agronomy

Article

Screening of Pre- and Post-Emergence Herbicides for Weed Control in *Camelina sativa* (L.) Crantz

Si-Zhe Dai [1], Yawen Wang [1], Min-Jung Yook [2], Hui-Zhen Wu [1], Min Chen [1] and Chuan-Jie Zhang [1,*]

1 College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, China; 1491443432dsz@gmail.com (S.-Z.D.); wangyw42@163.com (Y.W.); huizhenwu5289@163.com (H.-Z.W.); cmxzpaxl@163.com (M.C.)

2 Department of Agriculture, Forestry, and Bioresources, Research Institute of Agriculture and Life Sciences, College of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea; small6@snu.ac.kr

* Correspondence: chuanjiezhang@yzu.edu.cn or chuanjie.zhang@icloud.com

Abstract: Weed management has been one of the major challenges in camelina [*Camelina sativa* (L.) Crantz] production owing to the limited options for selective herbicides. The aim of this study was to evaluate and screen camelina-safe herbicides and establish an effective weed management program combining pre- and post-emergence herbicide application in camelina. There were 22 herbicides (6 herbicides registered as pre- and 16 herbicides registered as post-emergence herbicides) with various modes of action tested in this study. Greenhouse evaluation showed that, of the 22 herbicides tested, post-application of *s*-metolachlor and prodiamine (registered as pre-emergence herbicide), and clethodim, fluazifop-*p*, clopyralid, and quinclorac (registered as post-emergence herbicide) possessed adequate safety (~×4 of recommended doses) when used on the two camelina genotypes (CamC1 and CamK3) by evaluation of plant visual efficacy, seed weight, and plant biomass yield per plant. Herbicides from the ALS (e.g., flumetsulam), HPPD (e.g., mesotrione), IPP (e.g., clomazone), PPO (e.g., oxyfluorfen), and PS II (e.g., bentazon) groups caused severe camelina growth suppression and mortality. Field evaluation with greenhouse-selected herbicides demonstrated the superior weed control efficacy of sequential application combining pre- (*s*-metolachlor) and post-emergence (clethodim, fluazifop-*p*, or clopyralid) herbicides (84–90% reduction in weed biomass in camelina plots relative to untreated control) than the single application of those herbicides (68–83%). Clethodim and fluazifop-*p* provided good post-emerged grass weed control (e.g., crabgrass), whereas clopyralid effectively controlled the broadleaf weeds, such as common vetch and shepherd's purse. Camelina seed yields from *s*-metolachlor following clethodim, fluazifop-*p*, or clopyralid application were statistically comparable to the yield of the weed-free treatment (hand weeding) and were significantly greater than those of the untreated control, indicating the effective weed control efficacies provided by those herbicides. Sequential application of the above herbicides did not affect camelina seed oil content, the principal UFA concentrations (e.g., C18:1~3), UFA/SFA, and MUFA/PUFA. In summary, sequential application combining pre- (*s*-metolachlor) and post-emergence (clethodim, fluazifop-*p*, or clopyralid) herbicides shows effective weed control in camelina, thus providing a great opportunity to increase camelina production through herbicide-based weed management.

Keywords: herbicide safety; seed oil quality; seed yield; sequential application; weed control efficacy

check for updates

Received: 14 January 2025
Revised: 27 February 2025
Accepted: 28 February 2025
Published: 3 March 2025

Citation: Dai, S.-Z.; Wang, Y.; Yook, M.-J.; Wu, H.-Z.; Chen, M.; Zhang, C.-J. Screening of Pre- and Post-Emergence Herbicides for Weed Control in *Camelina sativa* (L.) Crantz. *Agronomy* **2025**, *15*, 640. https://doi.org/10.3390/agronomy15030640

1. Introduction

In recent years, camelina [*Camelina sativa* (L.) Crantz], also known as false flax or gold-of-pleasure, has received attention in North America and Europe due to its multiple food and non-food use potential (e.g., pharmaceutical, feeding, and biofuel values) [1–3]. It is a low-input oilseed crop with tolerance to drought, low temperature, and insect pests, making it widely adaptable to a range of arid and semi-arid environments [4–6]. Camelina is mainly planted in northern and northwest China, such as Gansu province, Xinjiang, as an alternative edible oil source with an estimated cultivation area of about 500–1500 ha [7,8]. Although camelina shows good competitive ability against weeds in some cases [9–11], weeds are the major constraint to camelina production [1,12,13]. The presence of weeds, such as annual foxtail [*Setaria viridis* (L.) Beauv.] and thistle (*Salsola tragus* L.), has resulted in low camelina seed yield and poor oil quality due to the competition between weeds and camelina for nitrogen, light, or water [14–16].

Compared with traditional manual or mechanical weeding, herbicide-based weed management has been considered an important approach for weed control in agricultural production, which can control weed growth in a short period of time, reducing the negative impact of weeds on crop growth and saving labor and time costs [17,18]. However, for camelina, there are only limited herbicide options available for weed control [1,12]. To date, sethoxydim and quizalofop-p-ethyl (ACCase inhibitor) are the only two registered herbicides for grass weed control in camelina in North America (the United States and Canada) [1,19]. By contrast, control of broadleaf weeds in camelina is rather challenging due to the sensitivity of camelina to most broadleaf herbicides [20]; thus, few herbicides have been approved for weed control in camelina. The studies reported that pre-emergence herbicides, such as dimethenamid-*p*, *s*-metolachlor, pendimethalin, etc., have been tested for their safety on camelina [12]. Among those herbicides, although *s*-metolachlor and pendimethalin caused plant damage when applied at the recommended doses, they did not reduce the camelina seed yield. Studies also showed that pre-application of quinclorac, a post-emergence herbicide, was safe for camelina [12]. Screening of safe herbicides for camelina has practical significance in increasing camelina production. Therefore, the objectives of this study were to (i) evaluate and screen camelina-safe herbicides from a diverse group of herbicides with various modes of action (post-application of 6 pre- and 16 post-emergence herbicides in a greenhouse study); (ii) further evaluate the weed control efficacy and camelina safety of those selected herbicides applied in single or combination in sequence (field study). Herbicide safety criteria on camelina included the effect of selected herbicides on the camelina plant density, 1000-seed weight, seed yield ha^{-1}, oil content, and the principal UFA composition and concentrations. Finally, a program combining the application of pre- and post-emergence herbicides for effective weed control in camelina was discussed and proposed.

2. Materials and Methods

2.1. Plant Materials and Herbicides Tested

In this study, two camelina genotypes, CamC1 and CamK3 (characterized as spring type) [21], were used. CamC1, originated from China, was provided by the Research Center for *C. sativa* Planting and Engineering Technology (RCCPET) at Anyang city, Henan province, China. CamK3, origin of Korea, was obtained from Yanbian University, Jilin province, China. Seeds of both camelina genotypes showed a germination >95% in a preliminary seed germination test. A total number of 6 pre- and 16 post-emergence herbicides with various modes of actions (e.g., ACCase, ALS, PPO inhibitors) were tested in the study. Detailed information on herbicide common names, formulations, standard application doses, mode of action, and manufacturer are provided in Table 1.

Table 1. Summary of the information of the 22 herbicides (the first 6 herbicides registered as pre-emergence herbicides and the other 16 herbicides registered as post-emergence herbicides) tested in this study.

Herbicide	CAS No.	Formulation [a]	Standard Application Dose (g a.i. ha^{-1})	Mode of Action	Manufacturer
Oxyfluorfen	42874-03-3	EC (240 g L^{-1})	144	Protoporphyrinogen-IX-Oxidase (PPO)	NanTong Jiahe Chemicals Co., Ltd., Nantong, China
Clomazone	81777-89-1	CS (360 g L^{-1})	648	Isopentenyl pyrophosphate (IPP) isomerase	FMC (Shanghai) Chemical Technology Co., Ltd., Shanghai, China
s-metolachlor	87392-12-9	EC (960 g L^{-1})	1440	Cell division	Hefei Xingyu Chemical Co., Ltd., Hefei, China
Pendimethalin	40487-42-1	EC (330 g L^{-1})	990	Microtubule assembly	Rotam CropSciences Ltd., Tianjin, China
Prodiamine	29091-21-2	WG (65%)	780	Microtubule assembly	Oriental (Luzhou) Agrochemical Co., Ltd., Luzhou, China
Flumetsulam	98967-40-9	WG (80%)	1212	Acetolactate Synthase (ALS)	Zhengzhou Lawngreen Biotechnology Co., Ltd., Zhengzhou, China
Bentazon	25057-89-0	AS (480 g L^{-1})	1080	Photosystem II (PSII)	BASF Crop Protection (Jiangsu) Co., Ltd., Nantong, China
Clethodim	99129-21-2	EC (120 g L^{-1})	63	Acetyl-CoA carboxylase (ACCase)	Innovation Meiland (Hefei) Co., Ltd., Hefei, China
Quizalofop-*p*-ethyl	94051-08-8	EC (10%)	90	ACCase	Hubei Best Agricultural Chemical Co., Ltd., Suizhou, China
Haloxyfop-*p*	95977-29-0	EC (108 g L^{-1})	48.6	ACCase	Jiangsu Flag Chemical Industry Co., Ltd., Nanjing, China
Fluazifop-*p*	83066-88-0	EC (15%)	67.5	ACCase	Wuhan Wulong pesticide Co., Ltd., Wuhan, China
Mesotrione	104206-82-8	OD (10%)	225	4-Hydroxyphenyl pyruvate dioxygenase (HPPD)	Shandong Vicome Greenland Chemical Co., Ltd., Jinan, China
Fomesafen	72178-02-0	AS (250 g L^{-1})	562.5	PPO	Hubei Best Agricultural Chemical Co., Ltd., Suizhou, China
Clopyralid	1702-17-6	SG (75%)	101.3	Synthetic auxin	Shandong Huimin Vanda Biological Technology Co., Ltd., Jinan, China
Quinclorac	84087-01-4	WP (50%)	225	Synthetic auxin	Anhui Province Shengdan Biochemical Co., Ltd., Anqing, China
Benazolin-ethyl	25059-80-7	SC (30%)	225	Synthetic auxin	Jiangsu Futian Agrochemical Co., Ltd., Nanjing, China
Nicosulfuron	111991-09-4	OD (40 g L^{-1})	42	ALS	Hebei Green Agricultural Science and Technology Group Co., Ltd., Cangzhou, China
Halosulfuron-methyl	100784-20-1	WG (75%)	33.8	ALS	Jiangsu Agrochem Laboratory Co., Ltd., Changzhou, China

Table 1. *Cont.*

Herbicide	CAS No.	Formulation [a]	Standard Application Dose (g a.i. ha^{-1})	Mode of Action	Manufacturer
Bispyribac-sodium	125401-92-5	WP (20%)	45	ALS	Shanghai Hulian Biopharmaceutical Co., Ltd., Shanghai, China
Pyrazosulfuron-ethyl	93697-74-6	WP (10%)	22.5	ALS	Qiqihar Tianfeng Chemical Co., Ltd., Qiqihar, China
Florasulam	145701-23-1	SC (50 g L^{-1})	3.8	ALS	Zhangye Dagong PesticideChemistry Co., Ltd., Zhangye, China
Imazethapyr	81385-77-5	AS (5%)	75	ALS	Shandong Cynda Chemical Co., Ltd., Jinan, China

[a] emulsifiable concentrate (EC); capsule suspension (CS); water-dispersible granules (WG); aqueous solution (AS); oil dispersion (OD); water-soluble granules (SG); wettable powder (WP); suspension concentrate (SC).

2.2. Experimental Procedure

The present study consisted of two experiments. The first one (pot experiment: greenhouse study) was conducted to evaluate the safety of post-application of 22 herbicides (6 herbicides registered as pre- and 16 herbicides registered as post-emergence herbicides, respectively) in camelina (Table 1). The second experiment (field study) evaluated the weed control efficacy of the selected herbicides (from the greenhouse study) that were applied in single or combination in sequence in the field.

2.2.1. Experiment 1. Greenhouse Study for Screening Camelina-Safe Herbicides

Greenhouse evaluation of the herbicide safety of the post-application of the 22 herbicides with two spring camelina genotypes (CamC1 and CamK3) was conducted in a greenhouse of Prataculture Science (32°23′ N, 119°25′ E, 12 m a.s.l.), Yangzhou University, Jiangsu province, China. On 24 September 2019, seeds of CamC1 and CamK3 were sown in 105-well multi-pots (hole size: 3.5 cm × 1.5 cm × 4 cm) filled with organic horticultural potting soil (Yiyuan Agriculture and Forestry Ltd., Suzhou, China). The greenhouse was maintained at 25/20 °C (day/night) with a 16/8 h photoperiod supplemented by an overhead sodium lamp. When the seedlings of both camelina genotypes formed 3–4 leaves (BBCH 11) [22], they were transplanted into a plastic pot (volume: 2.2 L with 12.5 cm height and 15 cm diameter) containing the same aforementioned potting soil. The plants were kept and grown in the same greenhouse up to 7–8 leaves (BBCH 15) before herbicide treatment. All of the 22 herbicides were foliar-applied to each camelina genotype at their application dose of ×1 (standard dose), ×2, and ×4 using a backpack sprayer with flat-fan nozzles (8004, TeeJet spraying system) to deliver 1000 L ha^{-1} at 145 kPa (Table 1). Each herbicide dose treatment (including untreated control) consisted of three replicates (each pot containing six camelina plants as one replicate). After herbicide treatment, the treated plants were maintained in the greenhouse with the same growth conditions as described above and arranged in a randomized complete block design (RCBD) (n = 3). To allow the full absorption of the herbicide, the regular watering regime was initiated at 24 h after herbicide application.

At 21 days after herbicide treatment, plant visual efficacy was assessed. Based on the observations, the responses of camelina plants generally fell into three categories; thus, a scale of 0 (completely killed or no green tissue)—3 (no damage and identical growth status to the untreated control) was used to assess the plant visual efficacy. At maturity (nearly

>95% of silicles dried up), all the plants were harvested and assessed for plant height (cm), aboveground plant biomass per plant (g), and seed weight per plant (g).

2.2.2. Experiment 2. Field Study to Evaluate Weed Control Efficacy and Camelina Safety of the Selected Herbicides

Experimental Site, Design, and Field Management

Following the greenhouse herbicide safety evaluation, a two-year field study (2020–2021 and 2021–2022) to further evaluate the herbicide safety and weed control efficacy of the selected herbicides (from *Experiment 1*) was conducted at two different field sites (32°40′ N, 119°40′ E, 10 m a.s.l.) at Yangzhou University Pratacultural Science Experimental Station, Jiangsu province, China. The climate of the experimental region is characterized as humid subtropics, and the mean annual temperature and accumulated precipitation were 15.7 °C and 1043 mm (long-term mean of 1981–2010), respectively. Both the two field sites had a sandy loam soil type with a pH of 6.5–7.2. Initial physico-chemical properties of the topsoil (up to 50 cm) of the field were as follows: average organic matter 18.5 g kg^{-1}, alkali-hydrolyzed nitrogen 75 mg kg^{-1}, available potassium 7 mg kg^{-1}, and available phosphorus 55 mg kg^{-1}. There were no previous crops cultivated at the two field sites. The two fields were hand-weeded, and the seedbed was manually prepared. All camelina seeding plots were fertilized with mineral fertilizer (N-P-K, 15–15–15) at a rate of 630 kg ha^{-1} before camelina field seeding. The first and second years of camelina seeding occurred on 10 November 2020 and 30 October 2021, respectively. At both sites, CamK3 was manually seeded in each 0.5×0.5 m^2 plot consisting of 4 rows 15 cm apart at a depth of 10 mm. The seeding rate was approximately 500 seeds m^{-2}. Six herbicides, including 2 pre-emergence (*s*-metolachlor and prodiamine) and 4 post-emergence (clethodim, clopyralid, fluazifop-*p*, and quinclorac) herbicides, that were safe to camelina plants (*Experiment 1*) were applied in single or combination in sequence to assess the weed control efficacy in the camelina field. The herbicide treatments included the single application of the 6 herbicides and combined application of *s*-metolachlor followed by the 4 post-emergence herbicides (*s*-metolachlor + clethodim, *s*-metolachlor + fluazifop-*p*, *s*-metolachlor + clopyralid, *s*-metolachlor + quinclorac). Controls included weed-free (hand weeding) plots and untreated plots (no herbicide treatment). All herbicides were applied at their standard doses using the same sprayer as in the greenhouse study. Immediately after camelina seeding, the pre-emergence herbicides were applied to the camelina plots. Irrigation was provided 2 days after camelina seeding to help improve seed germination and survival, then no irrigation was supplemented throughout the study, and all plants were grown under rain-fed conditions. Owing to the lower rate of weed occurrence during the winter season, the 4 post-emergence herbicides were applied to the plots at 60 days after camelina seeding (mean weed height in the plots about 3–5 cm). The growth stage of weeds was within the range of the efficacy of the herbicides applied. All camelina plots were arranged as RCBD with three replicated plots for each treatment. During the study period of 2020–2022, the meteorological data on daily mean, minimum, and maximum temperatures and precipitation were recorded and provided by the local Meteorological Administration. No insecticides or fungicides were applied during the study period.

Weed Control Efficacy and Camelina Yield

In early March of 2021 and 2022, all weeds present in the herbicide-untreated control plots (0.5×0.5 m^2) that were arranged randomly across the field plot were collected and identified to species level according to a weed identification handbook [23]. The relative abundance of each weed species was determined by dividing the number of individual weeds per species by the total number of weeds. In the middle of May of both years (camelina plants reaching harvest maturity), the weeds present in each plot (0.5×0.5 m^2)

were harvested at the soil surface, and their dry weights were determined after oven drying at 105 °C for 30 min and 65 °C until a constant weight was reached. The weed control efficacy of each treatment was calculated by dividing the total weed biomass in herbicide-treated plots by that of the total biomass of untreated plots. For camelina, at maturity, the total number of camelina plants in each plot (plant density) was determined and converted to plant density per m^2. Aboveground plant biomass was hand-harvested and dried in a forced-air oven at 65 °C until a constant weight was obtained. The silicles from the plants were threshed, and the resulting seeds were air-cleaned and oven-dried at 40 °C for 48 h before determination of dry matter of camelina seed yield per plant and $plot^{-1}$ (g). Additionally, the camelina 1000-seed weight (g) for each treatment was calculated.

Camelina Seed Quality Evaluation

Camelina seed oil content was determined using nuclear magnetic resonance (NMR) (CNMR-1000, Wuhan Chenmu Technology Co., Ltd., Wuhan, China) at the Laboratory of Grass Germplasm Resources Research and Utilization, Yangzhou University, China. The seed samples included about 30 g of camelina seeds obtained from each herbicide-treated plot of both years. The seeds were oven-dried at 130 °C for 1.5 h and cooled in a desiccator for 20 min. Prior to measurement, the NMR was calibrated with pure camelina oil (about 25 mL). The oil content was reported as a percentage (%).

Seeds obtained from all treatments in both years were analyzed for seed fatty acid composition by gas chromatography (GC9800, Shanghai Kechuang Chromatography Instruments Co., Ltd., Shanghai, China) at the Animal Nutrition and Feed Engineering Technology Research Laboratory, Yangzhou University, China. The preparation of fatty acid methyl esters (FAMEs) followed Augustin et al. (2019). The GC conditions, FAMEs sorting, and calculation of individual fatty acid content (%) were described as in a previous study [21]. Briefly, GC conditions were as follows: injector (270 °C) and detector temperatures (280 °C); initial temperature of 100 °C for 13 min; 10 °C min^{-1} to 180 °C (6 min); 1 °C min^{-1} to 200 °C (20 min); final with 4 °C min^{-1} to 230 °C (35 min). The concentrations of individual fatty acids were expressed as a percentage of total fatty acid content (%).

2.3. Statistical Analysis

All raw data obtained from this study were initially tested for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test). The tests showed the residuals of data were normally distributed and the variances were homogeneous. The data were then subjected to analysis of variance (ANOVA) to determine the factors effect (*Experiment 1*: camelina genotype, herbicide type, and dose; *Experiment 2*: year and herbicide treatment) and their interactions on camelina growth and yield potential (*Experiment 1*: visual efficacy, plant height, seed yield, and biomass per plant; *Experiment 2*: plant density, 1000-seed weight, seed yield ha^{-1}, and oil content). For data from the two experiments (greenhouse and field), when ANOVA indicated significant treatment effects ($p < 0.05$), the data were analyzed and reported individually. Nevertheless, if no significant treatment effects were revealed ($p > 0.05$), the data were pooled across the year (e.g., plant density, weed biomass). Means were separated using Tukey post-hoc tests at $p < 0.05$. All statistical analyses were conducted using R 3.2.4 [24].

3. Results

3.1. Greenhouse Study to Screen Camelina-Safe Herbicides

In the greenhouse study, post-application of 22 herbicides with various modes of action (6 herbicides registered as pre- and 16 herbicides registered as post-emergence herbicides, respectively) was tested for herbicide safety on the two camelina genotypes

(CamC1 and CamK3). The ANOVA revealed that camelina genotype and treatment significantly affected the four parameters evaluated, including visual efficacy, plant height, and seed and biomass yield per plant (Table 2). Of the 6 post-applied herbicides tested (registered as pre-emergence herbicides), *s*-metolachlor (cell division inhibitor) and prodiamine (microtubule assembly inhibitor) at the dose of ×1~×4 did not show visible damage or plant growth suppression on the two camelina genotypes relative to the untreated controls (Tables S1 and S2). The seed and biomass yield per plant of the two genotypes treated by the two herbicides at all application doses were statistically similar to the untreated controls (Figures 1 and 2). For example, CamC1 treated by *s*-metolachlor and prodiamine had a mean seed yield per plant ranging from 0.26–0.28 g and 0.27–0.28 g across the herbicide dose, respectively, which was comparable to the mean of the untreated control (0.27 g) (Figure 1A). The biomass yield per plant of CamC1 treated by the two herbicides across the dose ranged from 3.52–3.57 and 3.01–3.17 g, respectively, which was also statistically similar to that of 3.35 g for the untreated control (Figure 2A). For CamC1, the changing pattern of the seed and biomass yield per plant of CamK3 responding to the two herbicides was also similar (Figures 1A and 2B). Regarding the other herbicides, for instance, pendimethalin, another microtubule assembly inhibitor, they caused obvious visual damage, especially a reduction in plant height (Table S2). Oxyfluorfen (PPO inhibitor), clomazone (IPP inhibitor), and flumetsulam (ALS inhibitor) completely killed the two camelina genotypes at all applied doses (Figures 1 and 2; Tables S1 and S2).

Table 2. ANOVA results (*F* values) of the factors effects and interactions on camelina growth and production in the greenhouse (effect of different treatments on camelina visual efficacy, plant height, seed, and biomass yield per plant) and field studies (effect of different treatments on plant density at harvest, 1000-seed weight, seed yield ha^{-1}, and seed oil content).

Year of Study	Source of Variation	Significant Level Determined by *p* Value								
			Visual efficacy		Plant height		Seed yield per plant		Biomass per plant	
		DF	*F* values	*p*	*F* values	*p*	*F* values	*p*	*F* values	*p*
Greenhouse study (2019–2020)	Camelina genotype (CG)	1	>10^{20}	**	98	***	15	**	24	***
	Treatment (T)	66	>10^{20}	***	3080	***	168	***	362	***
	CG × T	66	>10^{20}	**	48	**	7	**	11	**
	Residual	268		–		–		–		–
			Final plant density		1000-seed weight		Seed yield ha^{-1}		Oil content	
		DF	*F* values	*p*	*F* values	*p*	*F* values	*p*	*F* values	*p*
Field study (2020–2022)	Year (Y)	1	133.5	NS	2.9	NS	126.3	***	187.6	*
	Treatment (T)	11	354.2	***	7.2	NS	86.4	***	46.9	***
	Y × T	11	297.8	*	23.2	**	234.2	***	3.5	NS
	Residual	48		–		–		–		–

NS: Not significant; *, **, and *** represent significant at 0.05, 0.01, and 0.001 probability level, respectively.

Among the post-emergence herbicides evaluated, the herbicides from the ACCase inhibitor, including clethodim, quizalofop-*p*-ethyl, haloxyfop-*p*, and fluazifop-*p*, and from synthetic auxins, including clopyralid and quinclorac, were relatively safe to the two camelina genotypes (Figures 1 and 2; Tables S1 and S2). The results showed that both of the two camelina genotypes treated by clethodim, fluazifop-*p*, and clopyralid at the dose of ×1~×4 had a statistically similar seed and biomass yield per plant compared to the untreated controls (Figures 1 and 2). For example, the mean seed and biomass yields per plant of CamC1 treated by clethodim were in the range of 0.26–0.27 g and 3.52–3.55 g across the three doses, respectively, which were statistically similar to those of the untreated control (seed yield per plant: 0.26 g; biomass yield per plant: 3.52 g). CamK3 also showed a similar seed (range: 0.19–0.21 g) and biomass yield per plant (range: 3.38–3.60 g) to the untreated control (0.21 and 3.40 g, respectively) when treated by clethodim at the three doses. Additionally, clopyralid, the synthetic auxin herbicide, showed good herbicidal safety on the plant growth and productivity of CamC1 (mean seed yield per plant ranging 0.21–0.23 g vs. the untreated control of 0.22 g; mean biomass yield per plant ranging 2.99–3.17 g vs. untreated control of 3.17 g) and CamK3 (mean seed yield per plant ranging 0.23–0.24 g vs. untreated control of 0.23 g; mean biomass yield per plant ranging 3.22–3.30 g vs. untreated control of 3.25 g). For other ACCase inhibitors, such as quizalofop-*p*-ethyl and haloxyfop-*p*, while they were safe for plant growth and seed productivity of camelina at a ×2 application dose, when applied at a ×4 dose rate, a reduction in seed yield per plant in CamK3 was observed compared to the untreated control (Figures 1 and 2). This is also the case for the synthetic auxin herbicide, quinclorac. Regarding herbicides from ALS (e.g., halosulfuron, imazethapyr), HPPD (mesotrione), PPO (fomesafen), and PS II (bentazone), they caused severe herbicidal damage to camelina plants and completely killed the plants within 3 weeks. Among the ALS inhibitors, although nicosulfuron application did not kill all the camelina plants, it resulted in a significant reduction in camelina seed yield and biomass yield per plant.

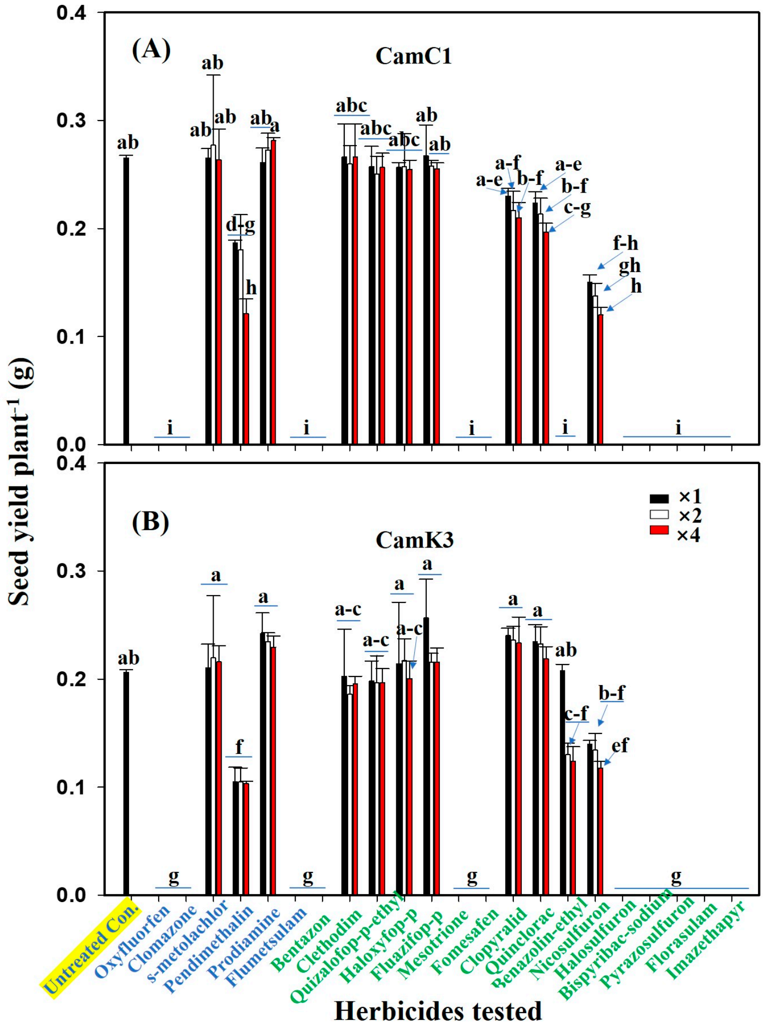

Figure 1. Greenhouse study for the evaluation of the effect of post-application of 22 herbicides at three application doses (×1, ×2, and ×4 of standard dose) on seed yield per plant (g) of CamC1 (**A**) and CamK3 (**B**), respectively. Of the 22 herbicides, 6 herbicides (e.g., oxyfluorfen, clomazone) are registered as pre-emergence herbicides, and the remaining 16 herbicides (e.g., bentazon, clethodim) are registered as post-emergence herbicides. The values reported are the mean of seed yield per plant (g) ± standard errors. Means with the same letter are not significantly different by Tukey post-hoc tests at $p < 0.05$.

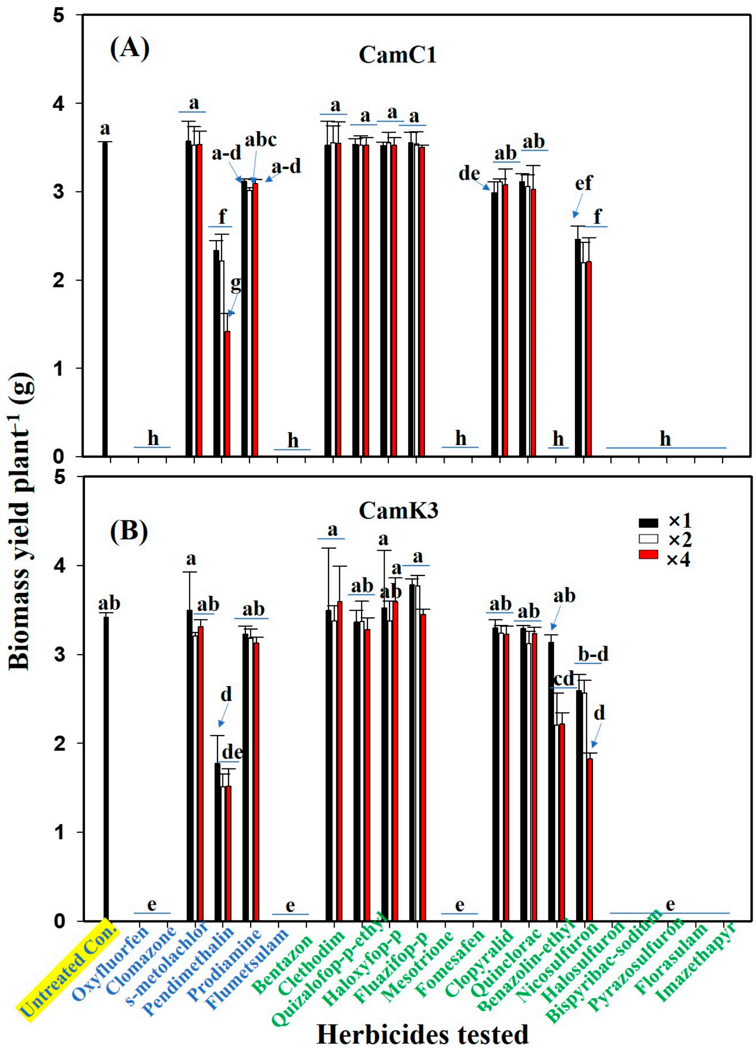

Figure 2. Greenhouse study for the evaluation of the effect of post-application of 22 herbicides at three application doses (×1, ×2, and ×4 of standard dose) on biomass yield per plant (g) of CamC1 (**A**) and CamK3 (**B**), respectively. Of the 22 herbicides, 6 herbicides (e.g., oxyfluorfen, clomazone) are registered as pre-emergence herbicides, and the remaining 16 herbicides (e.g., bentazon, clethodim) are registered as post-emergence herbicides. The values reported are the mean of biomass yield per plant (g) ± standard errors. Means with the same letter are not significantly different by Tukey post-hoc tests at $p < 0.05$.

Based on the evaluation results described above, two pre-emergence herbicides (*s*-metolachlor and prodiamine) and four post-emergence herbicides (clethodim, fluazifop-*p*, clopyralid, and quinclorac) were tested further for weed control efficiency and camelina safety under field conditions.

3.2. Field Study with Selected Pre- and Post-Emergence Herbicides

3.2.1. Weather Conditions During the Study Period

The weather data, including mean monthly air temperature and accumulated monthly precipitation during the study period, are shown in Table S3. Overall, the mean monthly air temperatures between the two experimental years were comparable (e.g., 3.7 vs. 3.9 °C in January and 5.3 vs. 4.0 °C in February of the two years, respectively), which were consistent with those of 2.9 (January) and 5.2 °C (February) over the past 30 years (1991–2020). During the study period, the lowest and highest temperatures were about 3 °C and 21 °C in December and May in the two years, respectively. Regarding the accumulated monthly precipitation, except for the higher precipitation of 144.3 mm compared to 15.5 mm in May, the values for the other months of the two years were consistent with the long-term means.

3.2.2. Weed Species and Abundance and Weed Control Efficiency

Weed species present in the camelina field plot and their abundance during the study period (2020–2022) are shown in Table 3. The annual weeds, including crabgrass [*Digitaria sanguinalis* (L.) Scop.] (mean abundance of 50.6%), common vetch (*Vicia sativa* L.) (mean abundance of 13.8%), and shepherd's purse [*Capsella bursa-pastoris* (L.) Medik.] (mean abundance of 10.2%), were the three most dominant weed species across the two years of investigation. Only one perennial weed, Chinese mugwort (*Artemisia argyi* Levl. et Vant.), was observed in the field with a mean abundance of about 1.5%. The abundance of the remaining weeds was about 23.6%.

Table 3. Weed species detected in camelina field plots (*Experiment 2*) and their relative abundances during the study period (2020–2021 and 2021–2022).

Weed Species	Family	Life Form	Abundance (%) of Individual Weed		Mean (%)
			2021	2022	
Crabgrass [*Digitaria sanguinalis* (L.) Scop.]	Poaceae	Annual	50.2% (31)	51.7% (37.0)	50.6%
Common vetch (*Vicia sativa* L.)	Fabaceae	Annual	10.3% (6.3)	17.3% (12.7)	13.8%
Shepherd's purse [*Capsella bursa-pastoris* (L.) Medik.]	Brassicaceae	Annual	14.4% (9.0)	6.1% (4.7)	10.2%
False cleavers (*Galium spurium* L.)	Rubiaceae	Annual	10.0% (6.3)	3.8% (2.7)	6.9%
Sticky chickweed (*Cerastium glomeratum* L.)	Caryophyllaceae	Annual	6.1% (4.0)	10.2% (7.3)	8.2%
Birdeye speedwell (*Veronica persica* Poir.)	Plantaginaceae	Annual	4.3% (3.0)	0 (0)	2.1%
Annual fleabane [*Erigeron annuus* (L.) Pers.]	Asteraceae	Annual	2.0% (1.3)	0 (0)	1.0%
Carolina geranium (*Geranium carolinianum* L.)	Geraniaceae	Annual	1.6% (1.0)	7.8% (5.7)	4.6%
Sun spurge (*Euphorbia helioscopia* L.)	Euphorbiaceae	Annual	1.5% (1.0)	0 (0)	0.8%
Chinese mugwort (*Artemisia argyi* Levl. et Vant.)	Asteraceae	Perennial	0	3.1% (2.3)	1.5%

Among the herbicides tested, sequential application of pre-emergence *s*-metolachlor followed by post-emergence clethodim, fluazifop-*p*, or clopyralid showed the best weed control efficacy compared to other herbicides during the two years' study. Those herbicides significantly reduced the percent weed biomass in herbicide-treated plots (up to 90%) relative to the untreated controls (mean: 112.3 g) (Figure 3). By contrast, single applications of those herbicides showed relatively lower weed control efficacy, with weed biomass reductions of 68–83% in the treated plots relative to the untreated controls. Compared to *s*-metolachlor, prodiamine applied as a pre-emergence herbicide showed poor weed control spectrum and efficacy (<40%) in both years of the study. Regardless of single or sequential application following *s*-metolachlor, quinclorac showed lower weed control efficacy (54–66%) compared to clopyralid (78–84%), both of which are synthetic auxin herbicides.

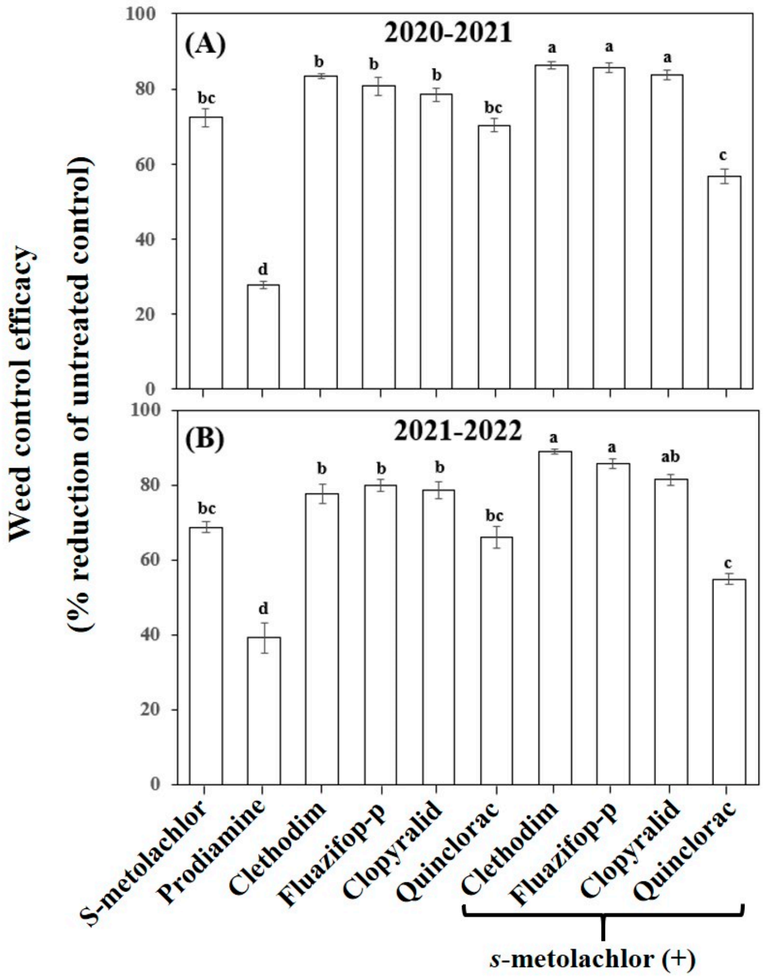

Figure 3. Weed control efficacy (% reduction in the untreated control) for CamK3 field plots treated with the screened herbicides (×1 of standard dose) in 2020–2021 (**A**) and 2021–2022 (**B**). Field herbicide treatments included the single pre-application of *s*-metolachlor and prodiamine, post-application of clethodim, fluazifop-*p*, clopyralid, and quinclorac, and sequential application combining *s*-metolachlor and post-emergence herbicides (clethodim, fluazifop-*p*, clopyralid, and quinclorac). The values reported are the mean of weed control efficacy ± standard errors. Means with the same letter are not significantly different by Tukey post-hoc tests at $p < 0.05$.

3.2.3. Camelina Plant Density, Seed, and Oil Yield Potential

To evaluate herbicide safety for camelina growth and yield potential, the camelina plant density (plant m^{-2}) in each plot at harvest, 1000-seed weight (g), and seed yield (kg ha^{-1}) were determined (Figures 4 and 5). As no significant year effect on camelina plant density at harvest and 1000-seed weight were observed (Table 2), the mean across the years was reported (Figure 4). In general, as shown in Figure 4A, camelina plots solely applied by clopyralid (223 plants m^{-2}) or sequentially applied by *s*-metolachlor following clethodim (210 plants m^{-2}), fluazifop-*p* (219 plants m^{-2}), or clopyralid (208 plants m^{-2}) had statistically similar plant densities to weed-free camelina plots (216 plants m^{-2}). By contrast, single pre-application of *s*-metolachlor (141 plants m^{-2}) or prodiamine (78 plants m^{-2}) showed much lower camelina plant densities. Single applications of clethodim (187 plants m^{-2}) or fluazifop-*p* (193 plants m^{-2}) showed reduced camelina plant density compared to sequential application following *s*-metolachlor. Either quinclorac applied on its own, or sequentially applied following *s*-metolachlor, showed the lower camelina plant density. Regarding 1000-seed weight, although the statistical difference was observed for various treatments, all of those values (range: 1.32–1.40 g across the treatments) were relatively consistent with the weed-free plots (1.37 g) (Figure 4B). The 1000-seed weight for sequential applications of clethodim (1.40 g), fluazifop-*p* (1.37 g), or clopyralid (1.37 g) following *s*-metolachlor was similar to the weed-free camelina plots.

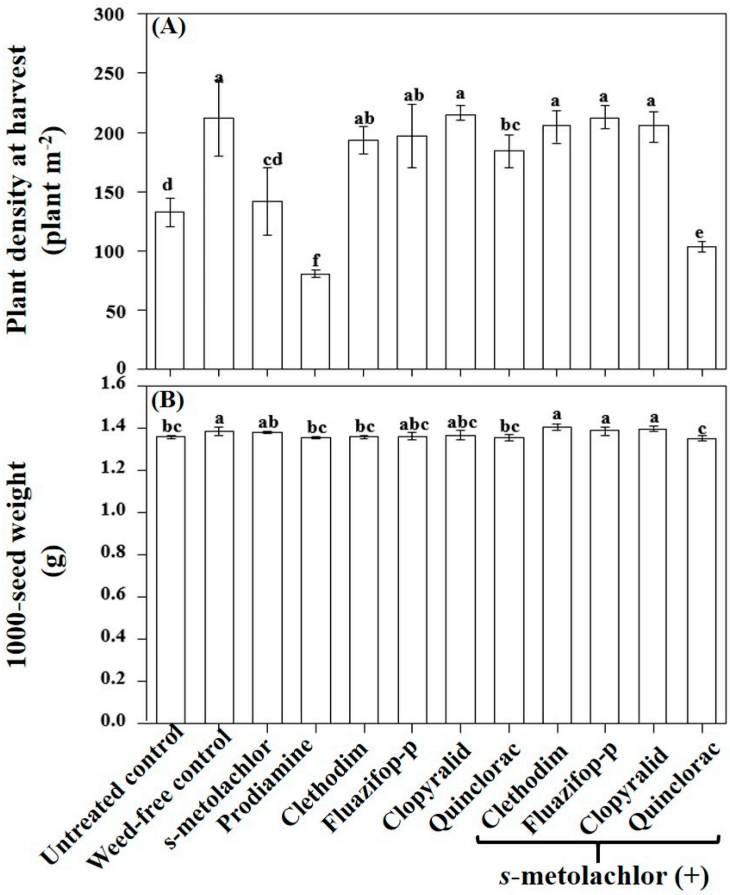

Figure 4. Plant density at harvest (plant m^{-2}) (**A**) and 1000-seed weight (g) (**B**) for CamK3 field plots treated with the screened herbicides (×1 of standard dose) during the two-year study of 2020–2022. Control groups included herbicide-untreated control and hand weeding (weed−free control) plots, respectively. Field herbicide treatments included the single pre-application of *s*-metolachlor and prodiamine, post-application of clethodim, fluazifop-*p*, clopyralid, and quinclorac, and sequential application combining *s*-metolachlor and post-emergence herbicides (clethodim, fluazifop-*p*, clopyralid, and quinclorac). The values reported are the mean of plant density or 1000-seed weight ± standard errors. Means with the same letter are not significantly different by Tukey post-hoc tests at $p < 0.05$.

Both the year and herbicide treatment showed a significant effect on the seed yield ha^{-1} and oil content (%) (Table 2). Camelina plots treated by *s*-metolachlor following clethodim (1924 and 1889 kg ha^{-1} in the two years, respectively), fluazifop-*p* (2043 and 1891 kg ha^{-1}, respectively), or clopyralid (1871 and 1962 kg ha^{-1}, respectively) showed statistically similar or increased seed yields when compared to the weed-free camelina plots (1867 and 1811 kg ha^{-1}, respectively) during the two years of study (Figure 5A,B). Single applications of clethodim, fluazifop-*p*, or clopyralid showed lower seed yield ha^{-1} compared to sequential application with *s*-metolachlor. Quinclorac applied on its own, or sequentially applied following *s*-metolachlor, showed lower seed yield production. The same case was also observed for prodiamine-treated camelina plots. In terms of the oil content, except for the lower values for prodiamine treatment in the first year (38.2%) and quinclorac treatment in both years (37.0–38.3%), the oil content values for the other treatments (range: 39.4–42.3%) were comparable to those of the weed-free camelina plots (mean: 41.8%) (Figure 5C,D). In both years, the sequential application of clethodim, fluazifop-*p*, or clopyralid following *s*-metolachlor showed a relatively greater seed oil content compared to the other treatments. It is worth mentioning that the oil content value for the sequential application of quinclorac following *s*-metolachlor in the first year of the study was unavailable due to crop harvest failure (Figure 5C).

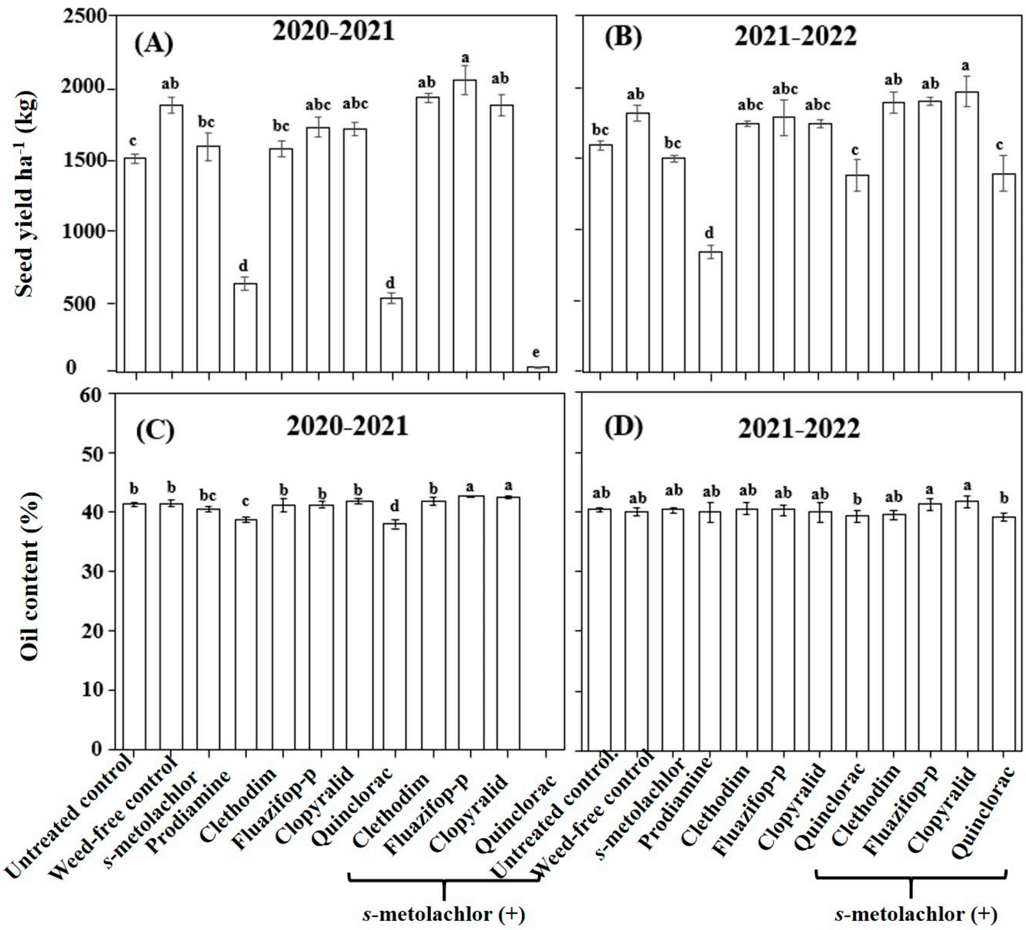

Figure 5. Seed yield ha^{-1} (kg) (**A**,**B**) and oil content (%) (**C**,**D**) for CamK3 field plots treated with the screened herbicides (×1 of standard dose) in 2020–2021 (**A**,**C**) and 2021–2022 (**B**,**D**). Control groups included herbicide-untreated control and hand weeding (weed-free control) plots, respectively. Field herbicide treatments included the single pre-application of *s*-metolachlor and prodiamine, post-application of clethodim, fluazifop-*p*, clopyralid, and quinclorac, and sequential application combining *s*-metolachlor and post-emergence herbicides (clethodim, fluazifop-*p*, clopyralid, and quinclorac). The values reported are the mean of seed yield ha^{-1} or oil content ± standard errors. Means with the same letter are not significantly different by Tukey post-hoc tests at $p < 0.05$.

3.2.4. Effect of Herbicide Treatments on Camelina Oil Quality

To further evaluate the effect of individual herbicides on camelina seed oil quality, the principal UFA compositions and concentrations of CamK3 collected from the two years of study were determined (Table 4). Overall, although the concentrations of individual UFA analyzed showed different changing patterns, the total SFA, MUFA, PUFA, and ratios of UFA/SFA and MUFA/PUFA were statistically comparable to those of the weed-free camelina plots (Table 4). For example, the contents of SFA, MUFA, and PUFA in camelina seed oil across the herbicide treatment ranged from 11.88–12.49%, 26.75–27.58%, and 57.18–58.41%, respectively, which were statistically similar to those of 11.96, 26.85, and 58.19% for the weed-free camelina plots. The consistent ratios of UFA/SFA and MUFA/PUFA further suggested that the current herbicide treatment did not affect the total amount of fatty acids and the ratio of the principal fatty acids in camelina seed oil. It is worth mentioning that no matter whether quinclorac was applied solely or sequentially following *s*-metolachlor, the contents of C18:1~3 in total fatty acids were significantly lower than the values for other herbicide treatments, which may explain the lower seed oil content that resulted from the quinclorac treatment (Figure 5C,D).

Table 4. The principal unsaturated fatty acids, concentrations (%), UFA/SFA, and MUFA/PUFA of CamK3 under various treatments.

Treatment	Principal Fatty Acid Concentration (%)								UFA/SFA Ratio	MUFA/PUFA Ratio
	C18:1	C18:2	C18:3	C20:1	C22:1	SFA	MUFA	PUFA		
Untreated control	13.67 d	21.34 c	32.03 de	10.29 ab	1.71 b	11.68 b	26.33 b	57.73 a	6.83 a	0.48 a
Weed-free control	14.03 bcd	22.45 b	33.09 a	10.28 ab	2.01 ab	11.96 ab	26.85 ab	58.19 a	7.20 a	0.49 a
s-metolachlor	14.36 abcd	22.18 b	32.68 abc	10.98 ab	1.91 ab	11.88 ab	26.82 ab	57.46 a	7.24 a	0.48 a
Prodiamine	14.65 abc	22.26 b	32.17 cd	10.93 ab	1.98 ab	12.04 ab	27.49 a	57.71 a	7.22 a	0.49 a
Clethodim	14.22 abcd	22.11 b	32.91 ab	10.58 ab	1.96 ab	12.49 a	27.58 a	57.41 a	7.37 a	0.48 a
Fluazifop-*p*	14.32 abcd	23.4 a	32.61 abc	11.29 a	2.08 a	11.98 ab	27.17 ab	58.41 a	7.26 a	0.48 a
Clopyralid	14.29 abcd	22.19 b	32.36 bcd	10.49 b	1.94 ab	12.64 a	27.13 ab	57.83 a	6.77 a	0.47 a
Quinclorac	13.87 cd	21.02 c	31.44 e	10.37 b	1.96 ab	12.21 ab	27.18 ab	57.54 a	7.06 a	0.48 a
s-metolachlor + clethodim	14.2 abcd	22.19 b	31.93 de	10.77 ab	1.91 ab	12.41 ab	26.78 ab	57.77 a	7.21 a	0.49 a
s-metolachlor + fluazifop-*p*	14.46 abcd	22.12 b	32.81 abc	10.93 ab	1.94 ab	12.26 ab	26.75 ab	57.98 a	7.29 a	0.48 a
s-metolachlor + clopyralid	14.85 a	22.14 b	32.27 bcd	10.59 ab	1.93 ab	12.21 ab	27.28 ab	58.03 a	7.15 a	0.48 a
s-metolachlor + quinclorac	14.07 bcd	21.98 b	31.84 de	10.52 ab	1.92 ab	12.27 ab	26.99 ab	57.18 a	6.99 a	0.49 a
Mean across treatments	14.25	22.12	32.42	10.66	1.94	12.17	26.97	57.76	7.12	0.48

Treatments mean with different letters within each column: significant different values ($p < 0.05$, Tukey post-hoc test). C18:1 (oleic acid); C18:2 (linoleic acid); C18:3 (linolenic acid); C20:1 (eicosenoic acid); C22:1 (erucic acid); SFA (saturated fatty acid); MUFA (monounsaturated fatty acid); PUFA (polyunsaturated fatty acid); UFA/SFA ratio = the sum of unsaturated fatty acid/the sum of saturated fatty acid; MUFA/PUFA ratio = the sum of MUFA/the sum of PUFA.

4. Discussion

Weed management has been one of the major challenges in camelina field production, even though camelina is claimed to be competitive and capable of suppressing weeds in some cases [1,25]. To the best of the authors' knowledge, there are only two grass herbicides (sethoxydim and quizalofop-*p*-ethyl), but no broadleaf herbicides registered for camelina production in the USA and Canada [12,15]. Previous studies have reported a broadleaf herbicide, dinitroaniline, that showed adequate safety for camelina plants, but many grass and broadleaf weeds in camelina field are not controlled by this herbicide. When this herbicide was applied at a rate of 1.26 kg ha^{-1}, camelina seed yield was significantly reduced by 31% [12]. Thus, the selection of safe herbicides for camelina and the development of effective herbicide-based weed management could be a potential way to increase camelina production. The present study was conducted to evaluate and screen safe pre- and post-emergence herbicides from a diverse group of herbicides with various modes of action to camelina and then test their weed control efficiencies in a camelina field plot.

4.1. Evaluation of Safe Herbicides for Camelina

The evaluations confirmed that the herbicides from cell division (e.g., *s*-metolachlor), synthetic auxin (e.g., clopyralid), microtubule assembly (e.g., prodiamine), and ACCase (e.g., clethodim, quizalofop-p-ethyl) inhibitor groups provided adequate safety for camelina plant growth and seed yield production. Pre-applications of *s*-metolachlor (1060–2140 g ai ha^{-1}) or pendimethalin (1060–2140 g ai ha^{-1}) have been recommended as safe herbicides for use in camelina [12]. Although both of them caused some visually evident injury symptoms to camelina, they did not reduce the camelina plant density and seed yield. In this experiment, the results of the greenhouse study confirmed that post-application of *s*-metolachlor (1440 g ai ha^{-1}) was safe for camelina growth, plant development, and seed production. When pendimethalin was post-applied (990 g ai ha^{-1}), it significantly reduced the seed and biomass yield per plant by as much as 50% in the two camelina genotypes. Although the phytotoxic effects of herbicides vary with camelina genotypes [20], attention should be paid during application of this herbicide in camelina (regardless of pre- or post-emergence). In contrast, prodiamine showed superior levels of crop safety in the two camelina genotypes (e.g., visual efficacy, seed yield) examined in this study compared to the same dinitroaniline family of pendimethalin, indicating its potential as an alternative pre-emergence herbicide for use in camelina. Apart from quizalofop-*p*-ethyl and sethoxydim [1], the other two ACCase inhibitors, clethodim and fluazifop-*p*, tested in this study showed reasonable herbicidal safety for the two camelina genotypes up to ×4 of standard application dose. Although clopyralid (90 g ai ha^{-1}) caused a certain degree of injury in several camelina genotypes [20], the present study suggested it was a safe herbicide for the two camelina genotypes when applied at 101 g ai ha^{-1}. As outlined in the previous report [12], quinclorac applied at 225 g ai ha^{-1} showed acceptable safety for the two camelina genotypes. Benazolin-ethyl, another synthetic auxin herbicide, showed obvious phytotoxic effects on the two camelina genotypes.

Consistent with previous studies [26], the ALS inhibitors tested in this study, including nicosulfuron, halosulfuron-methyl, and pyrazosulfuron-ethyl (sulfonylurea), bispyribac-sodium (pyrimidinyl benzoates), florasulam (triazolopyrimidine), and imazethapyr (Imidazolinone), showed severe herbicidal phytotoxic effects on camelina. Hulbert et al. also reported this crop species is highly sensitive to soil residual levels of many ALS inhibitors [27]. This is because ALS inhibitors can inhibit acetolactate synthase (ALS), a key enzyme in the biosynthesis of branched-chain amino acids in plants, which would cause the inhibition of protein formation, eventually killing the plant [26,28]. Additionally, this study showed that herbicides related to photosynthesis (e.g., bentazon, PSII inhibitor) or photosynthetic pigment synthesis (e.g., oxyfluorfen, PPO inhibitor; mesotrione and clomazone, HPPD inhibitor) caused severe herbicidal phytotoxic effects (e.g., bleaching symptoms) on camelina plants and completely killed the plant at any application dose. These herbicides can disrupt the photosynthetic process by binding to specific sites within the photosystem complex in plant chloroplasts, rapidly resulting in plant tissue necrosis and cell membrane destruction, and eventually leading to plant death [29,30].

4.2. Weed Control Efficiency, Camelina Yield, and Seed Oil Quality

Herbicide-based weed control efficacy mainly depends on weed species composition, the herbicidal response of weeds, and environmental factors [31–33]. Weed species present in camelina fields can be diverse. A three-year weed survey of a camelina field in Northeastern USA (Connecticut) showed that annual foxtail was the most abundant grass species, followed by several broadleaf weeds, such as common ragweed (*Ambrosia artemisiifolia* L.), lambsquarters (*Chenopodium album* L.), and common waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer]. Common weed species, such as shepherd's purse and field pennycress (*Thlaspi arvense* L.), occurred at low abundance [13]. Additionally, perennial weeds, such as morning glory (*Ipomoea purpurea* L.), Canada thistle (*Cirsium arvense* L.), and skeleton weed (*Chondrilla juncea* L.), were problematic for camelina [34]. In the experimental sites of this study, the annual crabgrass was the most abundant grass species (about 50%). The remaining weeds were annual or perennial broadleaf weeds, such as common vetch (about 13.8%), shepherd's purse (about 10%), and false cleavers (about 7%).

Grass weed species, such as foxtail, could be controlled by ACCase inhibitors (e.g., quizalofop-*p*-ethyl, fluazifop-*p*) due to their good selectivity between grass and dicot crops, including camelina [12,19]. By contrast, control of post-emerged broadleaf weeds in camelina is very challenging as camelina tolerates relatively few broadleaf herbicides. In practice, pre-emergence herbicides are usually used to reduce the emergence of broadleaf weeds from the soil seedbank. The previous studies outlined above coupled with the present study showed that *s*-metolachlor was safe for camelina and provided a wide spectrum of pre-emergence broadleaf weed control (e.g., waterhemp, lambsquarters) [35]. However, the efficacies of the herbicides were influenced by several factors, including the application time, application dose, and duration of herbicide residue [36]. The present study showed that pre-application of *s*-metolachlor effectively suppressed the weed emergence from fields, but no effect (herbicide residual effect) was found on the control of post-emerged weeds. Sequential application of pre- and post-emergence herbicides could compensate for each other, by providing a much wider spectrum of grass and broadleaf weed control. As revealed in this study, sequential application of *s*-metolachlor followed by post-emergence herbicides significantly reduced the weed biomass in the camelina field plot, and increased the camelina seed yield and plant biomass compared to the single applications of these herbicides. Among the effective herbicide combinations, ACCase inhibitors, such as clethodim and fluazifop-*p*, provided excellent weed control efficacy on post-emerged annual grasses (e.g., crabgrass), whereas clopyralid was effective for the control of broadleaf weeds, such as common vetch and shepherd's purse. To the authors' knowledge, clopyralid selected in this study is the first selective herbicide that effectively controlled broadleaf weeds in camelina. The herbicide cost for this sequential application was assessed based on herbicide application dosages and herbicide price provided by the manufacturers. If converted to the herbicide price ha^{-1}, the cost of *s*-metolachlor, fluazifop-*p*, and clopyralid ha^{-1} is about USD 22, USD 25, and USD 16.5, respectively (USD 63.5 for camelina ha^{-1}). It should be noted that USD 63.5 for camelina ha^{-1} is only an approximate price, and the actual prices may vary due to different regions, purchase channels, and market conditions. It is also worth mentioning that quinclorac, known as a post-emergence herbicide for grass and broadleaf weed control [37]), was reported to be adequately safe for camelina when applied as a pre-emergence herbicide [12]. In this study, although the post-application of quinclorac (225 g ai ha^{-1}) was safe for camelina plants in the greenhouse study, field application of this herbicide at the same rate significantly reduced the camelina seed yield, which was mainly attributed to the control failure of broadleaf weeds (e.g., false cleavers, Wilford's cranesbill). Thus, in agronomic practice, prior to the selection of herbicides, the weed species in a camelina field should be investigated for effective weed control.

The most important component of an oilseed crop, such as camelina, is the seed oil content and quality. The higher nutritional value of camelina oil is determined by its low level of SFA (8–13%) and high level of UFA (86–92%), mainly C18:1~3 (54.6–76.6%) [38–42]. To date, although there are no reports on the evaluation of herbicide effects on camelina seed oil quality, published studies have demonstrated that herbicide application (e.g., application timing, herbicide type, dose) can negatively affect seed oil concentrations in oilseed rape [43,44] or soybean [45–47]. In this study, the herbicides evaluated, except for quinclorac, showed little or no effect on the principal UFA concentration (e.g., C18:1~3), UFA/SFA, and MUFA/PUFA of camelina seed oil, indicating the safety of these herbicides for use in camelina. It is worth pointing out that the lower content of C18:1~3 for quinclorac-treated camelina could probably be caused by weed control failure in the camelina plot as a previous study reported the potential impact of weeds on the reduction in oil and meal quality in oilseed rape [48]. Again, the UFA compositions and concentrations demonstrate the importance and necessity of weed management in camelina to obtain higher seed yields and better oil quality. Currently, a new herbicide-tolerant camelina seed variety SES1154HR (branded NewGold) launched by Smart Earth Camelina Corp. (breeder: Christina Eynck) is available for Canadian growers in Canada [49]. This new variety is resistant to the

ALS inhibitor, thifensulfuron-methyl, and can facilitate the control of broadleaf weeds in camelina. However, caution should be paid regarding the potential for pollen-mediated gene flow from this herbicide-tolerant variety to traditional varieties or its relatives, which could generate subsequent weed management challenges [50–52]. Thus, related studies regarding the potential ecological risks of transgene flow (e.g., herbicide tolerance) to non-GM camelina or its relatives should be conducted to provide baseline information for mitigating transgene flow [53].

5. Conclusions

The objective of the present study was to evaluate and establish a program combining pre- and post-emergence herbicides for effective weed control in camelina. Increased seed yield was shown with the sequential application of pre- and post-emergence herbicides compared to the untreated controls, indicating the importance and necessity for weed control in camelina, even though camelina is claimed to be a very competitive crop. Among the herbicides tested, pre-emergence *s*-metolachlor was safe for camelina and effectively reduced weed seed emergence from the soil seedbank. Post-emerged grass weeds in camelina fields (e.g., crabgrass, foxtail) can be controlled by ACCase inhibitors (e.g., quizalofop-*p*-ethyl, fluazifop-*p*). For broadleaf weeds, clopyralid evaluated in this study provided adequate safety for camelina and showed good selectivity between the weeds (e.g., common vetch, shepherd's purse) and camelina; thus, clopyralid can potentially be used for broadleaf control. Finally, it is worth mentioning that the herbicide application program used in this study was effective for the distribution of weed species in the study region; however, control efficacies could vary with changes in weed species and abundance in different regions, especially in cases where other broadleaf weeds are dominant. Thus, further study is still needed to screen safer and more effective herbicides with various modes of action to provide a wider spectrum of broadleaf weed control in camelina.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy15030640/s1, Table S1: Visual efficacies of the herbicides tested on CamC1 and CamK3 genotypes at 21 DAT; Table S2: The mean plant height of the herbicides tested on CamC1 and CamK3 genotypes at 21 DAT; Table S3: Mean monthly air temperature and accumulated monthly precipitation during the study period in 2020–2021 and 2021–2022.

Author Contributions: Conceptualization and methodology, C.-J.Z., Y.W.; software and validation, C.-J.Z., Y.W., S.-Z.D., M.C., H.-Z.W.; visualization, investigation, and data curation, Y.W., S.-Z.D.; writing—original draft preparation, S.-Z.D., Y.W.; supervision, C.-J.Z., M.-J.Y.; project administration and funding acquisition, C.-J.Z.; writing—reviewing and editing, S.-Z.D., Y.W., M.-J.Y., H.-Z.W., M.C., C.-J.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This work was mainly supported by the National Science Foundation of China (Grant No. 32171670) and High-level Talents program of Yangzhou University to Chuan-Jie Zhang, and Natural Science Fund for colleges and university of Jiangsu Province (Grant No. 19KJB180034), China.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data will be made available on reasonable request.

Acknowledgments: We thank Lim SH, Kim DS, Min X, Yu J, Fan J, and Yan X for commenting and editing the manuscript.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviations

ACCase	Acetyl-CoA carboxylase;
ALS	Acetolactate synthase;
HPPD	4-Hydroxyphenyl pyruvate dioxygenase;
IPP	Isopentenyl pyrophosphate (IPP) isomerase
MUFA/PUFA	Monounsaturated fatty acid/polyunsaturated fatty acid
PPO	Protoporphyrinogen-IX oxidase;
PSII	Photosystem II
UFA/SFA	Unsaturated fatty acid/saturated fatty acid

References

1. Berti, M.; Gesch, R.; Eynck, C.; Anderson, J.; Cermak, S. Camelina uses, genetics, genomics, production, and management. *Ind. Crops Prod.* **2016**, *94*, 690–710. [CrossRef]
2. Vollmann, J.; Moritz, T.; Kargl, C.; Baumgartner, S.; Wagentristl, H. Agronomic evaluation of camelina genotypes selected for seed quality characteristics. *Ind. Crops Prod.* **2007**, *26*, 270–277. [CrossRef]
3. Zanetti, F.; Monti, A.; Berti, M.T. Challenges and opportunities for new industrial oil seed crops in EU-27: A review. *Ind. Crops Prod.* **2013**, *50*, 580–595. [CrossRef]
4. Hunsaker, D.J.; French, A.N.; Clarke, T.R.; El-Shikha, D.M. Water use, crop coefficients, and irrigation management criteria for camelina production in arid regions. *Lrrig. Sci.* **2011**, *29*, 27–43. [CrossRef]
5. Putnam, D.H.; Budin, J.T.; Field, L.A.; Breene, W.M. *Camelina: A Promising Low Input Oilsee*; Wiley: New Crops, NY, USA, 1993; p. 314.
6. Zhang, C.-J.; Gao, Y.; Jiang, C.; Liu, L.; Wang, Y.; Kim, D.-S.; Yu, J.-L.; Yu, L.; Yu, F.; Fan, Y.; et al. Camelina seed yield and quality in different growing environments in northern China. *Ind. Crops Prod.* **2021**, *172*, 114071. [CrossRef]
7. Du, S.; Li, X.; Yu, L.; Xu, M.; Zhang, H.; Zhang, H.; Zhang, H. Progress in characteristics and application of *Camelina sativa* L. *China Oils Fats* **2018**, *43*, 108–111. (In Chinese with English abstract)
8. Zhang, M. High-yielding cultivation technology of *Camelina sativa* L. in Heyin. *Rural. Econ. Technol.* **2012**, *12*, 123. (In Chinese)
9. Ghidoli, M.; Pesenti, M.; Colombo, F.; Nocito, F.F.; Pilu, R.; Araniti, F. *Camelina sativa* (L.) Crantz as a promising cover crop species with allelopathic potential. *Agronomy* **2023**, *13*, 2187. [CrossRef]
10. Neupane, D.; Lohaus, R.H.; Solomon, J.K.Q.; Cushman, J.C. Realizing the potential of *Camelina sativa* as a bioenergy crop for a changing global climate. *Plants* **2022**, *11*, 772. [CrossRef]
11. Obour, K.A.; Sintim, H.Y.; O'bend, E.; Jeliazkov, V.D. Oilseed camelina (*Camelina sativa* L. Crantz): Production systems, prospects and challenges in the USA great plains. *Adv. Plants Agric. Res.* **2015**, *2*, 00043. [CrossRef]
12. Jha, P.; Stougaard, R.N. Camelina (*Camelina sativa*) tolerance to selected preemergence herbicides. *Weed Technol.* **2013**, *27*, 712–717. [CrossRef]
13. Rizzitello, R.; Zhang, C.J.; Auer, C. Camelina (*Camelina sativa* L. Crantz) crop performance, insect pollinators, and pollen dispersal in the Northeastern US. *BioRxiv* **2019**. [CrossRef]
14. Jiang, Y.; Caldwell, C.D. Effect of nitrogen fertilization on camelina seed yield, yield components, and downy mildew infection. *Can. J. Plant Sci.* **2016**, *96*, 17–26. [CrossRef]
15. Leclère, M.; Jeuffroy, M.-H.; Butier, A.; Chatain, C.; Loyce, C. Controlling weeds in camelina with innovative herbicide-free crop management routes across various environments. *Ind. Crops Prod.* **2019**, *140*, 111605. [CrossRef]
16. Lenssen, A.W.; Iversen, W.M.; Sainju, U.M.; Caesar-TonThat, T.C.; Blodgett, S.L.; Allen, B.L.; Evans, R.G. Yield, pests, and water use of durum and selected crucifer oilseeds in two-year rotations. *Agron. J.* **2012**, *104*, 1295–1304. [CrossRef]
17. Anderson, R.L. A multi-tactic approach to manage weed population dynamics in crop rotations. *Agron. J.* **2005**, *97*, 1579–1583. [CrossRef]
18. Nath, C.P.; Singh, R.G.; Choudhary, V.K.; Datta, D.; Nandan, R.; Singh, S.S. Challenges and alternatives of herbicide-based weed management. *Agronomy* **2024**, *14*, 126. [CrossRef]
19. Enjalbert, J.; Johnson, J. *Guide for Producing Dryland Camelina in Eastern Colorado*; Colorado State University Extension: Fort Collins, CO, USA, 2011.
20. Sobiech, L.; Grzanka, M.; Kurasiak–Popowska, D.; Radzikowska, D. Phytotoxic effect of herbicides on various camelina [*Camelina sativa* (L.) Crantz] genotypes and plant chlorophyll fluorescence. *Agriculture* **2020**, *10*, 185. [CrossRef]
21. Gao, Y.; Jiang, C.; Zhang, Y.; Liu, L.; Wang, Y.; Kim, D.S.; Yue, J.; Diao, J.; Wu, N.; Chen, M.; et al. Agronomic performance of camelina genotypes selected for seed yield and quality characteristics in eastern China. *Ind. Crop. Prod.* **2022**, *184*, 115007. [CrossRef]

22. Martinelli, T.; Galasso, I. Phenological growth stages of *Camelina sativa* according to the extended BBCH scale. *Ann. Appl. Biol.* **2011**, *158*, 87–94. [CrossRef]
23. Xu, Z.; Zhou, G. *Identification and Control of Common Weeds*; Springer: Dordrecht, The Netherlands, 2022.
24. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2016. Available online: https://www.R-project.org/ (accessed on 10 March 2024).
25. Anderson, J.V.; Bigger, B.B.; Howatt, K.; Mettler, J.; Berti, M.T. Weed pressure, nutrient content, and seed yield in field grown sulfonylurea–resistant *Camelina sativa* and *Brassica napus*. *Agronomy* **2022**, *12*, 2622. [CrossRef]
26. Walsh, D.T.; Babiker, E.M.; Burke, I.C.; Hulber, S.H. Camelina mutants resistant to acetolactate synthase inhibitor herbicides. *Mol. Breeding* **2012**, *30*, 1053–1063. [CrossRef]
27. Hulbert, S.H.; Craine, W.; Pan, W.L. Registration of WA-HT1, a camelina line with resistance to residual levels of ALS inhibitor herbicides. *J. Plant Regist.* **2018**, *12*, 253–256. [CrossRef]
28. Zhou, Q.; Liu, W.; Zhang, Y.; Liu, K. Action mechanisms of acetolactate synthase-inhibiting herbicides. *Pestic. Biochem. Phys.* **2007**, *89*, 89–96. [CrossRef]
29. Battaglino, B.; Grinzato, A.; Pagliano, C. Binding properties of photosynthetic herbicides with the QB site of the D1 protein in plant photosystem II: A combined functional and molecular docking study. *Plants* **2021**, *10*, 1501. [CrossRef]
30. Leal, J.F.L.; Borella, J.; dos Santos Souza, A.; Langaro, A.C.; de Moura Carneiro, R.; de Souza da Silva, G.; de Oliveira Junior, F.F.; De Souza, F.R.; Machado, A.F.L.; De Pinho, C.F. Photosystem II- and photosystem I-inhibitor herbicides-driven changes in the dynamics of photosynthetic energy dissipation of *Conyza* spp. *Acta Physiol. Plant* **2023**, *45*, 94. [CrossRef]
31. Ryan, M.R.; Smith, R.C.; Mirsky, S.B.; Mortensen, D.A.; Seidel, R. Management filters and species traits: Weed community assembly in long–term organic and conventional systems. *Weed Sci.* **2010**, *58*, 265–277. [CrossRef]
32. Schumacher, M.; Dieterich, M.; Gerhards, R. Effects of weed biodiversity on the ecosystem service of weed seed predation along a farming intensity gradient. *Glob. Ecol. Conserv.* **2020**, *24*, e01316. [CrossRef]
33. Varanasi, A.; Prasad, P.V.V.; Jugulam, M. Impact of climate change factors on weeds and herbicide efficacy. *Adv. Agron.* **2016**, *135*, 107–146. [CrossRef]
34. Fleenor, R.A. *Plant Guide for Camelina (Camelina sativa)*; USDA-Natural Resources Conservation Service: Spokane, WA, USA, 2011; p. 99201.
35. James, W.; Peter, D.; Ray, D. *Weed Control and the Use of Herbicides in Sesame Production in Herbicides Theory and Applications Edited by Soloneski and Larramendy*; InTech: Houston, TX, USA, 2011. [CrossRef]
36. Bhowmik, P.C.; Bingham, S.W. Preemergence activity of dinitroaniline herbicides used for weed control in cool-season turfgrasses. *Weed Technol.* **1990**, *4*, 387–393. [CrossRef]
37. Song, D.; Jiang, X.; Wang, D.; Fang, S.; Zhou, H.; Kong, F. From the effective herbicide to the environmental contaminant: A review of recent studies on quinclorac. *Environ. Exp. Bot.* **2022**, *193*, 104706. [CrossRef]
38. Abramovič, H.; Abramn, V. Physico-chemical properties, composition and oxidative stability of *Camelina sativa* oil. *Food Technol. Biotech.* **2005**, *43*, 63–70. Available online: https://hrcak.srce.hr/file/162699 (accessed on 20 November 2024).
39. Eidhin, D.N.; Burke, J.; Beirne, D. Oxidative stability of ω3-rich camelina oil and camelina oil-based spread compared with plant and fish oils and sunflower spread. *J. Food Sci.* **2006**, *68*, 345–353. [CrossRef]
40. Kurasiak-Popowska, D.; Ryńska, B.; Stuper-Szablewska, K. Analysis of distribution of selected bioactive compounds in *Camelina sativa* from seeds to pomace and oil. *Agronomy* **2019**, *9*, 168. [CrossRef]
41. Mondor, M.; Hernández-Álvarez, A.J. *Camelina sativa* composition, attributes, and applications: A review. *Euro. Fed. Lipid* **2021**, *124*, 2100035. [CrossRef]
42. Zubr, J. Oil-seed crop: Camelina *sativa*. *Ind. Crops Prod.* **1997**, *6*, 113–119. [CrossRef]
43. Bodnar, V.R.; Lofton, R.; Manuchehri, M.R.; Zander, A.D. Impact of late-season herbicide applications on winter canola yield and seed quality. *Agrosyst. Geosci. Environ.* **2019**, *2*, 180053. [CrossRef]
44. Pilla, T.P.; Galon, L.; Brandler, D.; Bianchessi, F.; Tonin, R.J.; Toni, J.R.; Forte, C.T.; Vargas, L. Physical, physiological and sanitary quality of canola seeds after the application of maturing herbicides. *J. Agric. Sci.* **2020**, *8*, 721. [CrossRef]
45. Bellaloui, N.; Smith, J.R.; Ray, J.D.; Mengistu, A.; Gillen, A.M.; Fisher, D.K.; Singh, G. Responses of seed yield, quality, and composition to the harvest-aid paraquat in soybean grown in Mississippi. *Agrosyst. Geosci. Environ.* **2022**, *5*, e20262. [CrossRef]
46. Bradley, C.A.; Hartman, G.L.; Wax, L.M.; Pedersen, W.L. Quality of harvested seed associated with soybean cultivars and herbicides under weed-free conditions. *Plant Dis.* **2002**, *86*, 1036–1042. [CrossRef]
47. Nainwal, R.; Saxena, S.C. Effect of herbicides on plant growth and seed yield and quality of soybean (*Glycine max* L. Merr.). *Environ. Conserv. J.* **2023**, *24*, 77–82. [CrossRef]
48. Salisbury, P.A.; Potter, T.D.; Gurung, A.M.; Mailer, W.M.; Tei, W.F. Potential impact of weedy *Brassicaceae* species on oil and meal quality of oilseed rape (canola) in Australia. *Weed Res.* **2018**, *58*, 200–209. [CrossRef]
49. Canadian Food Inspection Agency (CFIA). 2021. Available online: https://active.inspection.gc.ca/english/plaveg/pbrpov/cropreport/caml/app00011240e.shtml (accessed on 1 January 2024).

50. Walsh, K.D.; Hills, M.J.; Martin, S.L.; Hall, L.M. Pollen-mediated gene flow in *Camelina sativa* (L.) Crantz. *Crop Sci.* **2015**, *55*, 196–202. [CrossRef]
51. Zhang, C.-J.; Auer, C. Hybridization between *Camelina sativa* (L.) Crantz and common Brassica weeds. *Ind. Crop. Prod.* **2020**, *147*, 112240. [CrossRef]
52. Zhang, C.-J.; Kim, D.-S.; Jiang, C.; Mahoney, J.; Liu, B.; Wang, Y.; Gao, Y.; Zhang, Y.; Sun, S.; Fan, J.; et al. Hourly pollen dispersal of *Camelina sativa* (L.) Crantz under different weather conditions and mitigation of wind-blown pollen dispersal using maize barrier. *Ind. Crop. Prod.* **2021**, *162*, 113318. [CrossRef]
53. Mallory-Smith, C.; Hall, L.M.; Burgos, N.R. Experimental methods to study gene flow. *Weed Sci.* **2015**, *63*, 12–22. [CrossRef]