weeds		26.8^{d}	26.8^{d}	41.7^{d}	122.2^{a}	96.1^{b}
Number of short-lived weed species		20^{a}	20^{a}	20^{a}	15^{b}	17^{ab}
Perennial						
Number of perennial weeds		4.5^{a}	2.0^{b}	6.8^{a}	1.8^{b}	4.7^{a}
Number of perennial weed species		7^{a}	6^{a}	3^{b}	6^{a}	5^{ab}
Total number of weed species		27^{a}	26^{a}	18^{b}	23^{a}	23^{a}

The significant differences are marked by letters in the table.

Table 4. Species composition and number of weeds species in maize in the 2–3 true leaves phase of the crop (BBCH 12–13) or 3–4 weeks after herbicide application (I term) depending on herbicide application variant – mean for research years (pcs.·m^{-2})

Specification		Herbicide application variant				
		100%	80% + A1	80% + A2	60% + A1	60% + A2
Short-lived						
Species	*Chenopodium album*	26.1^{bc}	24.4^{c}	28.6^{a}	26.4^{ab}	26.9^{ab}
	Galinsoga parviflora	23.1^{a}	20.8^{ab}	19.4^{b}	19.3^{b}	20.5^{ab}
	Echinochloa crus-galli	7.0^{ab}	3.8^{c}	3.6^{c}	4.6^{bc}	8.5^{a}
	Polygonum lapathifolium subsp. *lapathifolium*	3.4^{b}	4.5^{b}	3.9^{b}	9.9^{a}	4.6^{b}
	Veronica persica	3.0^{a}	3.5^{a}	2.4^{a}	2.3^{a}	2.8^{a}
	others	6.9^{b}	7.4^{b}	6.4^{b}	11.8^{a}	12.7^{a}
Number of short-lived weeds		69.5^{a}	69.5^{a}	64.4^{a}	64.3^{a}	74.3^{a}
Number of short-lived weed species		18^{a}	18^{a}	22^{a}	18^{a}	19^{a}
Perennial						
Number of perennial weeds		1.9^{a}	3.6^{a}	7.4^{a}	2.4^{a}	3.6^{a}
Number of perennial weed species		7^{a}	7^{a}	7^{a}	7^{a}	8^{a}
Total number of weed species		25^{a}	29^{a}	25^{a}	26^{a}	28^{a}

The significant differences are marked by letters in the table.

In 2019, treatment T2 showed the significantly highest number of weeds (270 pcs. per 1 m^2) compared to all the other treatments (Fig. 2). In the first study year, in treatments T0, T1, and T1 + T3, a significantly higher number of weeds was recorded compared to the corresponding plots in the next two years of the study.

During the first term of assessment, the greatest numbers of weeds were *Chenopodium album*, *Echinochloa crus-galli*, *Polygonum lapathifolium* subsp. *lapathifolium*, and *Galinsoga parviflora* (Tabs 3, 4). Depending on herbicide application date (Tab. 3), *C. album* was predominant in treatments T0, T1, T3 and T1 + T3. In contrast, in the plots where the herbicide was applied at stage BBCH 16 (T2), in turn, *G. parviflora* showed the quantitative prevalence. This species was also found in large numbers in treatment T3. *P. lapathifolium* subsp. *lapathifolium* occurred in greatest numbers in the plots weeded at stage BBCH 16 (T2) (12.3 pcs·m^{-2}). The numbers of *E. crus-galli* ranged from 2.5 pcs·m^{-2} (T1) to 10.2 pcs·m^{-2} (T3) and in the case of this species, an increasing trend could be observed with the delay in post-emergence herbicide application. The highest number of annual weeds was found in treatment T2, whereas the lowest one in T0. An opposite relationship was observed when the number of species was analyzed, which was 15 (T2) and 20 (T0), respectively. As in the case of annual species, the number of perennial species was the highest in the plots where the herbicide was applied earliest – before emergence (T0).

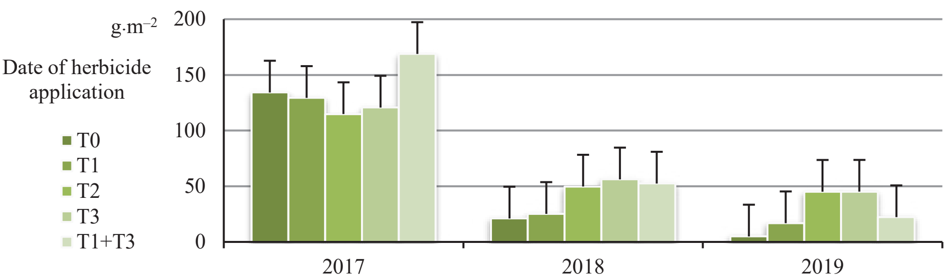
C. album was dominant in all herbicide application variants (Tab. 4), and the number of this species was similar in each variant. *G. parviflora* also showed similar numbers in each variant, but its greatest numbers were found in the plot weeded using a 100% dose

Table 5. Air-dry weight of weeds in maize in the 2–3 true leaves phase of the crop (BBCH 12–13) or 3–4 weeks after herbicide application (I term) – mean for research years (g·m^{-2})

Herbicide application variant	Date of herbicide application					Mean
	T0	T1	T2	T3	T1 + T3	
100%	55.39[a]	55.61[a]	56.67[a]	73.00[a]	81.62[a]	64.46[ab]
80% + A1	49.95[a]	42.98[a]	81.89[a]	75.78[a]	73.42[a]	64.81[ab]
80% + A2	50.52[a]	50.62[a]	70.49[a]	61.68[a]	69.65[a]	60.59[b]
60% + A1	41.64[a]	75.16[a]	72.90[a]	75.27[a]	85.69[a]	70.13[ab]
60% + A2	69.46[a]	61.25[a]	67.51[a]	84.27[a]	95.48[a]	75.60[a]
Mean	53.39[c]	57.13[bc]	69.89[ab]	74.00[a]	81.17[a]	–

Research years	mean
2017	133.57[a]
2018	40.92[b]
2019	26.85[c]

The significant differences are marked by letters in the table.

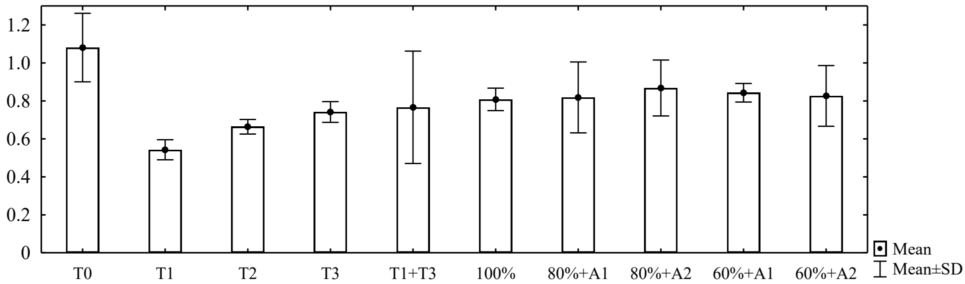


The significant differences on the figures are marked by a bar above graph bar

Fig. 3. Air-dry weight of weeds per 1 m^2 in maize in the 2–3 leaves phase of maize (BBCH 12–13) or 3–4 weeks after herbicide application (I term) – interaction of research years and herbicide application time

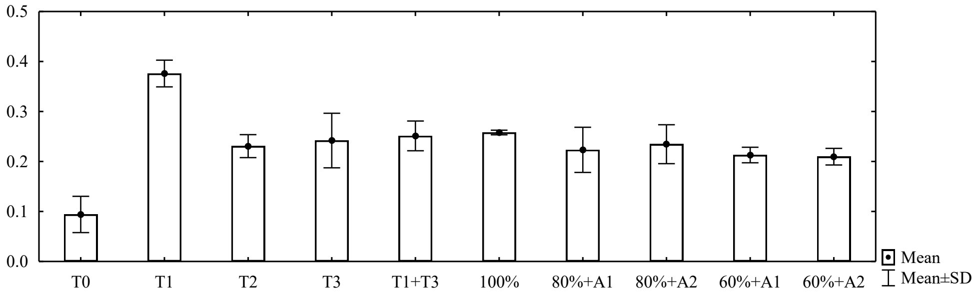
– 23.1 pcs·m^{-2}. The largest number of annual weed species was found in the variant 80% + A1, whereas perennial weed species were at a similar level in each herbicide application variant.

During the first term of weed infestation assessment, weeds produced the significantly highest biomass in the first year of the experiment (2017) – 133.57 g·m^{-2} (Tab. 5). The weed dry weight declined in the next years of the study, by 69.4% in 2018 and 79.9% in 2019. The weed biomass in the treatments where the herbicide was applied at time points T2, T3, and T1 + T3 was significantly higher than the dry weight of weeds from the plots where the herbicide was applied earliest (T0). When analyzing the effects of herbicide application variants on weed dry weight, significant differences were found only between



The standard deviation on the figures are marked by a bar above graph bar

Fig. 4. Shannon-Wiener's (H) diversity index of weed community in the 2–3 true leaves phase of the crop (BBCH 12–13) or 3–4 weeks after herbicide application (I term) – mean for research years



The standard deviation on the figures are marked by a bar above graph bar

Fig. 5. Simpson's (D) dominance index of weed community in the 2–3 true leaves phase of the crop (BBCH 12–13) or 3–4 weeks after herbicide application (I term) – mean for research years

the variants 80% + A2 and 60% + A2, in which the biomass was larger by 19.9% in the case of the lower dose.

In 2017, treatment T1 + T3 caused the significantly highest weed dry weight compared to the other treatments over the three-year study period – 168.8 g·m^{-2} (Fig. 3). The level of this parameter for the individual treatments in 2017 was significantly higher than for the corresponding treatments in the next study years.

The maize crop where the herbicide treatment was performed earliest (T0) was characterized by the highest value of the Shannon-Wiener species diversity index (H) – Figure 4 – and the lowest Simpson dominance index (D) – Figure 5. Post-emergence herbicide application at the BBCH 13 (T1) caused a reduction in most weed species' numbers but an increase in the percentage of *C. album* in weed infestation (Fig. 4). This contributed to a decrease in weed species diversity and an increase in the value of the dominance index (Fig. 5). As regards the herbicide application variant, the Shannon-Wiener species diversity index (H) – Figure 4 – and the Simpson dominance index (D) – Figure 5 – were at a similar level for the weed community in the maize.

The Sörensen index is used to assess the similarity of weed communities in quantitative and qualitative terms (Tabs 6, 7). When analyzing weed communities in the maize crop as modified by the different herbicide application dates, it can be observed that in quantitative terms, the greatest similarity of weeds was shown between treatments T1 and T1 + T3 as well as between T2 and T3 (Tab. 6). The same analysis proved that the treatment in which the herbicide was applied before emergence (T0) most differed in the number of weeds from treatment T2. The highest Sörensen qualitative index was found when treatments T1 and T0 were compared, which proves that they show the greatest similarity among all treatments studied.

When analyzing the effect of herbicide application variants on similarities between weed communities, it was proven that small quantitative and qualitative differences existed between the individual variants (Tab. 7). The Sörensen quantitative index ranged from

Table 6. Qualitative and quantitative Sörensen index in the 2–3 true leaves phase of the crop (BBCH 12–13) or 3–4 weeks after herbicide application (I term) depending on herbicide application date – mean for research years

	Qualitative Sörensen index					
Quantitative Sörensen index	date of herbicide application	T0	T1	T2	T3	T1 + T3
	T0	–	90.6	84.4	80.0	88.0
	T1	48.8	–	86.4	77.6	81.6
	T2	27.1	48.5	–	82.9	82.9
	T3	36.7	60.7	77.7	–	78.3
	T1 + T3	46.1	78.1	55.6	67.7	–

Table 7. Qualitative and quantitative Sörensen index 3 in the 2–3 true leaves phase of the crop (BBCH 12–13) or 3–4 weeks after herbicide application (I term) depending on herbicide application variant – mean for research years

	Qualitative Sörensen index					
Quantitative Sörensen index	herbicide application variant	100%	80% + A1	80% + A2	60% + A1	60% + A2
	100%	–	85.2	84.0	86.3	86.8
	80% + A1	87.7	–	88.9	94.5	94.7
	80% + A2	84.3	88.6	–	86.3	90.6
	60% + A1	82.6	86.7	84.2	–	88.9
	60% + A2	87.2	88.5	84.9	90.3	–

82.6% to 90.3% and the qualitative index was within a similar range, which is evidence of a high level of similarity between the weed communities.

Maize grain yield was negatively correlated to the number of weeds in the maize crop at the first term of weed infestation assessment (Fig. 6). The regression correlation model reveals that an increase in this trait by one unit caused a significant decrease in yield by about 0.119 dt·ha^{-1}. The value of the coefficient of determination (R2) shows that about 14% of the yield variation is explained by this model.

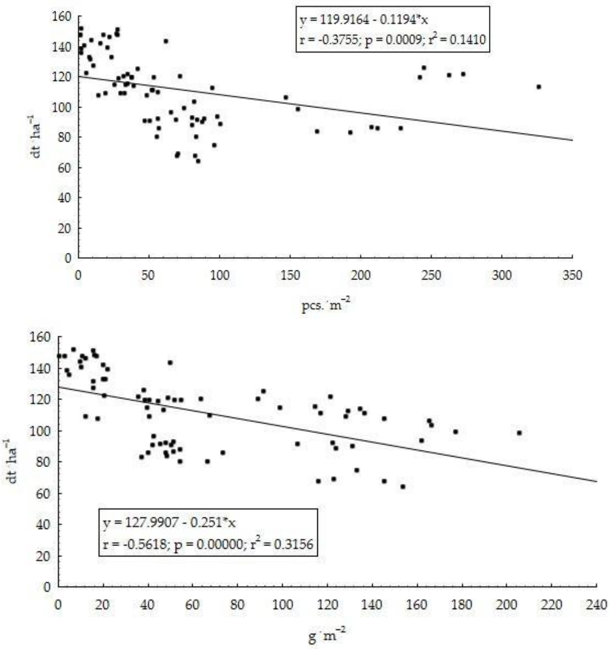


Fig. 6. Linear relationship model between maize grain yield and number and dry weight of weed per 1 m^2 in the 2–3 true leaves phase of the crop (BBCH 12–13) or 3–4 weeks after herbicide application (I term)

A negative correlation was noted between maize grain yield and weed dry weight at the first time point of weed infestation assessment (Fig. 6). The regression correlation model reveals that an increase in this trait by one unit caused a significant decrease in yield by about 0.251 dt·ha^{-1}. The value of the coefficient of determination (R2) shows that about 32% of the yield variation is explained by this model.

The second term of weed infestation assessment – before harvest

During the second term of weed infestation assessment, significantly more weeds were observed in maize in the first year of the experiment (2017) – Table 8. The treatments in which the herbicide was applied at stages BBCH 16 (T2) and BBCH 18 (T3) were characterized by the significantly highest number of weeds per 1 m^2 – 33.9 pcs. and 28.9 pcs. respectively. As far as the interaction of herbicide application date and variants is concerned, the highest number of weeds was recorded in treatment T2 after application

Table 8. Number of weeds in maize before harvest (BBCH 85–87) – mean for research years (pcs.·m^{-2})

Herbicide application variant	Date of herbicide application					Mean
	T0	T1	T2	T3	T1 + T3	
100%	10.4^{d}	15.6^{cd}	34.4^{ab}	23.8^{bcd}	16.2^{cd}	20.1^{a}
80% + A1	12.1^{d}	14.8^{cd}	28.2^{bc}	26.2^{bcd}	17.9^{cd}	19.8^{a}
80% + A2	11.3^{d}	13.9^{cd}	47.4^{a}	30.9^{b}	14.0^{cd}	23.5^{a}
60% + A1	12.5^{cd}	17.8^{cd}	26.5^{bcd}	34.6^{ab}	22.8^{bcd}	22.8^{a}
60% + A2	16.2^{cd}	17.6^{cd}	32.8^{ab}	28.8^{bc}	18.7^{bcd}	22.8^{a}
Mean	12.5^{b}	15.9^{b}	33.9^{a}	28.9^{a}	17.9^{b}	–

Research years	mean
2017	31.8^{a}
2018	24.2^{b}
2019	9.4^{c}

The significant differences are marked by letters in the table.

Table 9. Species composition and number of weeds in maize before harvest (BBCH 85–87) depending on herbicide application date – mean for research years (pcs.·m^{-2})

Specification		Date of herbicide application				
		T0	T1	T2	T3	T1 + T3
Short-lived						
Species	*Galinsoga parviflora*	2.2^{b}	3.8^{b}	10.6^{a}	11.4^{a}	8.5^{a}
	Amaranthus retroflexus	2.2^{a}	1.6^{a}	1.1^{a}	0.0^{b}	1.5^{a}
	Chenopodium album	1.8^{b}	6.5^{b}	15.5^{a}	12.7^{a}	4.1^{b}
	Galinsoga ciliata	1.3^{b}	2.4^{ab}	4.2^{a}	3.9^{a}	2.8^{ab}
	Echinochloa crus-galli	1.3^{a}	1.4^{a}	1.6^{a}	–	0.7^{b}
	others	1.2^{a}	0.1^{b}	0.6^{b}	0.4^{b}	–
Number of short-lived weeds		10.0^{b}	15.8^{b}	33.6^{a}	28.4^{a}	17.6^{b}
Number of short-lived weed species		7^{a}	6^{a}	6^{a}	5^{a}	5^{a}
Perennial						
Number of perennial weeds		2.5^{a}	0.1^{b}	0.3^{b}	0.5^{b}	0.3^{b}
Number of perennial weed species		4^{a}	1^{a}	2^{a}	4^{a}	2^{a}
Total number of weed species		11^{a}	7^{a}	8^{a}	9^{a}	7^{a}

– species not occurring; 0.0 number of weeds less than pcs.·m^{-2}
The significant differences are marked by letters in the table.

of the herbicide at a dose of 80% dose with the adjuvant Olejan 85 EC (80% + A2) – 47.4 pcs.·m^{-2} – and this number did not differ significantly only from the variant 100% (34.4 pcs.) and 60% + A2 (32.8 pcs.) at time point T2 and 60% + A1 at time point T3 (34.6 pcs.).

At the second term of weed infestation assessment, the dominant weed species were *G. parviflora*, *A. retroflexus*, *C. album*, and *G. ciliata* (Tabs 9, 10). *G. parviflora* was dominant in the plots where the herbicide was applied at two-time points (T1 + T3) and treatment T0, together with *A. retroflexus* (Tab. 9). *C. album* occurred in the largest numbers in treatments T1, T2, and T3. Before the harvest of maize, there was a smaller weed density and diversity than at the first term of weed infestation assessment. The T2 treatment, in which the herbicide was applied at stage BBCH 16, was characterized by the highest number of annual weeds – 33.6 pcs.·m^{-2}, whereas the treatment T0 showed the greatest weed diversity – 7 pcs.·m^{-2}. The largest number of perennial weeds was recorded in the T0 treatment, in which the herbicide was applied pre-emergence – 2.5 pcs.·m^{-2}, whereas the highest number of species was observed at treatments T0 and T3.

G. parviflora was predominant in the variants in which the herbicide was applied at a 100% dose and at 80% doses (80% + A1 and 80% + A2), while *C. album* dominated all variants with a 60% dose (60% + A1 and 60% + A2) – Table 10. At the second term of weed infestation assessment, the number of annual weeds and the number of annual weed species were at a similar level in all herbicide application variants. The highest number of perennial weed species was found in the variant in which the manufacturer's recommended dose was used (100%) – 4.

Table 10. Species composition and number of weeds in maize before harvest (BBCH 85–87) depending on herbicide application variant – mean for research years (pcs.·m^{-2})

Specification		Herbicide application variant				
		100%	80% + A1	80% + A2	60% + A1	60% + A2
Short-lived						
Species	*Galinsoga parviflora*	7.4^{b}	6.7^{c}	7.9^{ab}	6.1^{c}	8.2^{a}
	Chenopodium album	6.2^{b}	6.4^{b}	7.8^{b}	10.3^{a}	7.9^{b}
	Galinsoga ciliata	3.1^{ab}	3.4^{ab}	4.3^{a}	3.0^{ab}	2.5^{b}
	Amaranthus retroflexus	1.1^{a}	1.2^{a}	1.3^{a}	1.2^{a}	1.6^{a}
	Echinochloa crus-galli	0.7^{b}	1.2^{a}	1.0^{ab}	1.0^{ab}	1.3^{a}
	others	0.7^{a}	0.3^{b}	0.2^{b}	0.8^{a}	0.4^{b}
Number of short-lived weeds		19.2^{a}	19.2^{a}	22.5^{a}	22.4^{a}	21.9^{a}
Number of short-lived weed species		8^{a}	6^{a}	7^{a}	7^{a}	6^{a}
Perennial						
Number of perennial weeds		0.9^{a}	$0.^{6a}$	1.0^{a}	0.4^{a}	0.9^{a}
Number of perennial weed species		4^{a}	3^{a}	2^{a}	3^{a}	3^{a}
Total number of weed species		12^{a}	9^{a}	9^{a}	10^{a}	9^{a}

The significant differences are marked by letters in the table.

The highest weed dry weight was recorded in the second year of the experiment – 53.02 g·m^{-2}, while the lowest in its last year (2019) – 7.53 g·m^{-2} (Tab. 11). The treatment in which the herbicide was applied latest (T3) was characterized by the largest weed biomass and it was significantly higher than in the other treatments, except for T2. The plots treated with the herbicide at 60% of the manufacturer's recommended dose in combination with the adjuvant Trend 95 EC (60% + A1) were found to have a significantly higher weed dry weight (30.86 g·m^{-2}) in comparison with the variant 100% (21.49 g·m^{-2}) and 80% + A1 (21.29 g·m^{-2}).

The analysis of the interaction of herbicide application date and herbicide application variant proved that it significantly affected the dry weight of weeds per 1 m^2 of maize crop (Tab. 11). The plots where the herbicide was applied at two time points (T1 + T3) at the recommended dose (100%) showed by the lowest weed biomass – 10.04 g·m^{-2}. The herbicide treatments applied at time points T0, T1, T2, and T3 did not produce differences in weed biomass. In contrast, at two-time points, significant differences were noted after applying the herbicide between the full dose (10.04 g·m^{-2}) and the 80% dose with oil adjuvant (45.30 g·m^{-2}). Exuberant *C. album* plants occurring in the variant 80% + A2 in 2018 proved to be the reason for such a large dry weight.

At the second term of weed infestation assessment, the number of weeds in maize harmed grain yield (Fig. 7). The regression correlation model reveals that an increase in this trait by one unit caused a significant decrease in yield by about 0.861 dt·ha^{-1}. The value of the coefficient of determination (R2) shows that about 29% of the yield variation is explained by this model.

Maize grain yield and weed dry weight in maize were negatively correlated at the second term of weed infestation assessment (Fig. 7). The regression correlation model

Table 11. Air-dry weight of weeds in maize before harvest (BBCH 85–87) – mean for research years (g·m^{-2})

Herbicide application variant	Date of herbicide application					Mean
	T0	T1	T2	T3	T1 + T3	
100%	12.81^{de}	26.26^{bcde}	24.93^{bcde}	33.40^{abcd}	10.04^{e}	21.49^{c}
80% + A1	11.73^{de}	20.20^{cde}	27.04^{bcde}	28.68^{abcde}	18.82^{cde}	21.29^{c}
80% + A2	10.97^{de}	23.31^{bcde}	28.85^{abcde}	34.08^{abcd}	45.30^{ab}	28.50^{bc}
60% + A1	10.96^{de}	29.78^{abcde}	39.52^{abc}	51.22^{a}	22.83^{bcde}	30.86^{ab}
60% + A2	16.61^{cde}	24.71^{bcde}	29.42^{abcde}	32.84^{abcde}	21.11^{cde}	24.94^{bc}
Mean	12.61^{c}	24.85^{b}	29.95^{ab}	36.04^{a}	23.62^{b}	–

Research year	Mean
2017	15.70^{b}
2018	53.02^{a}
2019	7.53^{c}

The significant differences are marked by letters in the table.

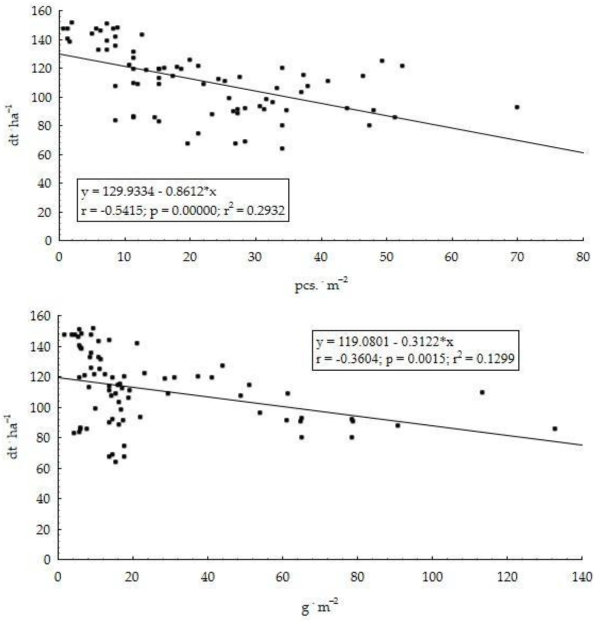


Fig. 7. Linear relationship model between maize grain yield and number and weed dry of weeds per 1 m^2 before harvest (BBCH 85–87)

reveals that an increase in this trait by one unit caused a significant decrease in yield by about 0.312 dt·ha^{-1}. The value of the coefficient of determination (R2) shows that about 13% of the yield variation is explained by this model.

DISCUSSION

A condition for the high effectiveness of herbicide treatment of a crop plant is the appropriate selection of the application date relative to the crop's growth stage and weeds [Kudsk 2008]. According to Krawczyk and Kaczmarek [2009], the high weed control efficacy of herbicides applied at lower doses can only be achieved until the 2 true leaf stage of weeds. Still, according to Dogan et al. [1999] the herbicide application time can be delayed to even the 4 true leaf stage of weeds. When reaching increasingly higher growth stages, weeds show greater tolerance to herbicides and additionally the amount of herbicide per unit of their surface area decreases with their growth [Sarabi et al. 2011]. In this research, on average over the three-year study period, the largest reduction of weed infestation in the maize crop was achieved by applying the foliar herbicide at the first application time, at the 3 leaf stage of maize when weeds were at the initial growth stages. In contrast, it was significantly lower at the next two times of herbicide application (T2 and T3). Yanev et al. [2021] showed a delay in the application time of mixtures of the following active ingredients: florasulam + aminopyralid, fluroxypyr + florasulam, and florasulam + mesotrione until stage BBCH 18 of maize caused a significant decrease in the efficacy of these herbicides towards species such as *C. album*, *A. retroflexus*, *Xanthium strumarium*, *Abutilon theophrasti*, and *Solanum nigrum* compared to their use at stage BBCH 15. In the opinion of Sarabi et al. [2011], however, the small surface area of plant tissue of weeds or large competition from other plants can be the reason for the low efficacy of herbicides applied at the 2 to 4 leaf stage of weeds.

A good solution to control weeds in maize is to apply pre-emergence herbicides, which protect a crop against weed competition from the beginning of its growth [Amosun et al. 2021]. On average, over the three-year study period, the lowest number and dry weight of weeds were found after applying the pre-emergence herbicide in the field. Nonetheless, it has been shown that the response of weeds to herbicide treatment varies. In a study by Andr et al. [2014], *A. retroflexus* was effectively controlled during both pre-emergence and post-emergence application of the same herbicide. In the study by Mehmeti et al. [2019], isoxaflutole applied both pre-emergence and post-emergence had more than 90% weed control efficacy.

The positive effects of adjuvants on the weed control efficacy of herbicides are widely documented in the literature of the subject [Khaffagy et al. 2020, Mahto et al. 2020]. In the study by Woźnica and Idziak [2015], the reduction of the dose of terbuthylazine applied pre-emergence and the dose of nicosulfuron applied pre-emergence as well as the application of these herbicides with adjuvant increased their effectiveness relative to the full dose from 24–68% to 74–95%. These authors also showed the herbicides to have higher efficacy after their application with oil adjuvant than with surfactant [Woźnica and Idziak 2010, Woźnica and Idziak 2015]. In the present experiment, on the other hand, the effectiveness of the action of the herbicides with the addition of both oil adjuvant and surfactant was found to be equally high. In the opinion of Woźnica and Idziak [2015] as well as Haliniarz [2019], application of nicosulfuron at a dose reduced by 50% with adjuvant shows equally high weed control efficacy as an application of this herbicide alone at the manufacturer's recommended dose. A study by Khaffagy et al. [2020], in turn, reveals that the use of a dose of nicosulfuron reduced by 50% together with adjuvant did not give satisfactory results, but the application of this herbicide at a dose reduced by 25% in combination with adjuvant caused a significant reduction in weed biomass in comparison with the dose of the herbicide applied alone. Nadeem et al. [2018] also demonstrated the rationale for reducing the dose of herbicide (atrazine + mesotrione + halosulfuron-methyl) by 25% applied in combination with an adjuvant. In this research, the doses of nicosulfuron reduced by 20% and 40% and applied with the adjuvants showed weed control effectiveness similar to that produced by the full dose of the herbicide in all years of the study. In the opinion of Haliniarz [2019], however, in crops sown in wide rows, such as maize, it is justified to use herbicides at doses reduced by 33% in combination with adjuvant. They reduce the number and dry weight of weeds at a level of application of the recommended herbicide doses.

A study by Mehmeti et al. [2019] shows the high biodiversity of segetal flora in maize grown in monoculture. In the present study, at the first term of weed infestation assessment, the maize monoculture was characterized by higher numbers and greater species diversity of weeds than before harvest, similar to a study by Haliniarz [2019]. The most frequent were: *C. album*, *E. crus-galli*, *P. lapathifolium* subsp. *lapathifolium*, *G. parviflora*, *G. ciliata*, and *A. retroflexus*. A high share of *C. album*, *E. crus-galli*, *A. retroflexus*, and *G. parviflora* in weed infestation in maize grown on different soils was also confirmed by Lehoczky et al. [2013], Haliniarz [2019], and Yanev et al. [2021]. According to Woźnica and Idziak [2015], species such as *C. album*, *E. crus-galli*, and *A. retroflexus* exhibit high competitiveness towards maize. In contrast, according to Landau et al. [2021] *C. album* and *A. retroflexus* are not highly competitive species.

CONCLUSIONS

1. The number of weeds in the maize crop varied between years and herbicide application date, while in the case of weed dry weight also between herbicide application variants., Pre-emergence application of the herbicide (BBCH 00) or early post-emergence application (BBCH 13) had the most beneficial effect on the level of weed infestation in the maize crop. Moreover, the lowest number and biomass of weeds were also found after post-emergence application at split doses (BBCH 13 + BBCH 18). Weeds produced the lowest biomass after application of an 80% rate of the herbicide together with the oil adjuvant.

2. The Shannon-Wiener species diversity index was highest when the herbicide was applied at the earliest – pre-emergence. As a result of the dominance of *Chenopodium album* in the plots where the herbicide was applied at the 3-leaf stage of maize, the Simpson dominance index had the highest value there.

3. The maize yield depends on the number and biomass of weeds assessed both at the maize's 2–3 leaf stage or 3–4 weeks after the herbicide application and before harvest.

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