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Article

Response of Industrial Hemp (*Cannabis sativa* L.) to Herbicides and Weed Control

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Abstract: Industrial hemp is a continuously expanding crop; however, there has been limited research on its herbicide selectivity and weed control. Pendimethalin, s-metolachlor and aclonifen at 1137.5, 960 and 1800 g a.i. ha^{-1}, respectively, were applied in field experiments in 2022 and 2023 in Greece to study the response of industrial hemp to pre-emergence (PRE) herbicides and record their efficacy on weeds. In 2023, each PRE herbicide was followed by the postemergence application of cycloxydim at 200 g a.i. ha^{-1} due to infestation of *Sorghum halepense*. In 2022, retardation in hemp growth was recorded by all PRE herbicide treatments, with there being a slight reduction in stand counts by pendimethalin and s-metolachlor and leaf yellowing by aclonifen in one the experiments. In 2023, no reductions in crop establishment and plant height were recorded, whereas leaf discoloration caused by aclonifen was less evident; cycloxydim did not affect hemp and perfectly controlled *S. halepense*. Despite the herbicide injury, hemp recovered and succeeded in higher biomass in both experiments at Thessaloniki and in higher seed production in the 2023 Thessaloniki experiment. This study showed that pendimethalin, s-metolachlor and aclonifen can be regarded as potential pre-emergence options with precautions in wet and light soils.

Keywords: chemical control; herbicide injury; herbicides; crop tolerance; weed management

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1. Introduction

Cannabis sativa L., also known as industrial hemp (hereinafter "hemp"), has been cultivated all around the world for centuries for the production of textile fibers, food and medicine purposes [1]. Over recent decades, hemp has also been cultivated as an energy crop due to its high biomass production and as a building and construction material [2]. Globally, hemp cultivation has emerged as a successful commercial crop due to its carbon-sequestering property and its phytoremediation capacity [3], being a sustainable source of cellulose for paper manufacturing and for generating zero waste because all parts of the plant can be further processed [4]. The crop was prohibited in the late 1960s [5] as consequence of marijuana illegalization [6]. The reintroduction of hemp cultivation in Greece started in 2016 and nowadays around 100 hectares are cultivated each year for it. According to European legislation, the cultivated varieties need to be registered as having a European Catalogue with Tetrahydrocannabinol (THC) content of <0.3%. The main hemp products from the crop in Greece are seeds for oil production and hemp flower buds [7]. In the European Community, the area dedicated to hemp cultivation has significantly increased in recent years from 20,540 hectares (ha) in 2015 to 33,020 ha in 2022 (60% increase), whereas its production increased from 97,130 tons to 179,020 tons (84.3% increase) [8].

Hemp is a very competitive crop against weeds due to its high growth rate (50 cm/month) and its ability to produce a higher amount of biomass [9,10], usually rendering weed control unnecessary [11,12]. In contrast, several reports have highlighted the necessity for weed control, particularly when shorter hemp cultivars are used or seeded at low densities due to the low competitive ability of hemp in the first weeks after emergence [13,14]. However, hemp has been reported to be sensitive to the direct application of herbicides or herbicide soil residues [12,15]. Till today, the registered herbicides for weed control for hemp, ethafluralin and quizalofop-p-ethyl, are restricted only in Canada [16,17]. Pendimethalin, acetochlor and s-metolachlor have been used in China [12,18–20]. In general, chemical weed control for hemp has received little attention [14]. As the cultivation of hemp is expanding, the lack of information regarding hemp's response to herbicides is of great concern for many hemp growers [15]. Among the preemergence (PRE) herbicides studied in previous years, pendimethalin, s-metolachlor and aclonifen were reported to cause less injury to hemp compared to other PRE herbicides tested such as clomazone, mesotrione, norflurazone and isoxaflutole [21–24], whereas cycloxydim applied postemergence (POST) has also been well tolerated by hemp [13]. Pendimethalin, a dinitroaniline herbicide assigned to Herbicide-Resistant Action Committee (HRAC) group 3, is a cell division inhibitor; s-metolachlor an a-chloroacetamide are assigned to (HRAC) group 15 that targets very long-chain fatty acid synthesis; aclonifen, a diphenyl ether herbicide listed in HRAC group 32, targets the solanesyl diphosphate synthase. Cycloxydim, an ACCase inhibitor, belongs to cyclohexanediones assigned to HRAC group 1 [25].

Due to limited research on chemical weed control in industrial hemp, the herbicides pendimethalin, s-metolachlor and aclonifen, already registered in Greece but not for the industrial hemp crop, were selected in this study to evaluate herbicide selectivity and weed control for hemp.

2. Materials and Methods

2.1. Plant Material

A field experiment was conducted in 2022 and 2023 in one location at the experimental farm of the Institute of Plant Breeding and Genetic Resources in Thessaloniki, Greece, in two different experimental fields with a short distance between them (40.536291 N, 23.005842 E) and (40.53654 N, 23.00174 E) due to crop rotation restrictions. In 2022, the same experiment was conducted in another location, at the experimental farm of the Department of Agriculture of the University of Ioannina in Arta (39.12233 N, 20.94592 E), Greece. Futura 75, a French-origin monoecious cultivar supplied by the Cooperative Centrale des Producteurs de Semensces de Chanvre of France, was chosen as the model crop. Futura 75 is characterized by a late vegetative cycle (<145 days), high biomass and seed production (i.e., 10–12 tn ha^{-1} and 0.8–1.0 tn ha^{-1}, respectively), with THC < 0.3% as required by EC regulation (No. 1173/2022) and for wide use by Greek farmers.

2.2. Crop Establishment and Growth Conditions

Hemp was hand-seeded on 14 April 2022, on 25 April 2023 in Thessaloniki and on 12 April 2022 in Arta, with a seed rate of 30 kg ha^{-1} in plots of 5 m length with 80 cm distance between rows. Each plot consisted of 4 rows of hemp. The soil type in Thessaloniki in 2022 experiment was sandy loam (52% sand, 14% clay, 34% silt) with pH 7.9, Electric Conductivity (EC) 0.451 mS/cm, 4.0% $CaCO_3$ and 1.2% organic matter content, whereas in 2023 it was loam (42% sand, 22% clay, 36% silt), with pH 7.7, EC 0.472 mS/cm, 3.5% $CaCO_3$ and 2.2% organic matter content. In both fields, wheat was the previous crop before hemp establishment. The soil type in Arta was clay (12% sand, 55% clay, 33% silt) with pH 8.0, EC 0.130 mS/cm, 12.9% $CaCO_3$ and 1.5% organic matter content. The field unit prior to hemp establishment was under fallow. In all experimental fields, the seedbed was prepared with a moldboard followed by disc-harrowing.

One day before sowing, basic fertilization was applied in all experiments that included nitrogen in the form of ammonium sulfate ($(NH_4)_2SO_4$), phosphorus as triple superphosphate ($Ca(H_2PO_4)_2$) and potassium as potassium sulfate (K_2SO_4) of the 20.5-0-0, 0-46-0 and 0-0-50 fertilizers, respectively (Table 1). Topdressing nitrogen as ammonium nitrate (NH_4NO_3) fertilization of the 33.5-0-0 fertilizer was applied on 1 June 2022 and on 16 June

2023 for each trial at Thessaloniki, respectively. Nitrogen as NH_4NO_3, phosphorus as P_2O_5 and potassium as K_2O at the rate of 180, 50 and 184 kg ha^{-1}, respectively, were applied in the Arta experimental field (Table 1).

Table 1. Herbicide active ingredients (ai) grouped by HRAC (Herbicide-Resistance Action Committee), time of application, Mode of Action (MoA) and information on the products used (trade name, type of formulation and manufacturer).

Herbicide Ai	HRAC Group	Time of Application	MoA	Product Information
pendimethalin	3	PRE *	Inhibition of microtubule assembly	Aqua Stomp 455 CS (45.5% ai) (BASF Hellas)
s-metolachlor	15	PRE	Inhibition of very long-chain fatty acid synthesis	Dual Gold 96 EC (96% ai) (Syngenta Hellas)
aclonifen	32	PRE	Inhibition of solanesyl diphosphate synthase	Challenge 600 SC (60% ai) (Bayer Hellas)
cycloxydim #	1	POST **	Inhibition of acetyl-CoA carboxylase	Focus 10 EC (10% ai) (BASF Hellas)

Cycloxydim was applied only in Thessaloniki experiment 2023. * PRE = pre-emergence; ** POST = post-emergence.

The total month rainfall and the average month temperature for the hemp growing season for the experiments in Thessaloniki and in Arta are presented in Figure 1 and in Figure 2, respectively. The PRE herbicides pendimethalin, s-metlolachlor and aclonifen (Table 1) were applied at 1137.5, 960 and 1800 g a.i. ha^{-1} in all experiments. The rates selected in this study were the lower or medium rates for each herbicide as stated on the label of each herbicide. The selection of these rates was based on preliminary pot trials with soil from the farm of the Institute of Plant Breeding and Genetic Resources. The PRE herbicides were applied 2 days after sowing (DAS) in both field experiments in Thessaloniki and 1 DAS in Arta.

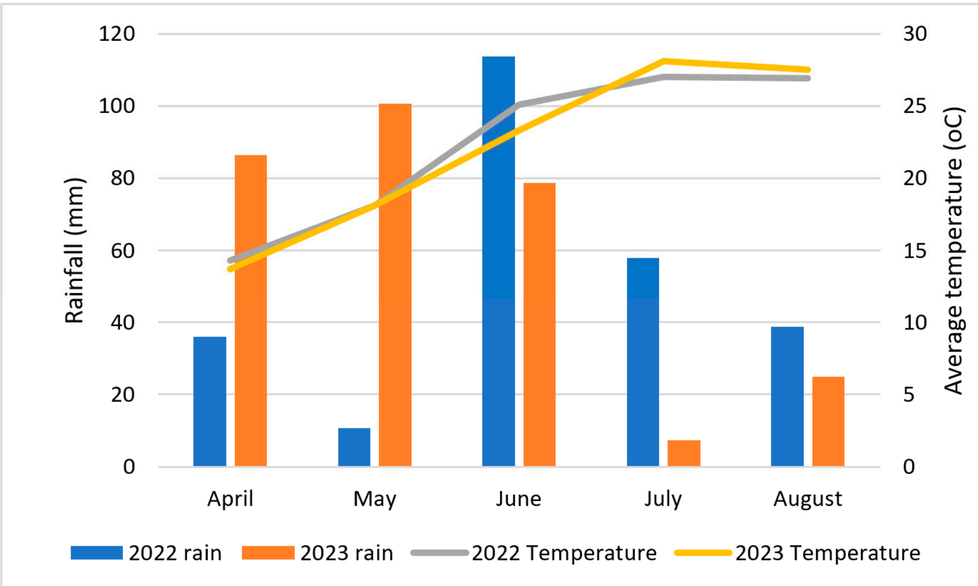

Figure 1. Average month temperature and total month rainfall from April to August for the Thessaloniki 2022 and 2023 experiments.

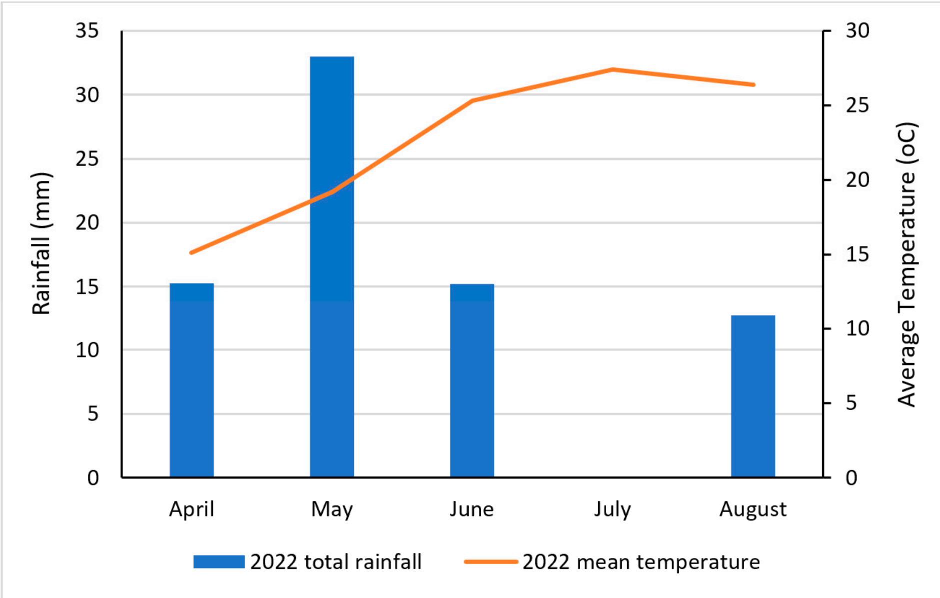

Figure 2. Average month temperature and total month rainfall from April to August for the Arta 2022 experiment.

In the 2023 Thessaloniki trial, in addition to PRE herbicides, the POST herbicide cycloxydim (Focus 10 EC, 10% a.i.) (Table 1) at 200 g ai ha^{-1} plus the adjuvant DASH HC 65 EC [oleic acid 5%, methyl oleate (palmitate) 37.5%, phosphate fatty alcohol polyalkoxylate 22.5%] at 750 mL ha^{-1} was applied at 30 DAS (i.e., 24 May 2023) to control johnsongrass (*Sorghum halepense* L.), due to high infestation of this weed species. Herbicides were applied with the same AZO handheld boom sprayer equipped with six twin flat spray nozzles (TeeJet® TTJ60-11002 Turbo Twin Tip, TeeJet Technologies, Glendale Heights, IL, USA) at 240 kPa pressure and at 400 l ha^{-1} water volume. Treatments also included an untreated control and a hand hoeing treatment to evaluate herbicide selectivity. Hoeing was applied once in both experiments in Thessaloniki, 40 DAS in 2022 and 30 DAS in 2023. In Arta, hand hoeing was applied four times due to extended weed infestation. The experiments in Thessaloniki were sprinkled irrigated until crop emergence and thereafter drip irrigation was established. In Arta, sprinkled irrigation was used throughout the growing period. Hemp fields were irrigated in all experiments, when needed. Plots were arranged in a randomized complete block design with 6 replications in 2022 (Thessaloniki and Arta) and with 4 replications in 2023 (Thessaloniki).

2.3. Assessments and Data Analysis

To evaluate the response of hemp to herbicides, stand counts, plant height, stem diameter, dry weight biomass, seed yield, thousand seed weight and symptoms of herbicide injuries were recorded in both experiments in Thessaloniki. All measurements and observations were performed on the plants of the two inner rows of each plot. Stand count assessment was performed at 30 DAS, whereas plant height was recorded twice at 50 and 80 DAS (initiation of flowering). Stem diameter and hemp dry biomass were recorded at 90 DAS after the crop's hand-harvesting from one meter of the 2nd and the 3rd row of each plot. Stem diameter was recorded (at growing stage code 2302) at the internode below the last pair of opposite leaves of plants with a digital caliper [26]. Hemp dry biomass was weighted after plant material was dried at 80 °C for 72 h using a forced-air oven. At seed maturity (130 and 115 DAS in 2022 and 2023, respectively), hemp inflorescences were harvested by hand from plants from one-meter crop row as described for the dry biomass assessment, air-dried for one week under glasshouse conditions and separated from other plant materials with shivers and an aspirator (Selecta Zig Zag, PETKUS Selecta, Hem, The Netherlands). Despite early seed shattering in some plots, seeds were recorded.

Healthy seeds (i.e., non-empty, shrunken or destroyed) were weighed using an analytical microbalance (Kern, ABS 320-4 N, Balingen, Germany) to determine seed production and thereafter their 1000-seed weight was evaluated. To assess herbicide efficacy, weed counts were performed between the two inner rows of each plot of the total length of the plot (4 m^2), whereas weed biomass was harvested from two m^2 area from the center of each plot at 90 DAS. Total weed dry weight was recorded after drying the weed biomass at 80 °C for 72 h and data were presented in g m^{-2}. The THC and the cannabidiol (CBD) concentrations of hemp fluorescence from each plot were determined by gas chromatography for each treatment and were assessed for both experiments in Thessaloniki as previously described [7].

In the Arta experiment, stand counts at 30 DAS, plant height at four different times (35, 49, 64 and 80 DAS), hemp fresh and dry biomass were recorded (130 DAS) to evaluate the response of hemp to herbicides. Due to *Sclerotinia sclerotionum* disease that damaged the crop, 10 randomly selected plants were collected from each plot for assessing fresh and dry weight of hemp at 127 DAS. Unfortunately, the hemp plant in hoeing treatment were severely injured and fresh and dry weight data could not be recorded. Moreover, due to early seed shattering and to crop injury by *S. sclerotionum*, seed yield was not enough to collect from the experiment in Arta. To assess herbicide efficacy, weed species counts were recorded twice (at 80 and 127 DAS) from randomly selected areas between the inner crop rows of each plot using a quadrat of 1 m^2.

Due to different weed species detected in each field and the application of the POST herbicide in 2023, ANOVA for mean comparison was applied separately for the three experiments. In the Arta experiment, weed counts were analyzed as a factorial experiment time by treatments in which time was used as a fixed variable since the population of naturally occurring weed flora follows, in most cases, a life cycle pattern that is species-specific. This was detected when weed counts for each species were analyzed in relation to sampling timing. Square root or log transformations were applied where necessary to fulfill the assumptions of ANOVA. Both transformed and non-transformed data are presented in tables when significant difference between means were detected. Data of all means were separated using the LSD test at 5% level of significance.

3. Results

3.1. Thessaloniki Experiment 2022

Herbicide injuries were recorded in this experiment. Pendimethalin and s-metolachlor resulted in lower stand counts of hemp (10.8 and 8.5 plants per row meter, respectively). However, these stand counts at 30 DAS were not statistically different to those of aclonifen, the untreated and the hoeing-weeded plots (13, 12 and 11 plants per row meter, respectively) (Table 2). Aclonifen, although it did not affect crop establishment, caused minor injuries forming yellowing discoloration across the central leaf vein of the hemp seedlings. These discoloration symptoms, however, were transient and disappeared within the following weeks. The three herbicides, however, affected the growth of the crop. In particular, at 50 DAS, the height of herbicide-treated hemp was significantly lower (1.12 to 1.26 m) compared to that of non-herbicide-treated plants (1.48 to 1.51 m). Nevertheless, at 80 DAS (i.e., beginning of flowering), the herbicide-treated plants were significantly higher (3.05 to 3.08 m) compared to the untreated control (2.79 m) and did not differ to the plants in the hoeing treatment (3.00 m) (Table 2). At 90 DAS, greater values in stem diameter were observed in the herbicide-treated hemp (10.27 to 11.78 mm) compared to those of the untreated control (7.47 mm) (Table 2). Hemp dry biomass at 90 DAS was similar across herbicide-treated plants ranging from 1033 to 1208 g per row meter and significantly greater compared to that of the untreated control (550 g per row meter). Pendimethalin-treated plants, though, resulted in greater hemp dry biomass compared to that of the hoeing-weeded plots (Table 2). The weed species recorded in the field in descending order were the dicots *Chenopodium album* L., *Amaranthus retrofelxus* L., *Tribolus terrestris* L., *Solanum nigrum* L. and *Portulaca olearacea* L. Infestation of *Ch. album* was even across the field, whereas *Convolvulus arvensis* L., *Cyperus* spp. and *Sorghum halepense* L. were spread and at lower densities. In the untreated control, the *Ch. album* density was recorded at 38.5 plants m^{-2}. *Ch. album* was reported to survive competition with hemp in field studies and dominate over other weeds, retaining its predominant position until harvest in the field trials of previous studies [27]. In the present study, *Ch. album* was totally controlled by pendimethalin along with *A. retroflexus*, *P. oleracea*, *T. terrestris* and *S. nigrum*. Aclonifen also exhibited high control of *C. album* along with that of *A. retroflexus*, *P. oleracea* and *T. terrestris*. However, in some plots, *S. nigrum* was not adequately controlled by aclonifen. Compared to both pendimethalin and aclonifen, s-metolachlor resulted in reduced control of *C. album* that averaged 9.9 weed plants m^{-2} weed density. Total weed dry biomass under pendimethalin and aclonifen treatments resulted in lower biomass of 12 and 26 g m^{-2}, respectively, followed by s-metolachlor with 97 g m^{-2} due to the reduced control of *Ch. album*, whereas the total weed biomass in the untreated control and in hoeing-treated plots was 262 and 8 g m^{-2} at 90 DAS (Table 2). The similar seed yield observed in all treatments (Table 2) may be challenging for reaching conclusions in terms of herbicide effect on hemp yield production since seed loss occurred before harvesting due to unexpected seed shattering. Regarding seed weight, the 1000-seed weight was not significantly different between treatments (Table 2). THC content was in line with the accepted limits for hemp cultivation, where CBD content (Table 2) was comparable to reported values [28].

Table 2. Stand counts at 30 DAS, hemp height at 50 and 80 DAS, stem diameter and hemp dry biomass at 90 DAS, grain yield and 1000-seed weight at 130 DAS, total weed dry biomass at 80 DAS, THC and CBD values in herbicide-treated, hoeing-treated and untreated-control (Thessaloniki experiment 2022).

Treatments	Rate g ai ha^{-1}	Stand Counts	Hemp Height		Hemp Stem Diameter	Hemp Dry Biomass	Hemp Seed Yield	1000-Seed Weight	Total Weed Dry Biomass	THC	CBD
		30 DAS (Plants per Row Meter)	50 DAS (m)	80 DAS (m)	90 DAS (mm)	90 DAS (g per Row Meter)	130 DAS (g per Row Meter)	130 DAS (gr)	90 DAS (gr m^{-2})	(%)	(%)
pendimethalin	1137.5	10.8	1.24 b	3.05 a	11.78 a	1208 a	1.99 [#] (108)	10.95	3.3 [##] a (12)	0.10	1.18
s-metolachlor	960	8.5	1.26 b	3.06 a	11.03 a	1058 ab	2.01 (123)	12.38	9.8 c (97)	0.10	1.20
aclonifen	1800	13.0	1.12 b	3.08 a	10.27 ab	1033 ab	1.90 (87)	10.63	5.2 b (26)	0.10	1.16
untreated check		12.0	1.51 a	2.79 b	7.47 c	550 c	1.81 (66)	11.83	16.1 d (262)	0.11	1.43
hoeing		11.0	1.48 a	3.00 a	8.70 bc	792 bc	1.95 (88)	12.25	2.7 a (8)	0.10	1.16
LSD (0.05)		ns	0.201	0.190	1.953	0.370	ns	ns	2.86	-	-

Within each column, mean values followed by different letter(s) are statistically significantly different at a = 0.05 ($p \leq 0.05$) according to Fisher's protected LSD criterion; non-transformed data for hemp seed yield and for total weed dry biomass are presented in parentheses. Abbreviations: DAS—days after sowing; ns—not significant; the dash symbol (-) indicates no statistical analysis performed; THC—Tetrahydrocannabinol; CBD—cannabidiol; [#] log(x) transformed data; [##] sqrt(x) transformed data.

3.2. Arta Experiment 2022

The herbicides applied did not affect hemp establishment. Stand counts at 30 DAS revealed no significant difference in crop emergence and an average hemp density of around 17 plants per row meter was detected for all treatments (Table 3). The absence of a herbicide effect on hemp was also evident since no herbicide injury was recorded throughout the study and plant height measured 35, 19, 64 and 80 DAS did not differ between treatments (Table 3). As already mentioned above, *S. sclerotionum* resulted in severe crop damage and did not allow for biomass collection from the plots where hoeing was applied. Regarding the herbicide-treated and the untreated plots, it was revealed that although the means of both fresh and dry weight biomass were greater in the herbicide-treated plots (1075 to 1113 g and 445 to 560 g for fresh and dry weight, respectively) compared to those of the untreated plots (867 g and 400 g for fresh and dry weight, respectively) no significant difference was revealed (Table 3). Regarding weed species, the herbicides did not show any significant efficacy; the field was dominated by the perennial grass weeds *Cynodon dactylon*

(L.) Pers. and *Sorghum halepense* L. that averaged 5.7 and 7.9 plants m^{-2}, respectively (Table 4). Dicots species such as *Abutilon theoprhasti* Medik, *Convolvulus arvensis* L., *Rumex crispus* L., *Solanum nigrum* L. and *Sonchus oleraceus* L. were recorded at lower densities. In addition, the interaction time by treatments was not significant and the results did not reveal any significant difference in weed counts at 80 and 127 DAS, although suppression of weed growth was observed in herbicide-treated plots. THC content was in line with the accepted limits for hemp cultivation, whereas CBD was comparable to reported values [28] (Table 3).

Table 3. Stand counts at 30 DAS, hemp height at 35, 49, 64 and 80 DAS, hemp fresh and dry biomass at 130 DAS, THC and CBD values in herbicide-treated, hoeing-treated * and untreated control (Arta experiment 2022).

Treatments	Rate g ai ha^{-1}	Stand Counts	Hemp Height				Hemp Fresh Biomass	Hemp Dry Biomass	THC	CBD
		30 DAS (Plants per Row Meter)	35 DAS (m)	49 DAS (m)	64 DAS (m)	80 DAS (m)	127 DAS (g) Mean from 10 Plants	127 DAS (g) Mean from 10 Plants	(%)	(%)
pendimethalin	1137.5	16.1	0.31	1.02	1.79	1.90	1113	560	0.13	1.79
s-metolachlor	960	18.4	0.33	0.99	1.78	1.93	1075	444	0.13	1.83
aclonifen	1800	17.8	0.28	0.95	1.72	1.84	1100	542	0.13	1.88
untreated check		17.5	0.35	1.09	1.79	1.90	867	400	0.15	2.53
hoeing		17.7	0.28	0.93	1.71	1.82	*	*	0.12	1.43
LSD (0.05)		ns	ns	ns	ns	ns	ns	ns	-	-

Abbreviations: DAS—days after sowing; ns—not significant; the dash symbol (-) indicates no statistical analysis performed; THC—Tetrahydrocannabinol; CBD—cannabidiol. * no hemp fresh and dry biomass was collected from the hoeing-treated plots due to the crop damage by the *S. sclerotionum* disease.

Table 4. Weed counts (plants m^{-2}) at 80 and 127 DAS as recorded in herbicide-treated and untreated control (Arta experiment 2022).

Treatments	Rate g ai ha^{-1}	DAS	ABU	CONAR	CYNDA	RUMCR	SOLNG	SONOL	SORHA
			Plants m^{-2}						
Pendimethalin	1137.5	80	0.0	0.3	3.2	1.8	0.7	1.2	10.0
		127	0.0	0.5	3.7	2.7	0.3	1.2	11.7
s-metolachlor	960	80	0.2	0.3	7.5	3.2	2.7	2.7	5.8
		127	0.3	0.5	6.8	3.2	2.0	2.5	7.0
Aclonifen	1800	80	0.3	1.3	9.0	1.2	0.2	1.5	5.5
		127	0.5	1.0	7.5	1.0	0.3	2.2	6.5
untreated control		80	0.0	1.0	3.5	2.0	1.5	2.7	7.7
		127	0.0	1.5	4.3	1.8	1.8	2.3	9.0
LSD (0.05)			ns	ns	ns	ns	ns	ns	ns

Abbreviations: DAS—days after sowing, ABU—Abutilon theoprhasti, CONAR—Convolvulus arvensis, CYNDA—Cynodon dactylon, RUMCR—Rumex crispus, SOLNG—Solanum nigrum, SONOL—Sonchus oleraceus, SOLHA—Sorghum halepense; ns—not significant.

3.3. Thessaloniki Experiment 2023

Due to limited data from the Arta experiment and the different responses of hemp to PRE herbicides, the experiment was repeated in 2023 in Thessaloniki. However, in this experiment, the POST herbicide cycloxydim followed each one of the PRE treatments (Table 1) to control *S. halepense* that was the dominant weed species in that field. In contrast to the 2022 results, pendimethalin and s-metolahlor did not cause any effect in hemp establishment. Stand counts at 30 DAS revealed no significant difference between treatments and hemp density was 14 plants per meter row on average (Table 5). Moreover, the herbicide injury symptoms reported on aclonifen-treated plants in 2022 were at a much lower extent and observed in a smaller number of plants. Similarly to the previous year, these symptoms were temporary and did not affect the growth and development of the crop. Cycloxydim did not cause any visual symptom; the plant height at 50 DAS revealed no difference among herbicides, hoeing and the untreated control, although slightly lower values recorded in pendimethalin were followed (fb) by cycloxydim (1.25 m) and aclonifen fb by cycloxydim (1.30 m) treatment. Height assessments at 80 DAS showed that all herbicide-treated plants had a similar height (2.42 to 2.55 m), which was slightly greater compared to that of the untreated control (2.39 m) (Table 5). Stem diameter recordings were similar for all treatments with values ranging from 8.56 mm in the untreated control to 11.18 mm for aclonifen fb by cycloxydim (Table 5). Regarding dry biomass, pendimethalin, s-metolachlor and aclonifen were all followed by cycloxydim, which revealed significant greater values (830 to 995 kg per row meter) compared to those of the untreated control (638 kg per row meter); the weed-hoeing plots revealed similar hemp biomass to that of the pendimethalin fb by that of cycloxydim and that of s-metolachlor fb that of cycloxydim-treated plots. Seed yield recordings showed greater values for all herbicide treatments (81 to 91 g per row meter) compared to those for the untreated control (45 g per row meter) (Table 5). The weed flora in the untreated control at 90 DAS consisted mainly of *S. halepense*, *Ch. album* and *Cynanhum laeve* (Michx.) Pers. at 4.3, 2.5 and 0.2 plants m^{-2}, respectively, followed by a lower density of *P. oleracea* and *Amaranthus* spp. (data not presented in Tables). The total dry weight in the untreated plots was 380 g m^{-2}. All herbicide treatments highly controlled the weeds; pendimethalin fb by cycloxydim resulted in a total weed dry weight of 22 g m^{-2}, s-metolachlor fb by cycloxydim in 14 g m^{-2}, whereas aclonifen fb by cycloxydim resulted in 23 g m^{-2}. THC content was in line with the accepted limits for hemp cultivation, whereas CBD content was comparable to the reported values [28] (Table 5).

Table 5. Stand counts at 30 DAS, hemp height at 50 and 80 DAS, stem diameter and hemp dry biomass at 90 DAS, hemp grain yield and 1000-seed weight at 115 DAS, total weed dry biomass at 90 DAS, THC and CBD values in herbicide-treated, hoeing-treated and untreated control (Thessaloniki experiment 2023).

Treatments *	Rate g ai ha^{-1}	Stand Counts	Hemp Height		Hemp Stem Diameter	Hemp Dry Biomass	Hemp Seed Yield	1000-Seed Weight	Total Weed Dry Biomass	THC	CBD
		30 DAS (Plants per Row Meter)	50 DAS (m)	80 DAS (m)	90 DAS (mm)	90 DAS (g per Row Meter)	115 DAS (g per Row Meter)	115 DAS (gr)	90 DAS (gr m^{-2})	(%)	(%)
pendimethalin	1137.5	13.3	1.25	2.42	9.40	995 ab	81 a	13.58	1.02 # a (22)	0.14	1.72
s-metolachlor	960	14.3	1.48	2.49	11.03	830 b	91 a	11.48	0.90 a (14)	0.12	1.40
aclonifen	1800	14.0	1.30	2.55	11.18	980 ab	87 a	11.25	1.31 a (23)	0.12	1.39
untreated check		14.3	1.43	2.39	8.56	638 c	45 b	13.10	2.48 b (380)	0.12	1.53
hoeing		14.9	1.42	2.51	9.03	1015 a	76 a	12.93	0.87 a (11)	0.11	1.33
LSD (0.05)		ns	ns	ns	ns	180	23.5	ns	0.807	-	-

Within each column, mean values followed by different letter(s) are statistically significant different at a = 0.05 ($p \leq 0.05$) according to Fisher's protected LSD criterion; non-transformed data for total weed dry biomass are presented in parentheses. Abbreviations: DAS—days after sowing; ns—not significant; the dash symbol (-) indicates no statistical analysis performed; THC—Tetrahydrocannabinol; CBD—cannabidiol; # log (x) transformed data; * cycloxydim (200 g ai ha^{-1}) followed all the PRE treatments for the control of *S. halepense.*

4. Discussion

The herbicide treatments resulted in effective weed control that increased hemp biomass and seed production compared to those of the untreated control, in the Thessaloniki experiments. Yet, in Arta, the herbicides resulted in higher hemp biomass, which was not significant different to that of the untreated control. The increased hemp biomass by herbicide treatments in both the Thessaloniki experiments and by hoeing in the 2023 experiment, along with the low hemp biomass obtained when weeds remained uncontrolled, clearly demonstrated the need for early weed management in hemp seeded at low densities. The earlier (30 DAS) management of weeds by hoeing in the Thessaloniki experiment 2023 compared to that of the later application of hoeing (40 DAS) in the first-year experiment possibly was the reason for the increased crop biomass and seed yield observed under hoeing in the second year of experimentation. That is in line with previous studies that highlighted the early weed control in hemp sown at low crop densities [13,14]. Moreover, the presence of difficult-to-control weed species such as *S. halepense* in the Arta field that were out of the range of the activity of the herbicides applied, along with the increased hemp biomass and seed yield by both the PRE and POST herbicides, indicated the need to control weeds species such as *S. halepense* that dominate the field.

Regarding the hemp response to herbicides, the reduction in stand counts by pendimethalin and s-metolachlor, the leaf discoloration by aclonifen and the shorter plants observed in the Thessaloniki experiment in 2022 should be under consideration. Soil moisture level, soil temperature and soil texture influence the selectivity of the soil-applied herbicides. The high sensitivity of hemp to pendimethalin and s-metolachlor applied at 2130 and 1790 g ai ha^{-1}, respectively, even at 0.125 times lower rates has been reported in a dose-respondent pot experiment [15]. In our opinion, the high sensitivity of hemp in this study might have been due to the high soil moisture that had occurred before spraying by watering the pots until soil saturation; moreover, the moisture level-to-field capacity maintained after daily herbicide application [15] might have further contributed to phytotoxic symptoms even at the low rates applied. Similarly, in the Thessaloniki experiment in 2022, rain occurred two days before herbicide application that, along with the high percentage of sand (52%) and the low clay (14%) and organic matter content (1.2%) of the soil, might have caused a decreased level of herbicide adsorption to soil colloids, thereby resulting in increased injury such as lower stand counts, discoloration and lower plant height. In contrast, no injury symptoms were recorded in the Arta experiment; in the Thessaloniki experiment in 2023, no reduction in stand counts or plant height were recorded; moreover, the leaf discoloration in aclonifen-treated plants was less pronounced and restricted to lower number of plants. In both of these experiments, no rain or irrigation occurred before herbicide treatments to affect herbicide soil adsorption, whereas the soils in Arta had 55% clay and 1.5% organic matter content, compared to the Thessaloniki soils that had 22% clay and 2.2% organic matter content. The heavier soil in Arta may have decreased herbicide efficacy, and for this reason weed control was restricted to weed suppression only. Increased herbicide injury under higher levels of soil moisture in sandy soils with lower sorption capacity has been reported for s-metolachlor, pendimethalin and aclonifen in sunflower [29]. S-metolachlor has the potential to leach in soils with low soil organic matter content (<2%), especially when rainfall occurs shortly after application leading to crop injuries such as the inhibition of seedling emergence or stunting of the emerged plants, [30,31]. As already stated, hemp has been reported as sensitive to herbicides [12,15]. Apart from the soil conditions, cultivar sensitivity may be another factor that may affect herbicide selectivity in hemp. A different response was reported between the CRS-1 and X-59 hemp cultivars when pendimethalin and s-metolachlor were applied [15]. Similarly, pendimethalin (Stomp Aqua 455 CS) at 1.6 kg a.i. ha^{-1} in the Uso 31 hemp cultivar resulted in shorter plants with less biomass compared to those of the untreated checks, whereas in the cultivar Fedora 17, it resulted in taller plants, although they were still shorter than plants subjected to hand hoeing [32].

Regarding the three herbicides tested, a study with s-metolachlor (Dual Gold 960 g L^{-1}) and aclonifen 600 (Challenge 600 g L^{-1}) at 3.0 L ha^{-1} reported that both of the active ingredients were safe for hemp with a selectivity ranking at grade 1 for them according to the European Weed Research Society (EWRS) 1–9 selectivity scale (1 for unaffected plant and 9 for the plant affected up to 80–100%) [13]. In another field study, no herbicide injury after pendimethalin (Prowl H_2O at 3 pt acre^{-1}) was observed, while s-metolachlor (Dual Magnum at 1.67 pt acre^{-1}) resulted in 8% temporary herbicide injury observed at 15 days after treatment; past that, no further injury was recorded [22]. In contrast, in another trial, pendimethalin (Prowl H_2O) at 1.6 kg a.i. ha^{-1} resulted in >50% injury and in a 32% stand count of the untreated check, however, with no seed yield differences when compared to the non-treated check [21]. That is in line with the results of the present study regarding the reduction in stand counts, the retardation in height and the injury symports observed, and hemp recovered from the herbicide effect and produced more yields. The same report revealed that s-metolachlor (Dual II Magnum at 1.6 kg a.i. ha^{-1}) resulted in 0% to less than 15% injury and in a stand count of 97% for the untreated check and was characterized as the safest PRE herbicide. In contrast, the same authors reported that both pendimethalin and s-metolachlor in the greenhouse study did not cause stand losses compared to those of the untreated control, revealing that only s-metolachlor caused plant height reduction, whereas both herbicides caused dry biomass reduction [21]. In a more recent study, pendimethalin (Stomp Aqua, 455 g L^{-1}) at 3.0 L ha^{-1}, s-metolachlor (Dual Gold 960 g L^{-1}) at 1.5 L ha^{-1} and aclonifen (Challenge 600 g L^{-1}) at 4.0 L ha^{-1} were evaluated among other treatments in hemp production [24]. This study reported very low phytotoxicity (1.67% and 3.00%) caused by aclonifen, 14.67% and 9.0% by pendimethalin and 20.33% and 25.33% for s-metolachlor at 2 and at 4 weeks after treatment. Plant height was similar for the three herbicide treatments and the untreated control at 8 weeks after treatment and the highest grain yield was achieved by aclonifen due to increased weed control coupled with low phytotoxiticy [24]. Regarding the hemp response to cycloxydim, low phytotoxicity of other ACCase inhibitors in hemp, such as clethodim and fluazifop-p-butyl, was reported with slight phytotoxic effects (<10%) during the first 2 weeks after treatment of both herbicides that gradually (at 8 weeks) became negligible (<1.33%) [24]. The absence of herbicide injury symptoms after cycloxydim application in our study indicated that cycloxydim was safe for use in hemp. This finding is further supported by a recent report that revealed the selectivity of cycloxydim at 10% (Stratos Ultra 100 g L^{-1}) at 2.0 L ha^{-1} for applied POST in hemp already treated PRE with s-metolachlor (Dual Gold 960 g L^{-1}) at 1.5 L ha^{-1}. For these treatments, no crop injury was detected and the selectivity ranked at grade 1 according to the EWRS 1–9 selectivity scale [13]. Different levels of phytotoxicity were reported for PRE herbicides between two different sites [23].

Different hemp responses to soil-applied herbicides may partially be due to other cultivation practices such as the planting depth of the hemp. Seeding depth closer to the soil surface may result in seed exposure to greater herbicide concentrations compared to that of deeper depth planting and this has led to inconsistent results regarding hemp tolerance to herbicides [31]. The different response of hemp to herbicides may be attributed to different cultivars used, different seeding rates, seeding depth and soil types, as well as to different environmental conditions [21].

5. Conclusions

Pendimethalin, s-metolachlor and aclonifen were safe PRE herbicides in industrial hemp, cultivar Futura 75, although injury symptoms were observed in one experiment. Precautions, however, should be taken in terms of reduced selectivity, particularly for s-metolachlor and pendimethalin if they have to be applied in wet and light soils as stand count reductions may be observed. Weed control is necessary when *Ch. Album* or *S. halepense* are present in the field and hemp is sown at low rates. Future research with different herbicide rates or different PRE herbicide mixtures under variable soil moisture levels along with POST herbicide treatments should increase knowledge about industrial hemp responses to herbicides.

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