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Effect of the Time of Herbicide Application and the Properties of the Spray Solution on the Efficacy of Weed Control in Maize (*Zea mays* L.) Cultivation

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Abstract: A field experiment was carried out in 2019–2021. The effect of an increased amount of iron in water and the addition of citric acid on the efficacy of herbicides applied in maize cultivation at various times was tested. In the pre-emergence treatment, thiencazabone-methyl + isoxaflutole were applied, while in the post-emergence treatment, nicosulfuron + tritosulfuron + dicamba were applied once in a full dose or in low dose system at two times in half of the recommended dose with the addition of an adjuvant. In selected combinations, $\text{FeSO}_4 \times 7\text{H}_2\text{O}$ and citric acid were added to the composition of the spray solution. The species composition of weeds and the efficacy of the herbicides used were determined. Plant stress caused by competition from weeds was investigated by measuring the plant chlorophyll fluorescence. The height of the cultivated plants and their yield level were also determined. The lowest efficacy of weed control was observed when the post-emergence herbicides were applied once. Increasing the iron content in water reduced the efficacy of the herbicides, but the addition of citric acid made it possible to decrease this problem.

Keywords: pre-emergence treatment; post-emergence treatment; full and split doses; iron; citric acid



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1. Introduction

Maize is one of the most important crops in the world [1]. Its significance encourages research in terms of both genetic characteristics and methods of cultivation, fertilization, and protection [2–5]. This plant is used in the production of food, animal feed, and for energy purposes [6]. It is characterized by a slow initial growth. Maize is a plant grown in wide inter-rows [7]. This contributes to the fact that it is highly exposed to the competitive influence of weeds [8]. Weeds can significantly reduce the yield of maize and show strong competition for environmental resources [9]. Weeds in maize cultivation appear at different times [10]. It is therefore important to choose the optimal time for herbicide application to control the entire weed species spectrum. Herbicides in maize cultivation can be applied both pre-emergence and post-emergence [11]. In the case of the pre-emergence treatment, the cultivated plant is not exposed to the competitive influence of weeds at the beginning of its development [12]. On the other hand, post-emergence treatments make it possible to adjust herbicides to the spectrum of weeds observed at the time of application [13]. However, it should be remembered that weeds should not be too advanced in growth [14,15]. In maize cultivation, weed management can also be achieved with split application of lower doses of herbicides [16]. This method allows for the selection of different treatment dates to the conditions in the field and for weed control in the period of their greatest sensitivity [17]. Herbicides are then applied in reduced doses and the adjuvant is added to the spray liquid [18]. The application of herbicides in reduced doses with their low efficacy may contribute to the selection of resistant biotypes of weeds [19]. However, the addition of adjuvants and the appropriate timing of the treatment allow

for high efficacy of low doses of herbicides [20,21]. Adjuvants contribute to lowering the surface tension and contact angle of spray liquid drops, better coverage of sprayed plants, and the penetration of herbicides into weed cells, thus increasing the efficacy of plant protection products [22].

Nicosulfuron and tritosulfuron are classified as sulfonylurea, while thien carbazone-methyl is classified as sulfonylaminocarbonyl triazolone chemical families. These substances are acetolactate synthase (ALS) or acetohydroxyacid synthase (AHAS) inhibitors [23]. Nicosulfuron and thien carbazone-methyl are used to control monocotyledonous and dicotyledonous weeds, and tritosulfuron is used to control dicotyledonous weeds [24–26]. Acetohydroxyacid synthase or acetolactate synthase, as catalysts, take part in the synthesis of branched-chain amino acids (isoleucine, valine, and leucine) [27]. Blocking of this process results in chlorosis, necrosis, and reduced plant growth. Sulfonylurea herbicides used to control a wide spectrum of weed species, show low toxicity towards animals and high selectivity towards crops [28]. Nicosulfuron and tritosulfuron show systemic activity, they are used post-emergence [29,30]. Thien carbazone-methyl has a systemic effect, it is used in pre-emergence and post-emergence treatments [31–33].

Dicamba is included in the benzoic acid chemical family [34]. This substance controls some dicotyledonous weeds [35]. It is a synthetic auxin herbicide (SAH) [36]. Due to the complexity of the auxin signaling pathways, the exact mode of action of substances with this mechanism of action is not fully known [37]. The symptoms of the action of these herbicides are tissue thickening, stem curling, inhibition of growth, chlorosis, and necrosis of the treated plants [38]. Dicamba has a systemic effect and is used post-emergence [39].

Isoxaflutol belongs to the isoxazole herbicide chemistry class [40]. It is used to control monocotyledonous and dicotyledonous weeds [41]. It is used pre-emergence and shows a systemic effect [42]. It belongs to the 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides [43]. It is an enzyme that is involved in the carotenoid synthesis pathway [44]. It takes part in the process of converting tyrosine to α -tocopherol and plastoquinone [45]. Blocking this process results in disturbances in the synthesis of carotenoids, and in a further stage in chlorophyll damage and plant bleaching [46].

The efficacy of herbicides is influenced by many factors [47]. It is possible to distinguish, the species composition of weeds occurring in the field and their development phase [48]. Weather conditions are also very important. In the case of herbicides applied to the soil, low soil moisture may contribute to a significant decrease in their efficacy [49]. The spraying solution properties also affect the efficacy of plant protection products [50]. The most important of them include the quantity of ions in the water used to prepare the spray solution [51]. One of the metals found in water is iron, the content of which should not exceed 0.2 mgxL^{-1} in drinking water [52]. However, there are reports of detecting significant amounts of this element in tap water [53,54].

Stress in crops can be caused by many factors [55]. One of them is competition from weeds [56]. Measurements of plant chlorophyll fluorescence allow for determining the condition of plants [57]. The parameters determined after darkroom adaptation include F_0 -minimal fluorescence of dark-adapted state, F_v -variable fluorescence, F_m -maximal fluorescence of dark-adapted state, and F_v/F_m -maximum quantum yield of PSII photochemistry [58].

The research hypothesis is that the timing of the herbicide application and the properties of the spray solution have an effect on the efficacy of weed control. The aim of the study was to assess the efficacy of weed control by herbicides applied at various times and in a spray solution modified by the addition of iron and citric acid.

2. Materials and Methods

The field experiment was conducted in 2019–2021 at the Poznan University of Life Sciences Research and Education Center (REC) in Brody ($52^\circ 25' \text{ N}$, $16^\circ 18' \text{ E}$), Poland. The soils of the test fields were classified as loamy sand, with a pH of 6.1–6.8 and an organic matter content of 1.2%. The experiment was performed in a randomized complete block

design, with four replications for each combination. The plots were $2.5 \text{ m} \times 9 \text{ m} = 22.5 \text{ m}^2$. Conventional cultivation was carried out in the experimental field. In each plot, four rows of maize were sown with a spacing of 70 cm. Maize of the "PR39H32" variety was sown annually during the last 10 days of April. The sowing depth was 4 cm. Mineral fertilization was planned considering the nutrient content in the soil and the nutritional needs of the plants. Phosphorus was used in autumn in the year preceding cultivation at the dose of $50\text{--}75 \text{ kg} \times \text{ha}^{-1}$. In the spring, before sowing the crop, $90 \text{ kg N} \times \text{ha}^{-1}$ was applied, in the 7–8 leaves phase of maize, and the second dose of nitrogen was applied at $70 \text{ kg} \times \text{ha}^{-1}$. The height of the maize was measured annually in September. The crops were harvested at the turn of September and October and converted to 15% grain moisture.

The characteristics of thermal and precipitation conditions were presented for decades and for whole months using the Sielianinow's hydrothermal index, calculated according to the following formula:

$$k = \frac{P}{0.1 \Sigma t}$$

k: Sielianinow's hydrothermal index

P: sum of atmospheric precipitation in mm

Σt : sum of air temperatures $>0 \text{ }^\circ\text{C}$

The obtained results were presented for 10 classes of the discussed coefficient (Table 1), in accordance with the methodology of Skowera and Puła [59].

Table 1. Classes of the Sielianinow's hydrothermal index.

K-Index Classes	Values
Extremely dry	$k \leq 0.4$
Very dry	$0.4 < k \leq 0.7$
Dry	$0.7 < k \leq 1.0$
Slightly dry	$1.0 < k \leq 1.3$
Optimum	$1.3 < k \leq 1.6$
Slightly humid	$1.6 < k \leq 2.0$
Humid	$2.0 < k \leq 2.5$
Very humid	$2.5 < k \leq 3.0$
Extremely humid	$k > 3.0$

The herbicides applied in the experiment were Nicogan 040 SC (nicosulfuron-40 g a.i. $\times \text{L}^{-1}$; Adama Polska Sp. z o.o., Warsaw, Poland), Mocarz 75 WG (tritosulfuron-250 g a.i. $\times \text{kg}^{-1}$ + dicamba-500 g a.i. $\times \text{kg}^{-1}$; BASF SE, Ludwigshafen, Federal Republic of Germany), and Adengo 315 SC (thiencarbazone-methyl-90 g a.i. $\times \text{L}^{-1}$ + isoxaflutole-225 g a.i. $\times \text{L}^{-1}$; Bayer SAS, Lyon, French Republic). The doses of individual substances per hectare are given in Tables 5–7. Treatment A was applied pre-emergence (BBCH 00–09), and treatments B and C in early (BBCH 12–13) and later (BBCH 15–16) post-emergence, respectively. The iron level in the water increased with the use of $\text{FeSO}_4 \times 7\text{H}_2\text{O}$ (Chempur, Piekary Śląskie, Poland) at $0.015 \text{ g} \times \text{L}^{-1}$. In selected combinations, citric acid ($\text{C}_6\text{H}_8\text{O}_7$, Archem, Lany, Poland) was added to the spray liquid at $0.25 \text{ g} \times \text{L}^{-1}$. In treatments where the plant protection products were applied at a split doses system, Break-Thru Vibrant adjuvant (Evonik Industries AG, Essen, Germany) was added to the composition of the sprayed liquid at 0.1% volume/volume. Herbicides were applied with CO_2 -pressurized sprayer equipped with flat fan nozzles of Tee Jet DG 11002-VS. The width of the sprayer boom was 2.5 m. The height from the soil surface in the pre-emergence treatment and from the height of the crops in the post-emergence treatment was 50 cm. The debit of the sprayer was $230 \text{ L} \times \text{ha}^{-1}$. Visual evaluation of the efficacy of herbicides was performed 21 days after the application of all herbicides. Efficacy was expressed according to a scale (0–100% of weed control compared to untreated check). Meteorological data during each application are presented in Table 2.

Table 2. Meteorological conditions for individual treatments.

Time of Herbicide Application	Date	Temperature [°C]	Time to the First Rainfall AA	Rainfall		
				1 Week BA	The First Rainfall AA	1 Week AA
				[mm]		
2019						
A	25 April 2019	17.8	3 days	0.1	4.4	7.1
B	21 May 2019	20.6	9 h	28.0	9.4	9.4
C	30 May 2019	12.8	2 days	17.9	0.1	0.1
2020						
A	27 April 2020	18.8	2 days	3.4	1.2	6.3
B	19 May 2020	14.4	4 days	0.0	8.4	17.0
C	1 June 2020	14.8	1 day	10.3	9.0	11.8
2021						
A	28 April 2021	12.0	1 day	0.0	0.6	26.9
B	24 May 2021	18.1	12 h	4.5	0.1	17.0
C	2 June 2021	23.4	1 day	22.7	0.1	0.1

BA—Meteorological data before application; AA—after application; treatment time: A—BBCH 00-09; B—BBCH 12-13; C—BBCH 15-16.

Weed infestation was determined at the beginning of July. All individual weed species were counted in two places (each 1 × 0.7 m²) for each plot, next they were averaged and expressed on a 1 m² basis. Analyses of the plant communities were carried out on permanent research plots, which were homogeneous plant patches of maize. The total number of species in all plots was determined, the weed species in the studied areas were marked, and the number of individuals belonging to respective taxon was determined and they were classified to the appropriate phytosociological system [60]. The species composition of weed communities and the number of plants of each species from the untreated control plots were used to assess the biodiversity by means of the Simpson (D), Shannon–Wiener (H'), and Margalef's (K) indexes [61,62] according to formulas: $D = 1 - \sum p_i$; $H' = -\sum p_i \ln p_i$; $H' = -\sum_{i=1}^k p_i \ln p_i$, where k is the number of categories and p_i is the share of each species in the sample; and $K = \log S / \log N$, where S is the number of species and N is the total number of individuals in the sample. To evaluate the proportion of each plant species, the constancy degree (Braun–Blanquet approach) was determined using the following scale: V—80–100% of all phytosociological relevés; IV—60–80%; III—40–60%; II—20–40%; I—0.01–20%. The cover coefficient was calculated using an expression of the sum of the average percentage of species cover that occurred in all the phytosociological relevés divided by the total number of phytosociological relevés and multiplied by 100 [63].

Measurement of chlorophyll fluorescence was performed using a Multi-Mode Chlorophyll Fluorometer (OS5p, Opti-Sciences, Inc., Hudson, NJ, USA). Each year of the research, the measurements were taken 37 days after the application of the last herbicides. The study was performed on randomly selected plants, on the youngest, fully developed leaves. Two measurements were made on each of the plots, which gave eight results for the combination in each year of the study. Prior to measurement, the leaves were dark adapted for 30 min with white clips to silence the photosynthesis. Before starting the study, the parameters were set so that the fluorescence signal was in the range of 150–250 counts and was stable. The manuscript presents the results for certain parameters, namely: F₀ is minimum fluorescence, F_m is maximum fluorescence, F_v is variable fluorescence, and F_v/F_m is the maximum photochemical efficiency of photosystem II.

The Shapiro–Wilk test was used to check the assumption of normality and the raw data needed no transformation. The data were subjected to ANOVA analysis, and next the means were separated by protected Tukey's LSD test with at p = 0.05.

3. Results

In all the years, the classes ranging from slightly dry to extremely dry dominated (Table 3). In 2020, none of the months was classified as an optimal.

Table 3. Results of the Sielianinov index for individual years.

Decade	Months					
	IV	V	VI	VII	VIII	IX
2019						
I	0.0	1.4	0.0	0.3	0.6	1.7
II	0.6	3.4	0.1	1.4	0.5	0.6
III	0.5	1.5	0.3	1.3	0.2	0.6
monthly	0.4	2.1	0.1	1.1	0.4	1.5
2020						
I	0.0	1.2	0.9	2.3	0.4	0.8
II	0.2	1.2	0.3	0.8	2.8	0.0
III	0.4	1.4	1.3	0.0	2.3	2.0
monthly	0.2	1.3	0.8	1.0	1.8	0.9
2021						
I	1.7	4.0	0.5	1.0	1.0	0.0
II	3.1	1.1	0.5	0.3	0.6	1.0
III	0.1	1.4	0.8	0.0	3.4	1.2
monthly	1.6	1.9	0.6	0.4	1.6	0.7

Classes of the Sielianinov's hydrothermal index: $k \leq 0.4$ —extremely dry; $0.4 < k \leq 0.7$ —very dry; $0.7 < k \leq 1.0$ —dry; $1.0 < k \leq 1.3$ —slightly dry; $1.3 < k \leq 1.6$ —optimum; $1.6 < k \leq 2.0$ —slightly humid; $2.0 < k \leq 2.5$ —humid; $2.5 < k \leq 3.0$ —very humid; $k > 3.0$ —extremely humid.

A total of only 14 weed species were identified in all of the study plots during study period (Table 4). Certain species like *Chenopodium album* L., *Capsella bursa-pastoris* (L.) Medik., *Echinochloa crus-galli* (L.) P. Beauv. classified to *Polygono-Chenopodietalia* Order, *Polygonum aviculare* L. classified to *Stellarietea mediae* Class, and *Fallopia convolvulus*; (L.) Á. Löve classified to other species were more prevalent in the community, whereas others, notably *Fumaria officinalis* L., *Lamium purpureum* L. (*Polygono-Chenopodion* Alliance), *Solanum nigrum* L. (*Polygono-Chenopodietalia* Order), and others were much less common. The constancy degree only indicated the presence of the species in the analyzed patch, but in no way indicated its competitiveness. The role that species play in a weed community is expressed by the cover coefficient values. The results of the study indicate a dominant role in the weed community, primarily of the species like *C. album* (5492.0–7491.1), *E. crus-galli* (184.7–2030.7), and *F. convolvulus* (248.6–2694.6).

The communities had the highest values of biodiversity indices in 2020 ($H' = 1.91$; $I = 1.22$) and the lowest index of domination ($D = 0.38$) in 2019. The lowest biodiversity ($H' = 1.29$; $K = 0.75$) and the highest domination ($D = 0.38$) were observed in 2019. The relationships were statistically significant only in case of H' (Figure 1).

The lowest number of weeds on the control plots was recorded in 2020, and the highest in 2021 (Table 5). In 2020, the lowest efficacy of the herbicides used in reducing the number of weeds was observed. In all of the study years, the lowest level of weed reduction was observed in combinations where a single post-emergence treatment was performed. The addition of an iron compound to the composition of the spray liquid contributed to a decrease in the efficacy of the applied plant protection products in terms of the reduction of the number of weeds, but it was not statistically confirmed in all variants.

The highest *C. album* and *E. crus-galli* control (Table 6) were observed for selected combinations in which the herbicides were applied at a low dose and in the pre-emergence system. The addition of an iron compound to the spray solution contributed to the reduction of control of dominant weed species. Treatment of N40 + T50 + D100 + iron had the poorest control (less than 80%) *C. album* and *E. crus-galli*. The use of citric acid allowed to limit the

negative influence of iron on the efficacy of herbicides and significantly improved weed control.

Table 4. Constancy degrees (Braun–Blanquet approach) and cover coefficient values of species in maize.

Species	2019	2020	2021
Species specific to the (ChAll.) <i>Polygono-Chenopodion</i> Alliance			
<i>Fumaria officinalis</i>			I ^{79.4}
<i>Lamium purpureum</i>		II ^{63.3}	
Species specific to the (ChO.) <i>Polygono-Chenopodietalia</i> Order			
<i>Capsella bursa-pastoris</i>	IV ^{85.4}	III ^{70.1}	III ^{207.6}
<i>Chenopodium album</i>	V ^{7491.1}	V ^{5917.1}	V ^{5492.0}
<i>Echinochloa crus-galli</i>	V ^{2030.7}	III ^{184.7}	III ^{250.8}
<i>Solanum nigrum</i>			III ^{65.7}
Species specific to the (ChO.) <i>Centauretalia cyani</i> Order			
<i>Anthemis arvensis</i>			III ^{52.9}
<i>Papaver rhoeas</i>		II ^{94.9}	
Species specific to the (ChCl.) <i>Stellarietea mediae</i> Class			
<i>Anchusa arvensis</i>		IV ^{192.0}	II ^{26.9}
<i>Polygonum aviculare</i>	III ^{59.4}	IV ^{338.5}	IV ^{1779.9}
<i>Viola arvensis</i>		II ^{184.7}	
Species specific to the (ChSCL.) <i>Galio-Urticenea</i> Class			
<i>Galium aparine</i>		II ^{260.0}	
Other species			
<i>Fallopia convolvulus</i>	IV ^{248.6}	V ^{2694.6}	V ^{2026.9}
<i>Polygonum lapathifolium</i> ssp. <i>brittingeri</i>	II ^{84.7}		

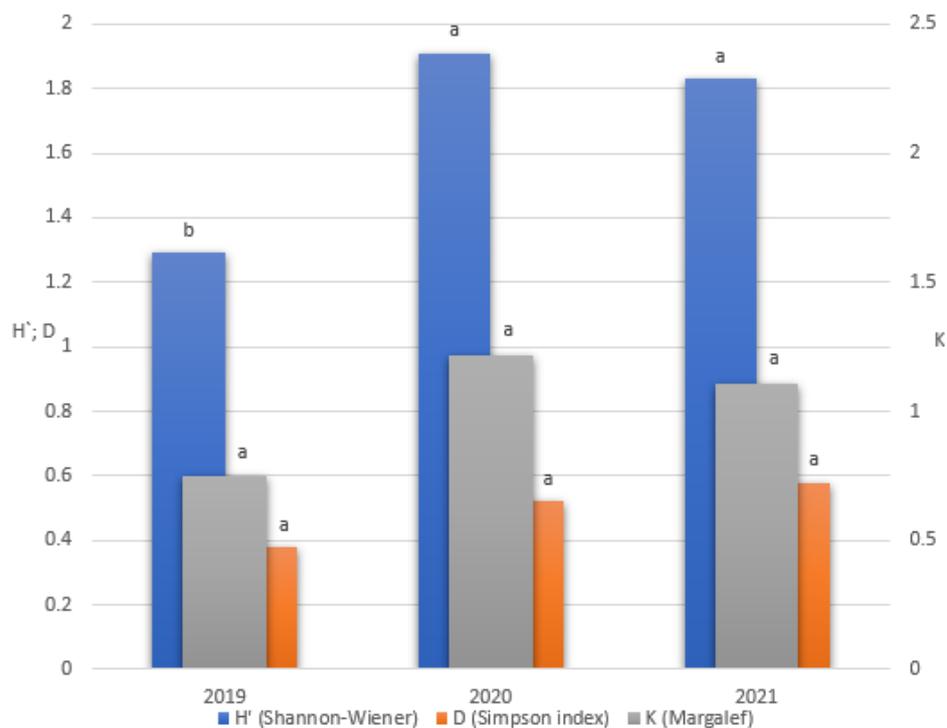


Figure 1. Shannon–Wiener (H'), Simpson's (D) indexes of weed diversity, and Margalef's richness index in maize. The same letters indicate that treatments were not significantly different according to the Tukey test.

Table 5. Number of weeds per m² and percentage of their reduction compared to control (untreated check) [%].

No.	Treatment	Time of Herbicide Application	Year		
			2019	2020	2021
1	Untreated check	-	124.7 (0.0%)	85.4 (0.0%)	137.9 (0.0%)
2	N40 + T50 + D100	C	12.9 ab (89.7%)	29.6 abc (65.3%)	40.7 ab (70.5%)
3	N40 + T50 + D100 + iron	C	15.4 a (87.7%)	44.3 a (48.1%)	47.5 a (65.5%)
4	N40 + T50 + D100 + iron + CA	C	8.9 abc (92.8%)	34.3 ab (59.8%)	41.8 ab (69.7%)
5	N20 + T25 + D50 + BT	B; C	4.3 c (96.6%)	27.9 bc (67.3%)	23.6 bcd (82.9%)
6	N20 + T25 + D50 + BT + iron	B; C	5.4 c (95.7%)	29.0 bc (66.1%)	41.8 ab (69.7%)
7	N20 + T25 + D50 + BT + iron + CA	B; C	9.3 abc (92.5%)	24.3 bc (71.5%)	31.8 abc (77.0%)
8	T29 + I74	A	2.5 c (98.0%)	15.7 c (81.6%)	5.0 d (96.4%)
9	T29 + I74 + iron	A	2.9 c (97.7%)	16.1 c (81.2%)	9.6 cd (93.0%)
10	T29 + I74 + iron + CA	A	2.2 c (98.3%)	15.0 c (82.4%)	8.6 d (93.8%)

N40 + T50 + D100—nicosulfuron 40 + tritosulfuron 50 + dicamba 100 g × ha⁻¹; N40 + T50 + D100 + iron—nicosulfuron 40 + tritosulfuron 50 + dicamba 100 g × ha⁻¹ with an increased amount of iron; N40 + T50 + D100 + iron + CA—nicosulfuron 40 + tritosulfuron 50 + dicamba 100 g × ha⁻¹ with an increased amount of iron and citric acid; N20 + T25 + D50 + BT—nicosulfuron 20 + tritosulfuron 25 + dicamba 50 g × ha⁻¹ + Break-Thru Vibrant adjuvant; N20 + T25 + D50 + BT + iron—nicosulfuron 20 + tritosulfuron 25 + dicamba 50 g × ha⁻¹ + Break-Thru Vibrant adjuvant + increased amount of iron; N20 + T25 + D50 + BT + iron + CA—nicosulfuron 20 + tritosulfuron 25 + dicamba 50 g × ha⁻¹ + Break-Thru Vibrant adjuvant + increased amount of iron and citric acid; T29 + I74—thiencarbazone-methyl 29.7 + isoxaflutole 74.25 g × ha⁻¹; T29 + I74 + iron—thiencarbazone-methyl 29.7 + isoxaflutole 74.25 g × ha⁻¹ with an increased amount of iron; T29 + I74 + iron + CA—thiencarbazone-methyl 29.7 + isoxaflutole 74.25 g × ha⁻¹ with an increased amount of iron and citric acid. A—BBCH 00–09; B—BBCH 12–13; C—BBCH 15–16. The same letters indicate that treatments were not significantly different according to the Tukey test.

Table 6. Visual assessment of the *Chenopodium album* (CHEAL) and *Echinochloa crus-galli* (ECHCG) control [%].

No.	Treatment	Time of Herbicide Application	CHEAL			ECHCG		
			2019	2020	2021	2019	2020	2021
1	Untreated check	-	0.0 f	0.0 f	0.0 f	0.0 e	0.0 f	0.0 f
2	N40 + T50 + D100	C	86.3 d	76.3 d	83.8 d	81.3 cd	80.0 d	78.8 d
3	N40 + T50 + D100 + iron	C	78.8 e	70.0 e	77.5 e	78.8 d	71.3 e	73.8 e
4	N40 + T50 + D100 + iron + CA	C	90.0 cd	82.5 c	88.8 c	82.5 cd	82.5 d	80.0 cd
5	N20 + T25 + D50 + BT	B; C	98.8 a	90.0 ab	97.5 a	93.8 ab	88.8 bc	92.5 ab
6	N20 + T25 + D50 + BT + iron	B; C	86.3 d	81.3 c	83.8 d	85.0 c	81.3 d	83.8 c
7	N20 + T25 + D50 + BT + iron + CA	B; C	97.5 a	88.8 ab	98.8 a	93.8 ab	90.0 b	92.5 ab
8	T29 + I74	A	91.3 bc	90.0 ab	88.8 c	97.5 a	91.3 ab	95.0 a
9	T29 + I74 + iron	A	81.3 e	87.5 b	78.8 e	91.3 b	86.3 c	88.8 b
10	T29 + I74 + iron + CA	A	95.0 ab	91.3 a	93.8 b	96.3 b	93.8 a	95.0 a

N40 + T50 + D100—nicosulfuron 40 + tritosulfuron 50 + dicamba 100 g × ha⁻¹; N40 + T50 + D100 + iron—nicosulfuron 40 + tritosulfuron 50 + dicamba 100 g × ha⁻¹ with an increased amount of iron; N40 + T50 + D100 + iron + CA—nicosulfuron 40 + tritosulfuron 50 + dicamba 100 g × ha⁻¹ with an increased amount of iron and citric acid; N20 + T25 + D50 + BT—nicosulfuron 20 + tritosulfuron 25 + dicamba 50 g × ha⁻¹ + Break-Thru Vibrant adjuvant; N20 + T25 + D50 + BT + iron—nicosulfuron 20 + tritosulfuron 25 + dicamba 50 g × ha⁻¹ + Break-Thru Vibrant adjuvant + increased amount of iron; N20 + T25 + D50 + BT + iron + CA—nicosulfuron 20 + tritosulfuron 25 + dicamba 50 g × ha⁻¹ + Break-Thru Vibrant adjuvant + increased amount of iron and citric acid; T29 + I74—thiencarbazone-methyl 29.7 + isoxaflutole 74.25 g × ha⁻¹; T29 + I74 + iron—thiencarbazone-methyl 29.7 + isoxaflutole 74.25 g × ha⁻¹ with an increased amount of iron; T29 + I74 + iron + CA—thiencarbazone-methyl 29.7 + isoxaflutole 74.25 g × ha⁻¹ with an increased amount of iron and citric acid; A—BBCH 00–09; B—BBCH 12–13; C—BBCH 15–16; The same letters indicate that treatments were not significantly different according to the Tukey test.

Measurement of plant chlorophyll fluorescence allowed for the evaluation of the stress in crops induced by the competition of maize for environmental resources with weeds (Figures 2–5). However, it was not statistically confirmed for all the parameters tested. In the case of F_0 , no statistically significant differences were observed between individual combinations in all years of the study. For the remaining parameters (F_m , F_v , and F_v/F_m), a statistically significant decrease in the values for the control combination was observed. In addition, a statistically significant reduction in the value of these parameters was recorded in 2020 for a combination in which a single post-emergence herbicide treatment was applied, and an iron compound was added to the composition of the spray solution.

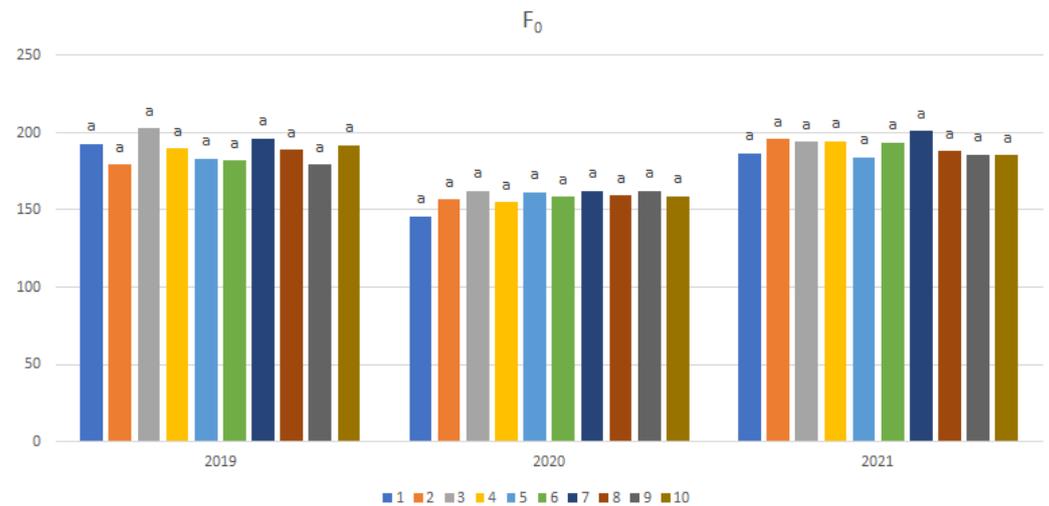


Figure 2. F_0 (minimum fluorescence) results for maize in individual years of research. Different letters indicate statistically different mean LSD (0.05): 2019 = 18.98; 2020 = 11.27; 2021 = 15.63. The combination numbers are as specified in Tables 5 and 6.

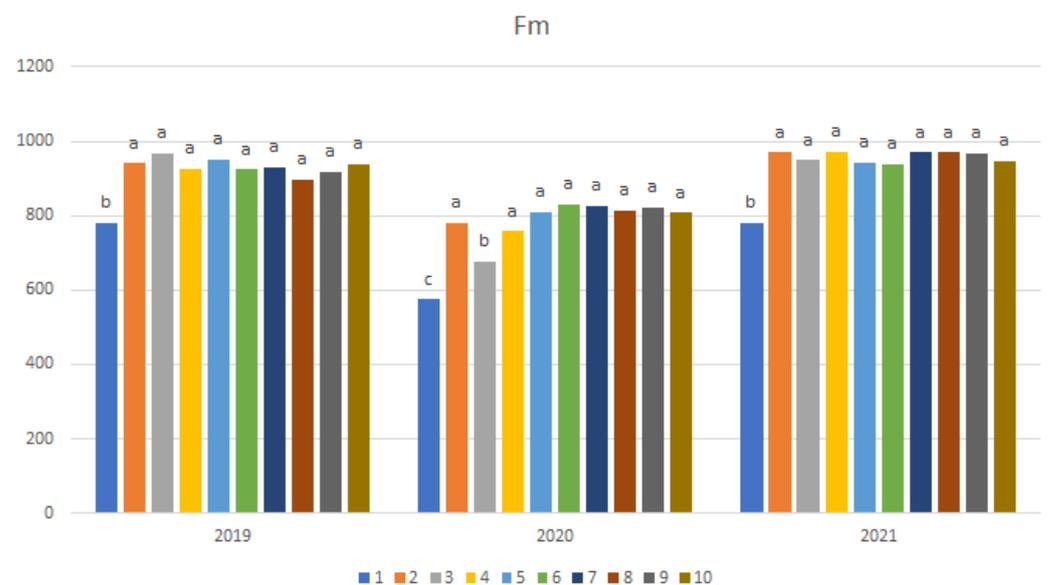


Figure 3. F_m (maximum fluorescence) results for maize in individual years of research. Different letters indicate statistically different mean LSD (0.05): 2019 = 85.29; 2020 = 62.31; 2021 = 102.31. The combination numbers are as specified in Tables 5 and 6.

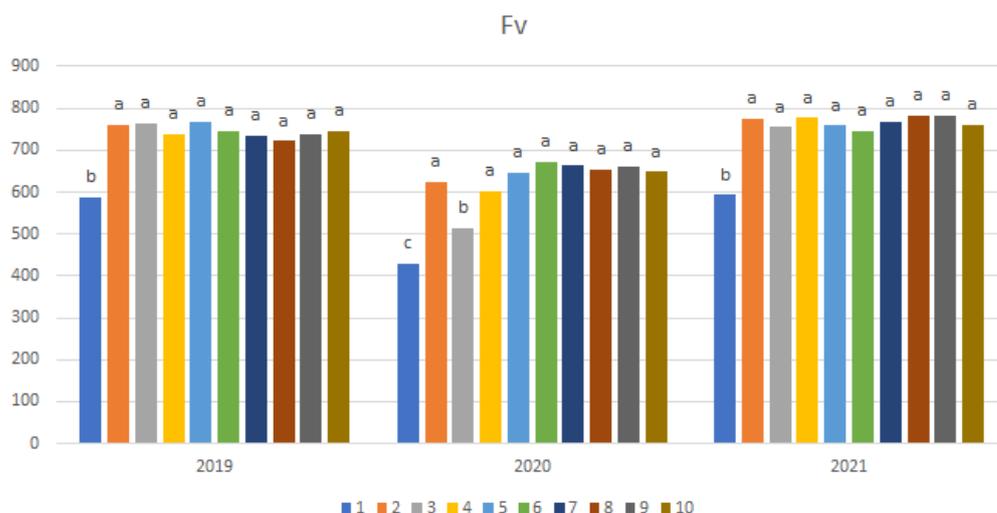


Figure 4. Fv (variable fluorescence) results for maize in individual years of research. Different let-ters indicate statistically different mean LSD (0.05): 2019 = 79.59; 2020 = 58.64; 2021 = 93.13. The combination numbers are as specified in Tables 5 and 6.

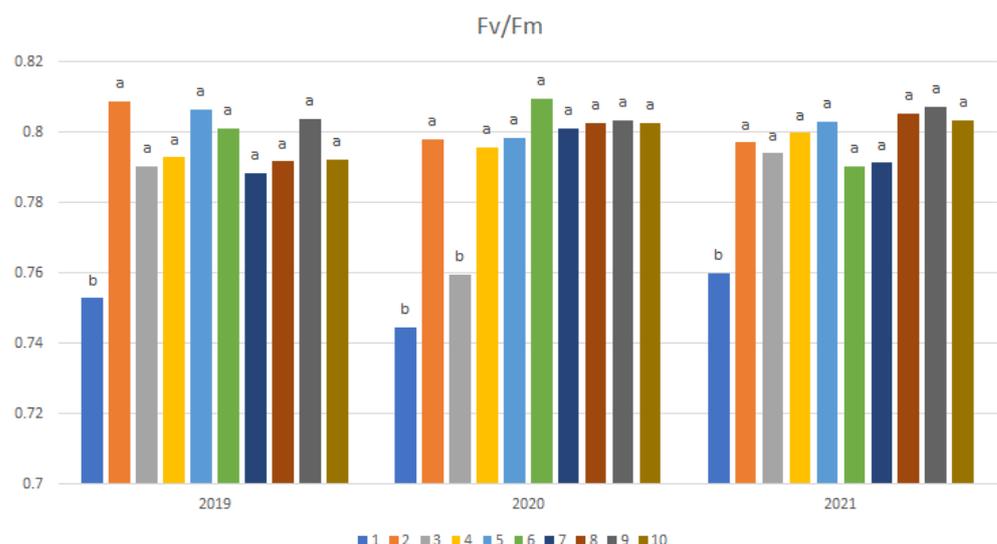


Figure 5. Fv/Fm (maximum photochemical efficiency of photosystem II) results for maize in individual years of research. Different letters indicate statistically different mean LSD (0.05): 2019 = 0.024; 2020 = 0.017; 2021 = 0.017. The combination numbers are as specified in Tables 5 and 6.

In 2019, no statistically significant differences in the height of crops between herbicide treatments were observed (Table 7), except in the untreated check where the plants were significantly lower. In 2020 and 2021, for combinations with herbicides, the lowest plants were observed for variants with a single post-emergence treatment. The herbicide application contributed in all combinations to the increase in maize height compared to the control.

The use of all herbicide variants contributed to a statistically significant increase in maize yield (Table 7). In 2019, the highest yield was observed within all periods of application of plant protection products. In the case of subsequent years, the highest level of yielding was recorded when herbicides were applied post-emergence at split doses and as a pre-emergence treatment. The lowest grain maize yield was recorded in 2020.

In all years of research, the use of plant protection products contributed to a statistically significant increase in the level of hectolitre mass (kg hL^{-1}) compared to the control (Table 8). In 2019 and 2021, there were no statistically significant differences in the weight of hectolitre

in combinations where herbicide protection was applied. In 2020, the highest value of the weight of hectolitre was recorded for combinations where the herbicides were applied in the low dose system and pre-emergence (without the addition of the iron compound and in the combination in which, in addition to the iron compound, citric acid was added to the spray solution composition).

Table 7. Impact of dose and time application on maize height and grain yield.

No.	Treatment	Time of Herbicide Application	Plant Height			Grain Yield		
			2019	2020	2021	2019	2020	2021
			[cm]			[t × ha ⁻¹]		
1	Untreated check	-	78.8 b	107.8 d	72.9 e	0.7 d	0.3 e	0.0 c
2	N40 + T50 + D100	C	167.5 a	177.2 c	196.8 d	9.6 abc	4.1 cd	9.3 b
3	N40 + T50 + D100 + iron	C	163.8 a	190.9 c	198.1 cd	9.2 bc	4.0 d	9.3 b
4	N40 + T50 + D100 + iron + CA	C	161.1 a	200.4 bc	201.3 bcd	10.0 ab	5.2 bc	9.5 b
5	N20 + T25 + D50 + BT	B; C	161.2 a	224.0 ab	208.6 abc	10.1 ab	6.2 ab	10.2 ab
6	N20 + T25 + D50 + BT + iron	B; C	163.2 a	227.1 a	209.5 ab	9.7 abc	6.5 a	9.9 ab
7	N20 + T25 + D50 + BT + iron + CA	B; C	160.8 a	231.9 a	206.6 abcd	10.5 a	6.6 a	9.9 ab
8	T29 + I74	A	159.8 a	231.2 a	210.8 ab	9.4 bc	6.4 a	10.6 ab
9	T29 + I74 + iron	A	160.4 a	228.4 a	213.7 a	8.9 c	6.2 ab	10.3 ab
10	T29 + I74 + iron + CA	A	159.0 a	224.2 ab	212.1 ab	10.1 ab	6.8 a	11.3 a

N40 + T50 + D100—nicosulfuron 40 + tritosulfuron 50 + dicamba 100 g × ha⁻¹; N40 + T50 + D100 + iron—nicosulfuron 40 + tritosulfuron 50 + dicamba 100 g × ha⁻¹ with an increased amount of iron; N40 + T50 + D100 + iron + CA—nicosulfuron 40 + tritosulfuron 50 + dicamba 100 g × ha⁻¹ with an increased amount of iron and citric acid; N20 + T25 + D50 + BT—nicosulfuron 20 + tritosulfuron 25 + dicamba 50 g × ha⁻¹ + Break-Thru Vibrant adjuvant; N20 + T25 + D50 + BT + iron—nicosulfuron 20 + tritosulfuron 25 + dicamba 50 g × ha⁻¹ + Break-Thru Vibrant adjuvant + increased amount of iron; N20 + T25 + D50 + BT + iron + CA—nicosulfuron 20 + tritosulfuron 25 + dicamba 50 g × ha⁻¹ + Break-Thru Vibrant adjuvant + increased amount of iron and citric acid; T29 + I74—thiencarbazone-methyl 29.7 + isoxaflutole 74.25 g × ha⁻¹; T29 + I74 + iron—thiencarbazone-methyl 29.7 + isoxaflutole 74.25 g × ha⁻¹ with an increased amount of iron; T29 + I74 + iron + CA—thiencarbazone-methyl 29.7 + isoxaflutole 74.25 g × ha⁻¹ with an increased amount of iron and citric acid; A—BBCH 00–09; B—BBCH 12–13; C—BBCH 15–16; The same letters indicate that treatments were not significantly different according to the Tukey test.

Table 8. Impact of dose and time application on hectolitre weight and weight of a thousand grains.

No.	Treatment	Time of Herbicide Application	Hectolitre Weight			WTG		
			2019	2020	2021	2019	2020	2021
			[kg × hl ⁻¹]			[g]		
1	Untreated check	-	35.4 b	34.0 d	0.00 b	272.4 b	214.7 b	0.0c
2	N40 + T50 + D100	C	71.7 a	66.5 bc	66.8 a	315.1 a	243.1 a	321.1 ab
3	N40 + T50 + D100 + iron	C	71.6 a	65.2 c	66.9 a	307.9 a	245.5 a	319.3 b
4	N40 + T50 + D100 + iron + CA	C	72.1 a	66.2 c	66.5 a	309.0 a	240.2 a	318.6 b
5	N20 + T25 + D50 + BT	B; C	70.3 a	67.5 abc	66.4 a	313.0 a	244.7 a	324.5 ab
6	N20 + T25 + D50 + BT + iron	B; C	72.0 a	69.2 ab	67.6 a	311.5 a	237.8 a	333.6 ab
7	N20 + T25 + D50 + BT + iron + CA	B; C	71.3 a	70.1 a	66.6 a	314.9 a	245.3 a	327.3 ab
8	T29 + I74	A	72.4 a	68.3 ab	66.0 a	307.0 a	246.5 a	337.0 a
9	T29 + I74 + iron	A	71.4 a	66.8 bc	67.0 a	307.6 a	239.5 a	329.2 ab
10	T29 + I74 + iron + CA	A	70.5 a	70.4 a	67.3 a	309.8 a	241.2 a	337.3a

WTG—weight of a thousand grains; N40 + T50 + D100—nicosulfuron 40 + tritosulfuron 50 + dicamba 100 g × ha⁻¹; N40 + T50 + D100 + iron—nicosulfuron 40 + tritosulfuron 50 + dicamba 100 g × ha⁻¹ with an increased amount of iron; N40 + T50 + D100 + iron + CA—nicosulfuron 40 + tritosulfuron 50 + dicamba 100 g × ha⁻¹ with an increased amount of iron and citric acid; N20 + T25 + D50 + BT—nicosulfuron 20 + tritosulfuron 25 + dicamba 50 g × ha⁻¹ + Break-Thru Vibrant adjuvant; N20 + T25 + D50 + BT + iron—nicosulfuron 20 + tritosulfuron 25 + dicamba 50 g × ha⁻¹ + Break-Thru Vibrant adjuvant + increased amount of iron; N20 + T25 + D50 + BT + iron + CA—nicosulfuron 20 + tritosulfuron 25 + dicamba 50 g × ha⁻¹ + Break-Thru Vibrant adjuvant + increased amount of iron and citric acid; T29 + I74—thiencarbazone-methyl 29.7 + isoxaflutole 74.25 g × ha⁻¹; T29 + I74 + iron—thiencarbazone-methyl 29.7 + isoxaflutole 74.25 g × ha⁻¹ with an increased amount of iron; T29 + I74 + iron + CA—thiencarbazone-methyl 29.7 + isoxaflutole 74.25 g × ha⁻¹ with an increased amount of iron and citric acid; A—BBCH 00–09; B—BBCH 12–13; C—BBCH 15–16; The same letters indicate that treatments were not significantly different according to the Tukey test.

In all of the years of research, the use of plant protection products contributed to a statistically significant increase in the weight of 1000 grains compared to the control (Table 8). In 2019 and 2020, there were no statistically significant differences between herbicide treatments. In 2021, the highest weight of thousand grain was recorded for the combination in which the herbicides containing N40 + T50 + D100 treatment were applied once (without any addition to the composition of the spray liquid) and in combinations where the herbicides were applied in a split doses system and pre-emergence.

4. Discussion

Agriculture is commonly considered to be one of the main threats to the biological diversity [64]. According to Gawęda et al. [65] and Płaza et al. [66], the richness of the field weed community depends on weed control methods, crop rotation, cultivation, selection of species and cultivars, sowing time, sowing quantity, row spacing, and soil mulching, and are called anthropogenic weed community arable fields [67]. Weed species, classified as an archaeophytes, e.g., *Viola arvensis*, naturally occur in arable land and are common throughout Poland. The values of the D and K indexes indicate a relatively persistent stability of weed communities for all of the years of the study. On the basis of the H' index, it was found that in the first year, the biodiversity of communities was lower, which was influenced by environmental conditions, mainly the weather conditions during the field study. H-index values in 2020 and 2021 were most strongly influenced by the presence in the weed community of species such as *Fumaria officinalis*, *Lamium purpureum*, *Solanum nigrum*, *Papaver rhoeas*, *Anthemis arvensis*, *Anchusa arvensis*, *Viola arvensis*, and *Galium aparine*, which did not occur in 2019.

In the conducted experiment, a high herbicidal efficacy was observed for the combinations in which pre-emergence herbicides were applied. These plant protection products made it possible to protect maize against competition from weeds from the very beginning of its development [68,69]. However, it should be remembered that the condition for achieving the appropriate efficacy of this type of treatment is adequate soil moisture [70]. In the conducted research, rainfall was noted in all years of the experiment, shortly after the application of the pre-emergence herbicide, which allowed for the uptake of the active ingredients by the germinating weeds. This made it possible to achieve the high efficacy for the applied herbicides.

The lowest efficacy of the applied plant protection products was observed when herbicides were applied once in the 5–6 leaves stage of the maize. Weeds were then advanced in growth, which made it difficult to control them effectively. The dominant species in all the years of research was *Chenopodium album*. As the species grew, it became covered with an increasingly thicker layer of wax, which reduced the level of herbicide penetration into the plant cells and their effect [71]. An additional factor contributing to the formation of a thicker wax barrier may be drought [72]. Therefore, in conditions unfavorable to the effects of herbicides, it is worth considering the application of herbicides in the low dose system.

The use of split doses allows for effective weed control. Additionally, it reduces their deposition in soil [73]. This technology is already well known in the cultivation of sugar beet [74]. Currently, guidelines have been introduced that place great importance on reducing the amount of chemical preparations used in agriculture [75]. The search for methods that allow for effective weed control is therefore a significant challenge in modern field protection [76]. The use of a split doses system allows for limiting the amount of herbicides that end up in the environment [77]. The low efficacy of herbicides used in the advanced development stage of weeds may result in the re-application of plant protection products in agricultural practice. An appropriately high efficiency of herbicides applied in the system of split doses system may eliminate this possibility.

One of the conditions for achieving the appropriate efficacy of herbicides is the use of water of an appropriate quality to prepare the spray solution [78]. This carrier is the main component of the spraying solution, so it can largely affect the efficacy of the treatments [79].

In the experiment, the increased content of iron ions contributed to the decrease in the efficacy of the herbicides used. The decrease in the herbicidal efficacy of plant protection products due to the increased content of iron ions and other metal ions has been described for various herbicides [80,81]. The addition of citric acid made it possible to eliminate the negative influence of the additional substance on the efficacy of weed control. Citric acid has sequestering properties; therefore, it inactivates the ions contained in water [82]. Thanks to this, it enables the prevention of precipitation of salts containing herbicide ions, and thus allows for maintaining the appropriate efficacy of the applied plant protection products.

There are many results of studies available in the literature on the stress of crops caused by the applied herbicides, which was demonstrated by measuring the plant chlorophyll fluorescence [83,84]. Sometimes, however, stress and damage caused by herbicides are transient and have no effect on yield [85,86]. It should be remembered that the competitive influence of weeds also contributes to plant stress [87,88]. Cultivated plants lack space, light, water, and nutrients [89]. The results of the plant chlorophyll fluorescence measurement showed maize stress on the control plots. In 2020, the measurement of this parameter also showed crop stress for the combination with the lowest level of weed control. It was demonstrated for most of the parameters tested. Only in the case of F_0 , no statistically significant differences were found between the individual combinations. The response of plants caused, *inter alia*, by water shortages or the content of nutrients, does not always significantly affect the value of this parameter [90,91].

Competition from weeds reduces the yield of maize [92]. In the case of late weeding, the cultivated plants are exposed to the competitive influence of weeds for a significant period of their development, which later translates into the yield level [93]. In our own experiment, the highest yield losses were observed for the combinations with the lowest level of weed control and for the control. A reduction in plant height was also observed with these treatments. Reducing the size of maize was disadvantageous from the point of view of the possibility of using this plant for animal feed or for energy purposes.

5. Conclusions

The weed communities had the highest values of biodiversity indices in 2020 and the lowest index of domination in 2019. In the experiment, the lack of weed control and its low level contributed to the stress on maize shown in the measurement of chlorophyll fluorescence (in F_v , F_m , and F_v/F_m parameters) and a decrease in the plant height and yield (grain yield, hectolitre mass, and weight of a thousand grains) of the crop. Late herbicide treatment turned out to be the least effective, and additionally, maize was exposed to competitive weeds for a long period of development. The addition of a compound containing iron ions to the composition of the spray solution contributed to a decrease in the efficacy of the applied plant protection products, while the use of citric acid allowed for reducing this effect. Research on the optimization of herbicides used is important both from the point of view of the desire to obtain high yields and the need to reduce the amount of chemicals that end up in the environment.

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