

## Article

# Assessing Herbicide Efficacy of Pelargonic Acid on Several Weed Species

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**Abstract:** Pelargonic acid is the most successful natural herbicide and can contribute to reducing synthetic herbicides, but information on its efficacy is contrasting. Given its high cost, a reduction of the rate could facilitate the spread of the use of this herbicide. Two greenhouse and three field experiments were conducted to evaluate the herbicidal efficacy of different doses of pelargonic acid on several weeds (*Abutilon theophrasti*, *Alopecurus myosuroides*, *Conyza sumatrensis*, *Lolium rigidum*, *Persicaria maculosa*, *Setaria pumila*, *Solanum nigrum*). Results show that the efficacy of pelargonic acid is partial both in the greenhouse and field since the sensitivity of weed species is very variable, yet significant weed biomass reduction was observed in field application. Grass weeds, in particular *A. myosuroides* and *L. rigidum*, were less sensitive to pelargonic acid, with reduced and transient symptoms even at the highest doses. A large difference in sensitivity was also observed between dicots weeds, with *P. oleracea*, *P. maculosa* and *A. theophrasti* being less sensitive than *C. sumatrensis* and *S. nigrum*. The efficacy of pelargonic acid in field conditions depends on the botanical composition of weed flora and environmental conditions. Hot and dry conditions can promote leaf traits that decrease weed sensitivity by reducing herbicide penetration inside leaves. Despite its high cost, pelargonic acid can be a useful tool in an integrated multi-tactic strategy for sustainable weed management, while its use as a stand-alone tactic is less recommendable.

**Keywords:** natural herbicides; pelargonic acid; botanicals; integrated weed management; herbicide efficacy; weed species sensitivity; sustainable weed management



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## 1. Introduction

Synthetic herbicides have been key tools in weed management strategies for decades, providing important economic and operational benefits [1,2]. However, a significant reduction in their use is expected in the coming years for many factors spanning from the evolution of herbicide resistance to the lack of discovery of new modes of action to the increased restrictions in herbicide registration and use [3]. Great interest has therefore arisen to identify more sustainable environmental-friendly alternatives for weed control to integrate or even substitute synthetic herbicides [3–5]. In addition to classical and innovative mechanical tools or cultural tactics for weed control, remarkable research efforts have been directed in the last 20 years to evaluate natural products as potential bioherbicides [6]. Studies have been focused particularly on the herbicidal activity of two main groups of natural chemicals: organic acids, such as acetic or pelargonic acid, and plant essential oils, such as pine and clove oils [7–10]. However, despite all the dedicated research, pelargonic acid is currently the only natural active ingredient with herbicidal effect currently available on the market, given that relevant flaws regarding crop selectivity, efficacy, and shelf-life are still hindering the technological development of other natural

herbicides [11,12]. Pelargonic acid (hereafter: PA) ( $\text{CH}_3(\text{CH}_2)_7\text{CO}_2\text{H}$ , n-nonanoic acid) is a saturated, nine-carbon fatty acid. PA is present as esters in the essential oil of species of the genus *Pelargonium* but can easily be produced from several vegetal oils. PA is basically a burndown herbicide, and its herbicide mode of action, similar to the other short-chain fatty acids, is cuticle destabilization, with the consequent rapid desiccation of plant tissues [13]. In particular, two subsequent actions occur during the phytotoxic action of PA: (i) induction of cellular membrane leakage due to intercalation of the acid and (ii) the light-driven peroxidative activity by singlet oxygen, with the consequent necrosis of plant tissues [14]. This phytotoxic effect can be observed a few hours after application, but only plant parts directly exposed to the spray droplets are damaged since PA is a contact herbicide and is not translocated into plants.

PA was initially studied as an additive for systemic herbicides such as glyphosate and glufosinate [15,16] and only later tested as a non-selective herbicide [10,17]. Despite several studies conducted in the last 15 years, reports on PA efficacy are still contrasting and case-specific. Good control of different weed species has been described in pot or greenhouse experiments [18–20], while erratic results have been reported regarding PA efficacy in field conditions. Kanatas et al. [21] achieved good control efficacy by combining PA application with stale seedbeds but only for annual weed species. Similarly, post-emergence application of PA in field vegetables showed different efficacy for dicots, grasses, and sedges [22,23]. This inconsistency in PA efficacy is also observable in field studies of perennial crops. Rowley et al. [24] reported high weed control in orchards but with four PA applications per growing season, while intermediate efficacy was observed with repeated applications in olive groves [25] and in vineyards [26]. Poor efficacy was reported against perennial weeds such as *Cyperus esculentus* L. or *Convolvulus arvensis* L. [27].

The site-specific environmental conditions, such as air temperature, solar radiation, and relative air humidity, at the moment of field application, could have been a significant factor behind the inconsistent herbicidal efficacy of PA observed so far. High relative air humidity, for example, has been proven to increase the efficacy of vinegar because it lengthens the persistence of spray droplets on the leaf surface, induces stomatal opening, and consequently increases vinegar penetration [28]. This can be relevant also to PA since acetic acid has the same mode of action. At the same time, different sensitivities to PA between weed species have been described. Travlos et al. [19] observed in a greenhouse experiment higher efficacy on *Galium aparine* L. than on *Avena sterilis* L. or *Lolium rigidum* Gaud. Similarly, PA applied in field vegetables resulted in lower efficacy against *Cyperus esculentus* than grasses and dicots [22,23]. Pannacci et al. [29] reported a large variation in the sensitivity to PA between the most (*Kickxia spuria* (L.) Dumort., *Echinochloa crus-galli* (L.) P.Beauv.) and the least sensitive species (*Portulaca oleracea* L., *Lolium multiflorum* Lam.) tested in their field studies. Therefore, the relative abundance of sensitive and tolerant species of a given weed flora can affect the overall efficacy in the field application of PA.

PA was authorized as a plant protection product in 2009, with Ireland as the designated rapporteur member state [30]. Commercial products containing PA are currently registered as herbicides in many member states of the European Union for several annual and perennial crops, but their use is still scarce due to their high cost per unit combined with the higher recommended doses. The recommended dose of PA for weed control is indeed  $16 \text{ L ha}^{-1}$  of a commercial product, corresponding to  $10,880 \text{ g a.i. ha}^{-1}$ ; given that the average price of the commercial products is around  $20 \text{ € L}^{-1}$ , the estimated cost for the sole herbicide is above  $300 \text{ € ha}^{-1}$ . Given that such recommended doses are meant to control weed plants up to 10 cm in height, lower doses could still ensure satisfactory control when applied on weed seedlings, as in the case of the stale seedbed technique. However, the application of lower doses could probably increase the influence of environmental conditions on PA efficacy and widen the differences between sensitive and tolerant weed species. Given this scientific and agronomic background, in order to enlarge knowledge of PA herbicidal activity, two greenhouse and three field experiments were performed with

doses equal or lower than that recommended on the label on specific weed species or mixed field weed flora.

## 2. Materials and Methods

### 2.1. Greenhouse Experiment

A greenhouse experiment was conducted, with two identical independent trials to ensure experimental repetition, to evaluate the herbicidal efficacy of PA on some important weed species: *Abutilon theophrasti* Medik. (velvetleaf, ABUTH, *Malvaceae*), *Alopecurus myosuroides* Huds. (black-grass, ALOMY, *Poaceae*), *Conyza sumatrensis* (Retz.) E. Walker (tall fleabane, CONSU, *Asteraceae*), *Lolium rigidum* Gaud. (rigid ryegrass, LOLRI, *Poaceae*), *Persicaria maculosa* Gray (ladysthumb, POLPE, *Polygonaceae*), *Setaria pumila* (Poir.) Roem. & Schult. (yellow foxtail, SETPU, *Poaceae*), and *Solanum nigrum* L. (black nightshade, SOLNI, *Solanaceae*). These species are key weeds for several main crops in Europe. In particular, *A. myosuroides* and *L. rigidum* are important weed species for winter cereals, while *A. theophrasti*, *P. maculosa*, *S. pumila*, and *S. nigrum* are common weeds in spring crops such as maize and soybean. Finally, *C. sumatrensis* is a key weed species in perennial crops such as orchards and vineyards.

This range of species enabled the testing of PA herbicidal activity on weeds belonging to different botanical families with different morphological traits. Seeds of *A. theophrasti*, *A. myosuroides*, *C. sumatrensis*, *P. maculosa*, *S. pumila*, and berries of *S. nigrum* were collected in summer and autumn in fields at the experimental farm “L. Toniolo” of the University of Padova, Italy (45°21'04" N 11°57'02" E, 8 m asl). Seeds of *L. rigidum* originated from plants cultivated in a greenhouse on the same farm. Seeds were collected from at least 50 mother plants per species to maintain intra-population variability by gently shaking inflorescences to collect only fully ripened seeds and fruits. Seeds of *S. nigrum* were then manually extracted from the berries. Seeds of the different species were cleaned, left to dry at room temperature (20 °C) for 2 weeks, and stored afterward in paper bags at 4 °C until the start of the experiments.

In order to break dormancy and promote germination, seeds were sown in Petri dishes on moistened peat substrate and exposed to various chilling treatments according to the specific requirements of each species. Seeds of *A. myosuroides* and *L. rigidum* were vernalized in a fridge at 4 °C in dark conditions for 3 days. Seeds of *A. theophrasti*, *P. maculosa*, *S. pumila*, and *S. nigrum* were maintained at the same conditions for 7 days. On the contrary, seeds of *C. sumatrensis* did not require any dormancy-breaking treatment and were directly incubated for germination. After the chilling treatment, seeds were incubated in germination chambers at an alternate temperature regime of 25 (light) – 15 (dark) °C and a 12 h light photoperiod, with neon tubes providing a photosynthetic photon flux density (PPFD) of 15–30  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Given that the length of germination and seedling early-growth phase depends on species, petri dishes were maintained in the germination chamber for periods ranging from 4 days for *A. myosuroides* and *L. rigidum* to 14 days for *C. sumatrensis* and *P. maculosa*, respectively, to obtain enough seedlings of the adequate growth stage for transplant. For each species and replicate, 15–20 seedlings were transplanted into rectangular plastic pots (160 × 160 × 200 mm) filled with a standard potting mix (60% silty loam soil, 15% sand, 15% perlite, and 10% peat). Pots were transferred in the greenhouse with regular irrigation to maintain optimal water availability for plants throughout the experiment. Light in the greenhouse was provided with metal halide lamps (400 W), 14 h photoperiod, PPFD~160  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . During the experiment, the minimum and maximum temperatures in the greenhouse varied from 20 to 23 °C and from 25 to 30 °C.

The experimental layout was a completely randomized design with three replicates and included three doses of PA (commercial Beloukha herbicide, pelargonic acid 680 g a.i. L<sup>-1</sup>, Belchim Crop Protection Italia S.p.A, Rozzano, MI, Italy). Applied doses of PA were: 10,880, 8160, and 5440 g a.i. ha<sup>-1</sup>, corresponding to 16, 12, and 8 L ha<sup>-1</sup> of the commercial Beloukha herbicide, respectively (hereafter: PEL16, PEL12, PEL8). PEL16 is the recommended field dose of this herbicide for crop seedbed cleaning. Untreated control pots

were also included for all species. The total number of treatments was 4 (three herbicide doses + untreated) \* 7 (weed species) = 28. The experiment was repeated two months after the first run. PA was applied when weed seedlings reached the stage of 1–2 tillers or BBCH 21–22 [31] for grasses (*A. myosuroides*, *L. rigidum*, *S. pumila*) and of 4–6 true leaves or BBCH 14–16 for dicots (*A. theophrasti*, *C. sumatrensis*, *P. maculosa*, *S. nigrum*). The application was performed using a precision bench sprayer equipped with three flat fan hydraulic nozzles (TeeJet TP11001-VH, Glendale Heights, IL, USA), with a spray volume of 200 L ha<sup>-1</sup> applied at a pressure of 215 kPa and speed of 0.6 m s<sup>-1</sup>. This spray volume is the minimum of the range (200–400 L ha<sup>-1</sup>) recommended for field application of PA and was selected in order to maximize PA concentration in the spray solution and, consequently, its herbicidal activity. This was feasible since these experimental conditions, that is, PA application performed with a precise bench sprayer on non-stressed weed plants at the right stage ensured optimal droplet coverage and persistence on weed leaves.

The survival and biomass of treated plants were evaluated 3 weeks after treatment (3 WAT). Plant survival was expressed as a percentage of the alive plants counted before the treatment in each pot. For each species, the above-ground biomass of the three untreated replicates was collected, and the total plant fresh weight was measured; then, the average plant weight was calculated by dividing the total plant weight by the number of live plants before herbicide application. For each species, to assess biomass reduction, the average plant weight of treated replicates was then expressed as a percentage of the average plant weight of the untreated. A value of 100% for a given replicate means that its biomass is the same as the untreated. The mean and standard error were calculated for all treatments for both response variables (Plant survival, Biomass fresh weight).

A factorial ANOVA ( $p < 0.05$ ) was performed using JASP software ([www.jasp-stats.org](http://www.jasp-stats.org), accessed on 15 January 2023) to test the effect of the factors trial, dose (hereafter: P Dose), species, and their interaction on the response variables plant survival and biomass fresh weight. This first analysis determined whether data from the two trials could be pooled and analyzed together. Otherwise, factorial ANOVA was performed for each trial as a single experiment with a completely randomized design. Assumptions, that is, data normality and homogeneity of variances, were tested for each ANOVA performed. Tukey's HSD test ( $p < 0.05$ ) was then applied to identify significant differences between treatment means.

## 2.2. Field Experiment

A field experiment was conducted with three identical independent trials to ensure experimental repetition in spring, summer, and autumn 2022 to simulate the conditions of PA application for seedbed cleaning or stale seedbed technique for spring-summer or autumn sown crops. The experiments were set up at the experimental farm "L. Toniolo" of the University of Padova, Italy. This farm is located at Legnaro (45°21'04" N 11°57'02" E, 8 m asl) and has silt-loamy soil. Three doses, corresponding to those included in the greenhouse experiment, of PA (commercial Beloukha herbicide, pelargonic acid 680 g a.i. L<sup>-1</sup>, Belchim Crop Protection Italia S.p.A, Rozzano, MI, Italy) were tested: PEL16, PEL12, and PEL8. Untreated control plots were also included. The experimental layout was a complete randomized block design with 3 replicates, each corresponding to a 10 m<sup>2</sup> plot. Weather data (daily air maximum, mean and minimum temperature, daily precipitation) were collected throughout the experiment from a nearby weather station managed by the Regional Agency for Prevention and Environmental Protection ([www.arpa.veneto.it](http://www.arpa.veneto.it), accessed on 20 December 2022). Soil tillage was performed for seedbed preparation for crop sowing. Given the scarcity of rainfall during 2022, sprinkler irrigation for a total of 25 mm was performed at the beginning of each trial to promote weed germination and seedling establishment.

Weed emergence was monitored, and a few days before herbicide application, weed seedlings were identified and counted in two 30 \* 30 cm sampling areas per replicate. PA was applied on weeds at the initial growth stages (from 2–3 true leaves to 2 tillers, BBCH 12–22). Field application of PA was conducted using a back-pack sprayer (MOD. 40007

Fox Sprayers; nozzle 8261036, color code: light blue, RS 110-10.) with a spray volume of 350 L ha<sup>-1</sup>. This spray volume is significantly larger than the one adopted for greenhouse PA application (200 L ha<sup>-1</sup>) and was selected in order to maximize leaf coverage and persistence of the spray solution to ensure a satisfactory penetration inside weed leaves under the specific field conditions (high temperature and solar radiation, low air relative humidity, and presence of a dust layer on weed leaves). The application of a lower spray volume, even with a higher concentration of PA, could have significantly decreased the persistence of spray droplets and eventually limited the herbicide's efficacy.

The herbicidal efficacy of the different doses of PA was evaluated with a second weed assessment 2 weeks after PA application. During that assessment, weeds were collected in four 30 \* 30 cm sampling areas per replicate, and fresh weight was measured. The specific dates of the two weed assessments and of PA application in the three field trials are reported in Table 1.

**Table 1.** Field experiment. Dates of weed assessments and PA application in the three field trials.

Trial	First Weed Assessment	PA Application	Second Weed Assessment
Spring	25 May	30 May	14 June
Summer	8 July	12 July	26 July
Autumn	2 November	3 November	17 November

A factorial ANOVA ( $p < 0.05$ ) was first performed using JASP software ([www.jasp-stats.org](http://www.jasp-stats.org), accessed on 15 January 2023) to test the effect of the factor trial, block, and pelargonic acid dose (hereafter: P Dose), on weed biomass. This first analysis would show whether data from the three trials could be pooled and analyzed together. Otherwise, factorial ANOVA was performed for each trial as a single experiment with a completely randomized block design. Assumptions, that is, data normality and homogeneity of variances, were tested for each ANOVA performed. Tukey's HSD test ( $p < 0.05$ ) was then applied to identify significant differences between treatment means.

### 3. Results

#### 3.1. Greenhouse Experiment

Application of PA caused phytotoxicity in a few hours, such as extensive leaf necrosis and wilting, with a visible sensitivity gradient between weed species. *Conyza sumatrensis* and *S. nigrum* showed intense and prolonged symptoms, while *L. rigidum* and *S. pumila* were almost undamaged. Anyhow, different phytotoxicity of PA was observed in the two greenhouse trials. In the first trial, most treated plants survived and quickly recovered from the initial damage, producing new leaves and continuing their growth. In the second greenhouse trial, the application of PA caused the same symptoms on treated plants but with a higher magnitude both in terms of plant survival and biomass fresh weight. This was confirmed by the first factorial ANOVA ( $p < 0.05$ ), which identified a significant effect of the factor trial on plant survival and biomass fresh weight (Tables 2 and 3). It was not possible to test at this level the effect of the interaction species \* P Dose on plant survival due to the lack of variance across replicates, given that most replicates have the same value (100%). The results of the two greenhouse trials were analyzed separately as a completely randomized design.

In the first greenhouse trial, most treated plants survived PA without any relevant damages visible at 3 WAT. Indeed, five (*A. theophrasti*, *A. myosuroides*, *L. rigidum*, *P. maculosa*, and *S. pumila*) out of the seven tested weed species showed plant survival above 90% for all tested doses (Figure 1). Regarding the two more sensitive species, *C. sumatrensis* and *S. nigrum*, plant survival were reduced only with PEL16, with survival values of  $51 \pm 12.2\%$  and  $78 \pm 8.20\%$ , respectively (Figure 1). Hence, it was not possible to perform factorial ANOVA to test the effect of P Dose and species on plant survival because the variance between replicates was too low, having most replicates with the same value (100%). On the

contrary, the application of PA caused a visible reduction of biomass measured at 3 WAT for all treated species. Factorial ANOVA identified the significant effect of the factors P Dose, species, and their interaction on biomass fresh weight (Table 4). Biomass progressively decreased across the tested doses of PA, but the extent of this reduction varied between species (Figure 1). No significant differences in biomass fresh weight were detected between treatments for *L. rigidum* and *S. pumila*, with values close to 80% compared to untreated, even at the highest dose of PA (PEL16). *A. theophrasti*, *A. myosuroides*, and *P. maculosa* had intermediate sensitivity to PA, with values of biomass at PEL16 being around 60% compared to untreated. Finally, PA caused a marked reduction of biomass fresh weight for *S. nigrum*, with values of  $58 \pm 7.8\%$  and  $29 \pm 8.2\%$  compared to untreated at PEL12 and PEL16, and for *C. sumatrensis*, with values of  $41 \pm 14.3\%$  and  $14 \pm 12.2\%$  compared to untreated at PEL12 and PEL16, respectively.

**Table 2.** Greenhouse experiment. Factorial ANOVA to test the effect of species, trial, and pelargonic acid dose (P Dose) on the response variable plant survival.

Cases	Sum of Squares	df	Mean Square	F	$p^1$
Species	15,322	6	2554	11.571	<0.001 **
Trial	5033	1	5033	22.806	<0.001 **
P Dose	4145	3	1382	6.261	<0.001 **
Residuals	34,649	157	221		

<sup>1</sup> Significance level (\*\*  $p < 0.01$  highly significant).

**Table 3.** Greenhouse experiment. Factorial ANOVA to test the effect of species, trial, pelargonic acid dose (P Dose), and their interaction on the response variable biomass fresh weight.

Cases	Sum of Squares	df	Mean Square	F	$p^1$
Species	29,612	6	4935	11.232	<0.001 **
Trial	6102	1	6102	13.888	<0.001 **
P Dose	63,551	3	21,184	48.210	<0.001 **
Species * P Dose	15,768	18	876	1.994	0.014 *
Residuals	61,078	139	439		

<sup>1</sup> Significance level (\*\*  $p < 0.01$  highly significant, \*  $p < 0.05$  significant).

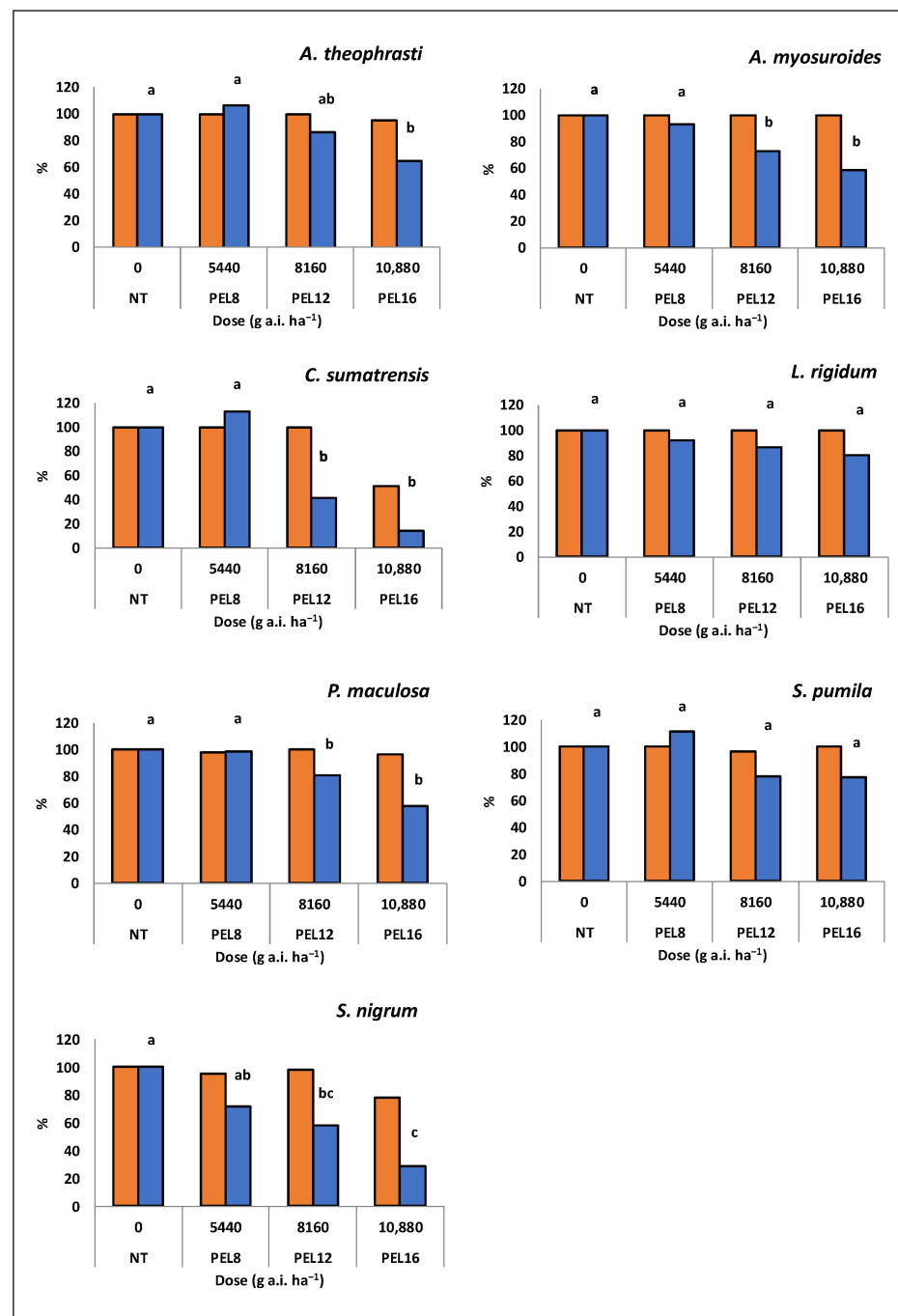
**Table 4.** First greenhouse trial. Factorial ANOVA to test the effect of the factors species, pelargonic acid dose (P Dose), and their interaction on the response variable biomass fresh weight.

Cases	Sum of Squares	df	Mean Square	F	$p^1$
Species	8781	6	1463	6.152	<0.001 **
P Dose	29,757	3	9919	41.695	<0.001 **
Species * P Dose	10,695	18	594	2.498	0.005 **
Residuals	13,322	56	238		

<sup>1</sup> Significance level (\*\*  $p < 0.01$  highly significant).

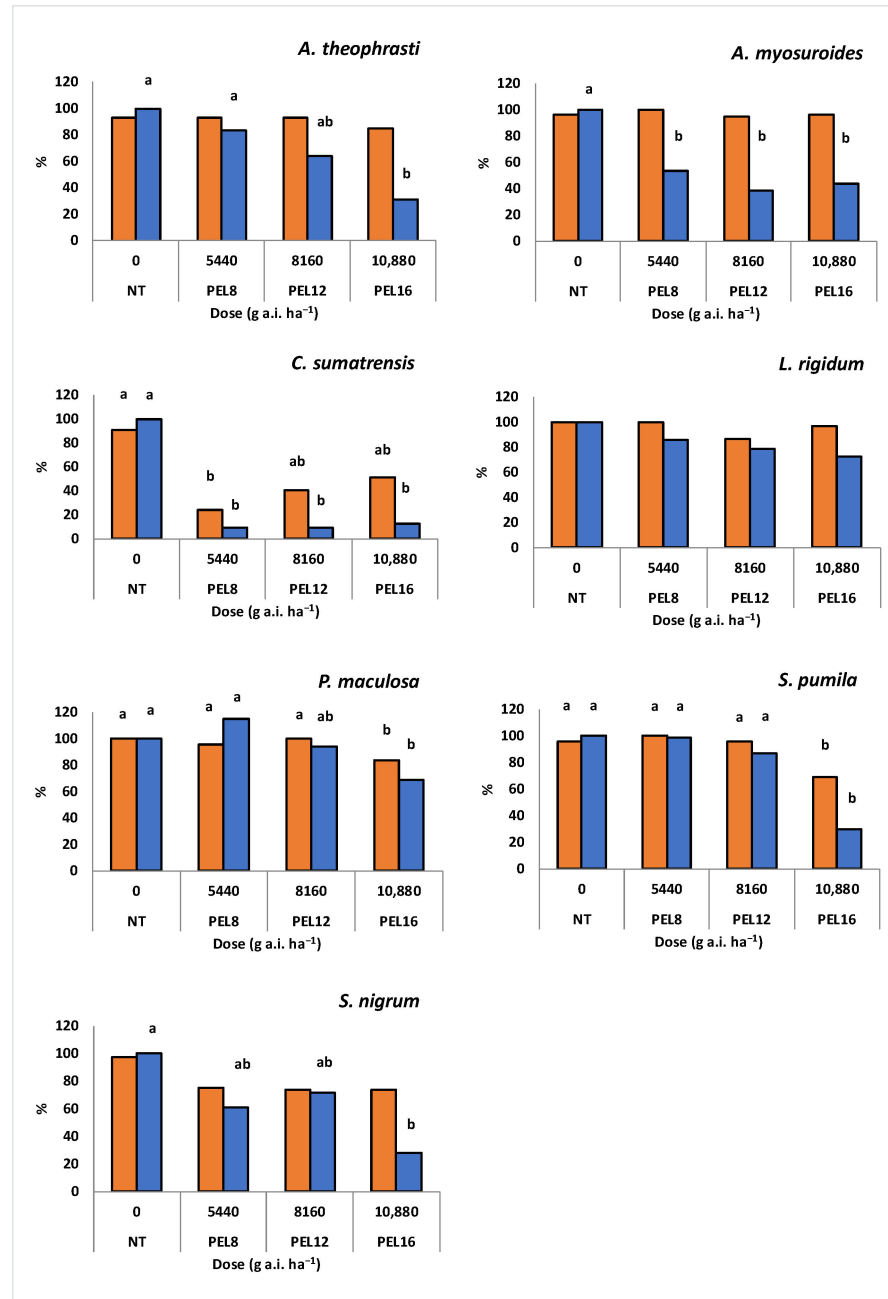
Regarding the second greenhouse trial, factorial ANOVA detected significant effects of P Dose, species, and their interaction on the response variables plant survival and biomass fresh weight (Tables 5 and 6). As observed in the first greenhouse trial, PA exerted phytotoxicity most by the reduction of plant biomass than by plant survival. Plant survival was overall lower than that in the first trial; however, the large majority of treated plants survived PA even if species-specific response was again observed (Figure 2). *Abutilon theophrasti*, *A. myosuroides*, *L. rigidum*, and *P. maculosa* showed the highest percentages of plant survival, with values above 85% even at PEL16. An intermediate level of response was observed for *S. pumila* and *S. nigrum*; for both species, Plant survival decreased to 70–75% at PEL16. Finally, *C. sumatrensis* was the most sensitive species, with a percentage of plant survival below 60% at all doses of PA. The application of increasing doses of PA reduced biomass progressively, but the extent of this reduction varied between species

(Figure 2). Anyhow, significant differences were detected between the value of untreated control and PEL16 for all species apart from *L. rigidum*. *Lolium rigidum* and *P. maculosa* were the least affected species, with a biomass fresh weight of about 70–75% compared to untreated at PEL16. Limited reduction of biomass fresh weight was observed for *S. pumila* at PEL8 and PEL12, while it significantly decreased to around 30% compared to untreated at PEL16. A more marked reduction of biomass was observed across all the doses of PA for *A. theophrasti*, *A. myosuroides*, and *S. nigrum*, even if values achieved at PEL16 were again in the 30–40% range compared to untreated. Finally, *C. sumatrensis* confirmed its high sensitivity to PA, given that an almost 90% reduction of biomass was achieved with PEL8 and PEL12.



**Figure 1.** First greenhouse trial. Plant survival (red bar) and biomass fresh weight (blue bar) observed for the different weed species at the different doses of pelargononic acid. Plant survival is expressed as

% of the initial plant while biomass as % of the mean value of the untreated control. Values are the mean of three replicates; letters identify significant differences between means of the same species according to Tukey’s HSD test ( $p < 0.05$ ). No letters are displayed if no significant differences were detected for a given species.



**Figure 2.** Second greenhouse trial. Plant survival (red bar) and biomass fresh weight (blue bar) observed for the different weed species at the different doses of pelargonic acid. Plant survival is expressed as % of the initial plant while biomass as % of the mean value of the untreated control. Values are the mean of three replicates; letters identify significant differences between means of the same species according to Tukey’s HSD test ( $p < 0.05$ ). No letters are displayed if no significant differences were detected for a given species.



**Table 5.** Second greenhouse trial. Factorial ANOVA to test the effect of the factors species, pelargonic acid dose (P Dose), and their interaction on the response variable plant survival.

Cases	Sum of Squares	df	Mean Square	F	$p^1$
Species	18,617	6	3109	15.980	<0.001 **
P Dose	3323	3	1108	5.705	0.002 **
Species * P Dose	8010	18	445	2.292	0.009 **
Residuals	10,873	56	194		

<sup>1</sup> Significance level (\*\*  $p < 0.01$  highly significant).

**Table 6.** Second greenhouse trial. Factorial ANOVA to test the effect of the factors species, pelargonic acid dose (P Dose), and their interaction on the response variable biomass fresh weight.

Cases	Sum of Squares	df	Mean Square	F	$p^1$
Species	29,051	6	4842	11.790	<0.001 **
P Dose	37,411	3	12,470	30.367	<0.001 **
Species * P Dose	17,996	18	1000	2.435	0.006 **
Residuals	22,997	56	411		

<sup>1</sup> Significance level (\*\*  $p < 0.01$  highly significant).

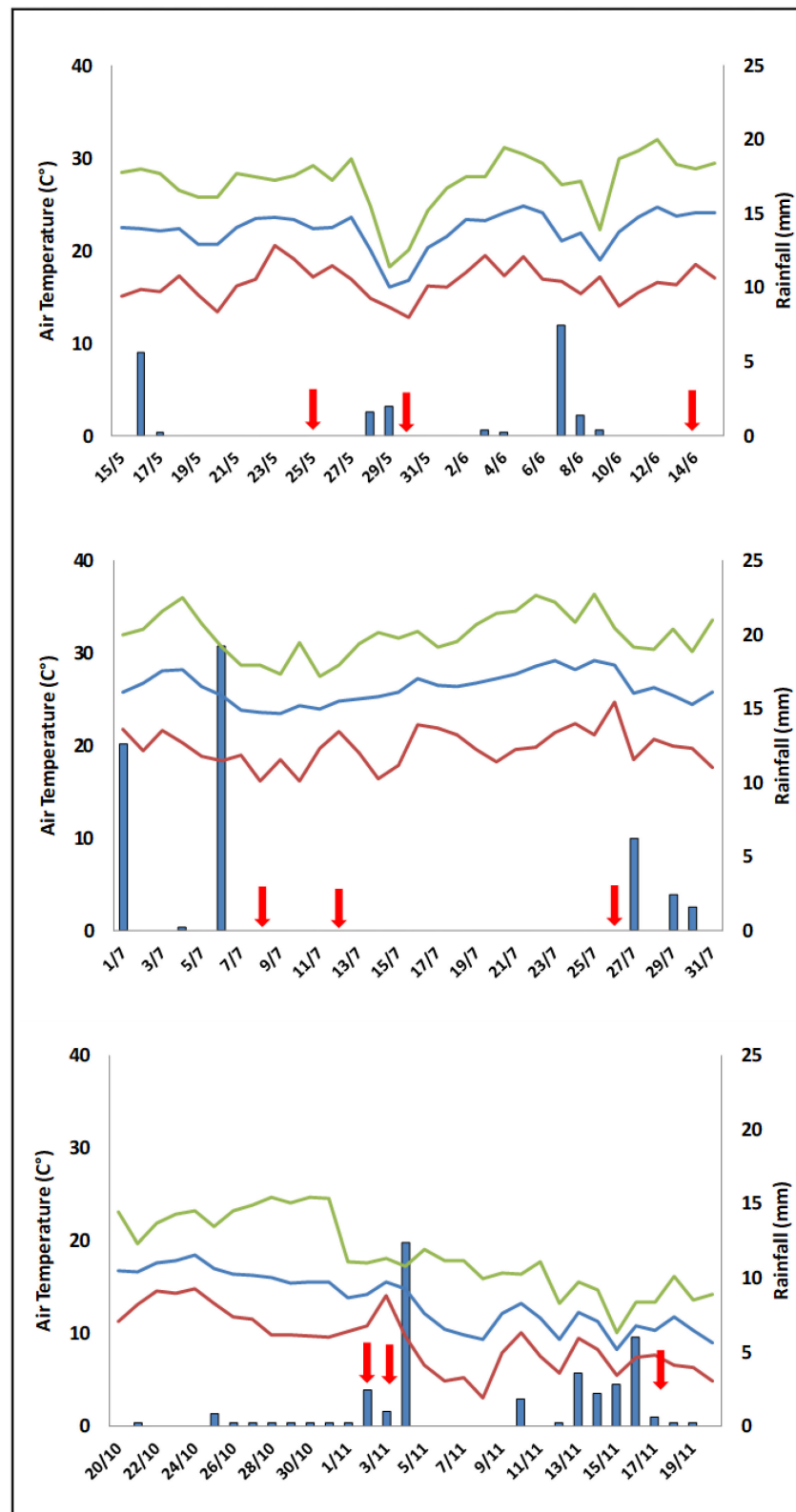
### 3.2. Field Experiment

#### 3.2.1. Weather Conditions

Environmental conditions varied across the three field trials, but weather conditions were warmer and drier than the local average during the whole duration of this study (Figure 3). In particular, daily air mean temperature remained around or above 20 °C throughout the spring trial with less than 20 mm of rainfall, while local weather is usually milder and wetter during this season. Similarly, during the summer trial, daily air mean temperature persisted around 25 °C, and about 40 mm of rain occurred. The weather was again abnormally warm and dry during the first part of the autumn, with daily air mean temperature around 15 °C and no precipitations until November. Temperatures then decreased, with daily air mean temperature fluctuating around 10 °C till the end of the experiment. Total precipitation during the autumn trial was lower than 40 mm.

#### 3.2.2. Weed Botanical Composition and Density

Weed communities varied in botanical composition and plant density across the three field trials and between plots of the same trial. Anyhow, weed density was rather high in all field trials. In the first field experiment in spring 2022, weed density ranged between 300 and 1000 plant m<sup>-2</sup> (Table S1A). Grasses were the main group of weeds, with *Digitaria sanguinalis* (L.) Scop. as the largely dominant species. Other common grasses were *S. pumila* and *Echinochloa crus-galli* (L.) P. Beauv. The most frequent dicots were *Chenopodium album* L. and *Portulaca oleracea* L. In the second experiment in the summer of 2022, weed density varied between 90 and 600 plant m<sup>-2</sup> (Table S1B). *Portulaca oleracea* was the dominant species this time thanks to its tolerance to hot and dry summer conditions, even if the three grass species (*D. sanguinalis*, *E. crus-galli*, and *S. pumila*) were also abundant. In the third experiment in autumn 2022, weed density ranged between 250 and 700 plant m<sup>-2</sup> (Table S1C). Given the exceptionally warm conditions of autumn 2022, weed flora was a mixture of summer and autumn-emerging species. *Echinochloa crus-galli* was indeed the dominant species, and other summer weeds, such as *D. sanguinalis* and *P. oleracea*, were common; however, autumn emerging species, such as *Capsella bursa-pastoris* (L.) Medik. and *Stellaria media* (L.) Vill. were also abundant.



**Figure 3.** Weather conditions during the field trials (spring trial upper graph, summer trial middle graph, autumn trial lower graph). Daily air temperature (Tmax green line, Tmean blue line, Tmin red line) and rainfall (blue bar) are reported. Red arrows indicate the moments of the first weed assessment, pelargonic acid application, and second weed assessment, respectively.

### 3.2.3. Herbicidal Efficacy of Pelargonic Acid

Weeds treated with PA showed first symptoms shortly after application, confirming what was observed in the greenhouse experiment, but many plants recovered, so optimal control was not achieved even with the highest dose of PA (PEL16). The different weed species naturally occurring in the three field trials presented variable levels of sensitivity to PA. *Digitaria sanguinalis* showed higher sensitivity than *S. pumila* or *E. crus-galli* since it turned brownish and stopped growth after herbicide application, while the other grasses remained green, showing little symptoms. PA was also poorly effective against *P. oleracea*, causing only limited and temporary symptoms such as small circular lesions on the leaves. Treated *P. oleracea* plants usually recovered within a few days and showed no biomass reduction in comparison with the untreated.

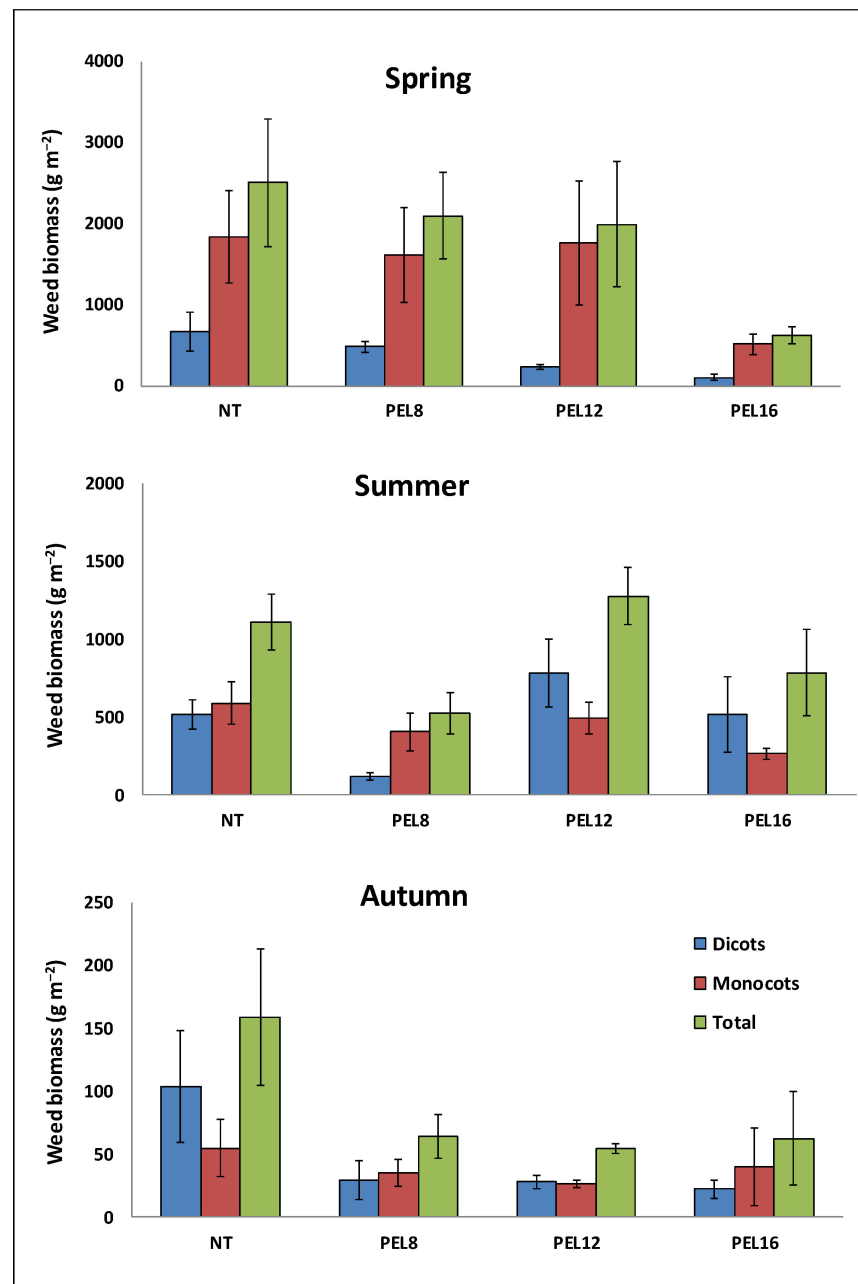
The first factorial ANOVA ( $p < 0.05$ ) identified a significant effect of the factor trial on weed biomass (Table 7). The results of the three field trials were therefore analyzed separately as a completely randomized block design.

**Table 7.** Factorial ANOVA to test the effect of the factors pelargonic acid dose (P Dose), trial, and block on the response variable weed biomass.

Cases	Sum of Squares	df	Mean Square	F	$p^1$
P Dose	$30 \times 10^{+5}$	3	$10 \times 10^{+5}$	2.466	0.083 <sup>ns</sup>
Trial	$18 \times 10^{+6}$	2	$88 \times 10^{+5}$	21.805	<0.001 <sup>**</sup>
Block	$27 \times 10^{+5}$	2	$14 \times 10^{+5}$	3.360	0.049 <sup>*</sup>
Residuals	$11 \times 10^{+6}$	28	$41 \times 10^{+4}$		

<sup>1</sup> Significance level (\*\*  $p < 0.01$  highly significant, \*  $p < 0.05$  significant, ns non-significant).

A significant effect ( $F_{3,6} = 5.791$ ,  $p = 0.033$ ) of the PA dose (P Dose) on weed biomass was detected for the spring trial; however, significant differences were detected only between the untreated (NT) and the treatment with PEL16. The fresh weight of weed biomass of the untreated and the treatments with the two lowest doses of pelargonic acid (PEL8 and PEL12) was around 2000–2500 g m<sup>-2</sup>, while it decreased to slightly above 600 g m<sup>-2</sup> for the treatment PEL16 (Figure 4). Spring-emerging grasses (*D. sanguinalis*, *E. crus-galli*, and *S. pumila*) were the most abundant weeds, accounting for more than 70% of total weed biomass across all treatments. No significant effect ( $F_{3,6} = 2.809$ ,  $p = 0.108$ ) of the PA dose (P Dose) on weed biomass was detected for the summer field trial, with the fresh weight of weed biomass ranging from around 500 g m<sup>-2</sup> for PEL8 to almost 1300 g m<sup>-2</sup> for PEL12, respectively. It is worth mentioning that the plots with the highest value of weed biomass, that is, those of treatment PEL12, were colonized by a large number of *P. oleracea* plants (Table S1B). The density of this weed was particularly high in plots belonging to Block 2 and Block 3, with above 180 and 400 plants m<sup>-2</sup>, respectively. No significant effect ( $F_{3,6} = 2.228$ ,  $p = 0.186$ ) of the PA (P Dose) on weed biomass was detected for the autumn field trial; nevertheless, the fresh weight of weed biomass of treatments (PEL8, PEL12, and PEL16) was less than a half (approximately 55–65 g m<sup>-2</sup>) than the untreated (approximately 160 g m<sup>-2</sup>). Finally, it is interesting to underline that weed biomass in the autumn trial was overall 10-fold lower than in the spring and summer.



**Figure 4.** Weed biomass measured for the different treatments in the three field trials (spring trial, upper graph; summer trial, middle graph; autumn trial, lower graph). The fresh weight of biomass of monocots species (blue bar), dicots species (red bar), and total biomass (green bar) are reported. Values are the mean of three replicates; bars represent standard error. NT means untreated, while PEL16, PEL12, and PEL8 correspond to 10,880, 8160, and 5440 g a.i. ha<sup>-1</sup> of pelargonic acid.

#### 4. Discussion

Lower herbicidal efficacy of pelargonic acid was observed in the present study in comparison with previous studies conducted under similar greenhouse conditions. Kanatas et al. [20] reported indeed that pelargonic acid decreased by 70–75% fresh weight in *E. crus-galli* and *Sorghum halepense* (L.). Travlos et al. [19] described analogous levels of efficacy on *L. rigidum* and *Avena sterilis* L. However, in these studies, the herbicidal effect was evaluated at 10–14 DAT (Days After Treatment), while in the present study, this assessment was performed at 21 DAT. This was performed in order to fully appreciate the regrowth of weeds after treatment since it is already known that pelargonic acid usually achieves

only temporary control against weeds. In the present study, the weeds recovered about 7–14 days after treatment, as already observed under field conditions [10]. Postponing the efficacy assessment from 10 to 21 DAT gave the treated plants enough time to recover and reduce the biomass gap with the untreated plants. For example, several *S. nigrum* plants, whose shoot tip was severely damaged by pelargonic acid application, were able to produce new stems and leaves from the axillary buds.

Pelargonic acid obtained a higher effect on both plant survival and biomass fresh weight in the second greenhouse trial compared to the first. This could be caused by the higher temperatures inside the greenhouse during the second experiment, given that high temperatures, in the absence of water stress, are known to increase the herbicidal efficacy of organic acids [28]. After the initial destabilization of the leaf cuticle provoked by PA, high temperatures indeed accelerate the light-driven peroxidative activity and enlarge uncontrolled water transpiration from damaged tissues. This can increase plant mortality and hinder the recovery of damaged plants, particularly in the case of the most sensitive species, such as *C. sumatrensis*. At the same time, higher temperatures accelerate the growth of untreated plants in control pots, widening differences in biomass production with treated plants that suffered at least a temporary growth stop after the exposition to pelargonic acid. However, the higher temperatures during the second greenhouse trial did not modify the response of the least sensitive species, such as *L. rigidum*, probably because pelargonic acid did not provoke any relevant damage to the leaf cuticle of those plants. Overall, a satisfactory level of control was not achieved for most of the tested species in the second greenhouse trial, even at the highest PA dose, which is the recommended rate on the label. Different levels of species-specific sensitivity to pelargonic acid were indeed observed, as already reported in previous greenhouse and field studies. Travlos et al. [19] described in a greenhouse experiment higher efficacy on *Galium aparine* L. than on *A. sterilis* or *L. rigidum*. Similarly, applications of pelargonic acid in field vegetables achieved lower control of *Cyperus esculentus* L. than of grasses and dicots [22,23]. Biological and morphological traits are the main driving factors of species-specific sensitivity to pelargonic acid. Perennials are more tolerant since pelargonic acid, having no systemic effect, and are not able to reach and damage their vegetative organs, such as rhizomes or tubers. In the present experiment, the three types of grass (*A. myosuroides*, *L. rigidum*, and *S. pumila*) were generally less affected by pelargonic acid than the dicots. Grasses have narrow, elongated, and erect leaves, and these traits can reduce leaf coverage by spray droplets during herbicide application and their successive persistence on the leaf surface. Moreover, meristems in grasses are protected by the basal leaf sheaths and not exposed on the shoot tips as in dicots. These traits can decrease the efficacy of contact, non-systemic herbicides such as pelargonic acid. Different levels of sensitivity were also observed among the dicot species included in the experiment, with *A. theophrasti* and *P. maculosa* being notably more tolerant than *S. nigrum* and *C. sumatrensis*, even at the highest pelargonic acid dose. Several morphological traits, such as leaf shape or the presence of wax and hairs on the leaf surface, can affect the sensitivity of weeds. Evans et al. [32] observed that the obtuse leaf blade angle in *A. theophrasti* facilitates the spray droplets' movement on the leaf surface away from the shoot tip and towards the leaf tip, thus increasing dripping and reducing herbicide action. Similarly, seedlings of *P. maculosa* have narrow, convex, wax-covered leaves, and this can reduce the coverage and persistence of spray droplets on the leaf surface. On the contrary, large concave leaves with horizontal or acute blade angles, such as those of *S. nigrum* and *C. sumatrensis*, can increase the leaf coverage and persistence of the spray droplets or even facilitate their displacement towards the meristem on the shoot tip, thereby intensifying the herbicidal effect of pelargonic acid.

In field application efficacy of pelargonic acid varied among the field trials. Overall, it was incomplete, confirming what was observed in the greenhouse experiment and what was already reported in previous field experiments conducted on spontaneous weed flora [27,29,33]. The botanical composition of weed flora can significantly affect the efficacy of pelargonic acid, and remarkable inter-specific sensitivity differences have been

largely described [22,23]. Pannacci et al. [29] reported large variations in the sensitivity to pelargonic acid, expressed as ED<sub>50</sub> value, between the most (*Kickxia spuria* (L.) Dumort., ED<sub>50</sub> = 2600 g ai ha<sup>-1</sup>, *E. crus-galli*, ED<sub>50</sub> = 3400 g ai ha<sup>-1</sup>) and the least sensitive species (*P. oleracea*, ED<sub>50</sub> > 18,700 g ai ha<sup>-1</sup>, *Lolium multiflorum* Lam., ED<sub>50</sub> > 21,800 g ai ha<sup>-1</sup>) included in their field studies. The dominance in weed communities of sensitive or tolerant species can reasonably lead to contrasting levels of control efficacy for pelargonic acid, as also observed in the present experiment. The dominant weed species in the spring field trial was *D. sanguinalis*, which seemed even more sensitive to pelargonic acid than *E. crus-galli*. As a consequence, the application of pelargonic acid at the highest dose achieved a relevant reduction of weed biomass. On the contrary, *P. oleracea* was the prevalent species in many plots of the summer field trial, and poor control level was observed in those areas. Finally, weed flora in the autumn field trial was dominated by *E. crus-galli*, and overall large weed biomass reduction was achieved with the application of pelargonic acid.

Weather conditions during field experiments could have been another important factor affecting the efficacy of pelargonic acid in different ways. The dry, hot conditions, such as those occurring in the spring and summer trials, could have enhanced drought-tolerance traits on weed leaves, such as increased deposition of wax in the cuticle, increased leaf hairiness, and limited stomatal opening. These traits also hinder herbicide leaf penetration and adsorption, leading to lower herbicide activity. Moreover, dry, hot weather conditions at the moment of field application can lessen the persistence of spray droplets on the leaf surface and, at the same time, reduce the stomatal opening. This can limit herbicide penetration, adsorption, and consequently its efficacy; control efficacy of vinegar-based herbicide, which has the same mode of action as pelargonic acid, has been indeed reported to be positively correlated with high air relative humidity [28]. Thus, it can be supposed that the combination of dry, hot weather effects on weed sensitivity and herbicide leaf penetration reduced the efficacy of pelargonic acid in the spring and summer trials. This led to unsatisfactory control levels, particularly in the case of the summer trial, due to the massive presence of *P. oleracea*, which is highly tolerant to pelargonic acid.

## 5. Conclusions

It may be concluded that weed control levels obtained with field application of pelargonic acid can significantly vary according to the botanical composition of weed communities and environmental conditions. Full and persistent weed control is hardly achievable with the sole application of this herbicide. Pelargonic acid can therefore be a valuable tool for specific uses, such as the stale seedbed technique, within multi-tactics weed management strategies, while it does not seem reliable as a stand-alone weed control tactic. Choosing the appropriate timing for field application, i.e., when the air temperature is lower and relative humidity is higher, as in the early morning or evening, is crucial to ensure pelargonic acid efficacy.

Given the current high cost of pelargonic acid herbicides, reducing the applied dose would provide relevant economic benefits; however, the application of low doses of this herbicide caused a decrease in the control level with amplification of inter-specific variability in both greenhouse and field experiments. Field application of low doses would probably achieve only temporary weed control and, if repeated over time, progressively lead to the spread of the most tolerant species, such as perennials, grasses, and *P. oleracea*. This is an undesirable effect since it is known that *P. oleracea* can quickly become a dominant species in dry, hot conditions, especially in case of inadequate management of late emergence and post-harvest weeds [34]. Dose reduction does not seem to be a recommendable practice in the case of broadcast field applications. In order to reduce the dose of pelargonic acid applied per hectare and consequently the corresponding cost, it seems more promising to switch from broadcast to localized application. Tactics already tested with other herbicides, such as band application along crop rows [35] or patch-spraying [36], can allow reductions in the field area sprayed with pelargonic acid, consequently decreasing its dose per hectare, and maintaining a high herbicide dose in the sprayed areas.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy13061511/s1>, Table S1: Weed botanical composition and density in the field trials.

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