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Evaluate the Efficacy of pelargonic acid and lemongrass essential oil for the control of different weed species

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ABSTRACT

Natural herbicides can contribute to reduce the use of chemical herbicides but information on their efficacy is still limited. Greenhouse and field experiments were conducted to evaluate the herbicidal efficacy of pelargonic acid and lemongrass essential oil on several weed species under different environmental conditions. Lemongrass essential oil had almost no phytotoxic effect, but it was not possible to assess whether this inactivity was due to the specific chemical composition or formulation of the essential oil used in this study.

Pelargonic acid achieved a partial herbicidal effect, with different species-specific sensitivity levels among the tested weeds. Grass weeds, in particular *A. myosuroides* and *L. rigidum*, were more tolerant to pelargonic acid, with no mortality and limited biomass reduction even at the highest application doses. The large difference in sensitivity was observed also among dicots weeds, with *P. oleracea* and *A. theophrasti* being more tolerant than *C. sumatrensis* and *S. nigrum*, on the base of specific leaf traits as leaf angle, leaf hairiness, or wax layer on the cuticle. Furthermore, environmental conditions have been proven to affect the herbicidal efficacy of pelargonic acid in different simultaneous ways. Hot and dry conditions can indeed promote weeds leaf traits that reduce sensitivity to pelargonic acid and reduce the persistence of spray droplets on the leaf surface, limiting herbicide penetration inside weed leaves and consequently hindering its efficacy.

Despite its high cost, pelargonic acid can therefore be a useful tool in a multi-tactic strategy for sustainable weed management while its use as a stand-alone tactic is less recommendable.

Keywords: natural herbicides; pelargonic acid; lemongrass essential oil; weeds

1. INTRODUCTION

1.1 Importance of weed management

Weeds are plants that interfere with human activity in crop and non-crop areas. Weeds compete with crops for soil nutrients, water, and light. They also harbor insects and plant pathogens that are harmful to crop plants, and they may be toxic to crop plants through their root exudates and/or leaf leachates. Additionally, weeds can hinder farming operations, such as making crop harvest more expensive and difficult. Additionally, crop products are frequently contaminated by weed seeds during harvest. Thus, the presence of weeds in crop fields decreases the efficiency of inputs like fertilizer and irrigation water, increases the density of other pest organisms, and ultimately results in significantly lower crop yield and quality. On the overall, weeds are estimated to cause, in absence of adequate control, an average of 30-50% yield loss for the main field crops worldwide [1]–[4].

Herbicides are the dominant tool used for weed control in modern agriculture; they are highly effective and economical [5] [6]. but crops can be stressed by herbicides, which can also make them susceptible to other pests [7]. Environmental pollution, toxicity for non-target organisms, and herbicide residues in the water and soil are additional issues frequently reported as consequences of the excessive use of herbicides [8]. determining in the public opinion increasing concerns for the safety and health of people and the environment [9]–[11] As a consequence, stricter regulations on pesticide registration and use have been progressively enacted and policymakers are increasingly asking for a relevant reduction of pesticide use, as stated by European Commission within the 'Farm to Fork' strategy [12].

Meanwhile, the repeated use of the same herbicides, or of herbicides with the same Mode of Action, has provoked continuous selection pressure on weed communities, leading to the evolution of resistant weed biotypes. In the past 20 years herbicide resistance has been reported across the main agricultural areas worldwide and has affected all the main annual and perennial crops. 513 unique cases of herbicide resistance, involving 267 weed species, have been currently reported in the most updated surveys, affecting all the most used herbicides, such as acetyl-CoA carboxylase inhibitors Acetolactate Synthase inhibitors, triazines, and acetyl-CoA carboxylase inhibitors moreover, many harmful weed species, such as A*maranthus, Conyza, Echinochloa*, and *Lolium* spp. have evolved multiple resistance to a wide range of herbicide sites of action [13]–[17]. Herbicide resistance is therefore another significant problem linked to the excessive and exclusive use of synthetic herbicides [18]. that is currently hindering the sustainable intensification of food production.

New approaches to weed management and "greener" products are in high demand in the society and this necessity has been largely acknowledged by the scientific community [19]–[21]. One alternative

to reducing the stress that synthetic herbicides promote in crops and all their negative effects on the environment is the use of natural products as bioherbicides. The development of organic acids- or essential oil-based natural herbicides may lessen these adverse effects. They have different mechanisms of action that can prevent the evolution of herbicide-resistant weed biotypes, are less persistent than synthetic herbicides, and are more environmentally friendly [22][23]. In order to control weeds in organic and sustainable agriculture systems, organic acids, essential oils, crude botanical products, and other naturally occurring substances derived from plant tissues can be used as bio-herbicides and IPM programs might include bioherbicides as a cutting-edge weed control strategy [24]. Such natural substances face several opponents among European Commission members, as there are concerns about natural product registration processes due to a lack of relevant toxicological data for commercial use [25]. Despite these reservations, natural herbicides are often less hazardous to the environment and human health than commercial synthetic herbicides [26]. There is indeed evidence that most essential oils and their constituents are not necessarily genotoxic or harmful to human health [27]. Toxicity tests on non-target organisms such as birds, fish, and honeybees revealed little or no toxicity in the case of organic acids such as pelargonic acid [28]. However, since the acid is an irritant to the skin and eyes, product labels provide instructions on how consumers should use the products [29] Anyhow, users must take precautions, such as avoiding windy days and using large spray droplets, to reduce drift and potential harm to non-target plants even when they are applying natural herbicides.

1.2 Organic Acids and pelargonic acids

Organic acids have been investigated as natural herbicides since decades [30]–[33] Pelargonic acid (PA) ($CH_3(CH_2)_7CO_2H$, n-nonanoic acid) is a saturated, nine-carbon fatty acid (C9:0) that naturally occurs as esters in the essential oil of *Pelargonium* spp. and can come from the tissues of different plant species [34]–[36]. Pelargonic acid, in combination with its salts and emulsifiers, is used as a nonselective herbicide for weed control in gardens and professional fields worldwide [23], [34]. They are used as contact burndown herbicides, which attack cell membranes, causing cell leakage and membrane acyl lipid breakdown [35], [36]. The phytotoxic effects of pelargonic acid are visible very quickly after spraying, and the symptoms include phytotoxicity for the plants and their cells, which rapidly begin to oxidize, and necrotic lesions on the aerial parts of plants [37]. Pelargonic acid's potential use as a bioherbicide represents an appealing non-chemical weed control option that can be effectively integrated with other eco-friendly weed management strategies in important crops such as soybean [38]. Good herbicidal efficacy of pelargonic acid has been obtained on different weed species in pot or greenhouse experiments, [23], [39], [40] while contrasting results have been reported regarding control efficacy of pelargonic acid under field conditions [41] reported good control efficacy

of pelargonic acid in combination with stale seedbed before soybean crop but only for annual weed species. Similarly, different levels of control for dicots, grasses, and sedges were observed with pelargonic acid application in field vegetables [41], [42]. Regarding perennial crops, [43]reported high control efficacy against *Chenopodium album* in orchards but with four pelargonic acid applications per growing season. Intermediate weed control level was observed with repeated pelargonic acid applications in olive groves [44] while only partial control of *Conyza bonariensis* was obtained in vineyards [45]. [46]reported only short-term efficacy in controlling road-side vegetation and finally several studies observed poor weed control was achieved with pelargonic acid application on developed mixed weed flora [48]. This inconsistency across the different studies could be related to the strong influence of environmental conditions, such as temperature, solar radiation, soil humidity, on the herbicidal efficacy of pelargonic acid. On the other hand, a different level of sensitivity to pelargonic acid across the different weed species (or the different stages of the same species) should be considered as well [47], [48].

In order to ensure a more reliable and effective herbicidal action of pelargonic acid, studies have been conducted testing mixtures with other natural herbicides such as essential oils [39], [40]. but data about this topic are still limited.

Commercial products containing pelargonic acid are currently registered as herbicides in the European Union, even if they are not authorized in organic agriculture, but their use is still limited mainly due to their high cost. This is mainly due to the high doses of commercial herbicide, that is 16 L/ha, recommended for field applications. Anyhow, recommended doses of pelargonic acid had been calibrated to control weed plants up to 10 cm in height. Maybe lower doses could be enough to achieve satisfactory control on weed plants at the seedling stage (from emergence to 3-4 true leaves, BBCH 10-14); weeds are at this stage at the moment of early control operations, for example during the false seedbed technique. However, the application of lower doses could probably increase the differences among weed species in the sensitivity to pelargonic acid.

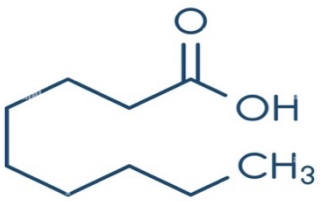

Figure 1. Pelargonic acid

1.3 Essential oils

The unique properties of essential oils-natural mixtures of volatile compound-give them specific pharmacological properties, including their well-known antibacterial, antioxidant, anti-inflammatory, and cancer chemoprotective effects, as well as their repellent, herbicidal, and insecticidal biological activities [49]-[52]. Essential oils derived from aromatic, biomass, invasive, or food crop plants have also been shown to have the potential as natural non-selective herbicides [24], [43], [53]. which have produced useful applications in the fields of agriculture, food, cosmetics, and human health. Some essential oils have already been shown to have an impact on weed seed germination and seedling growth [54], [55] Similar to how pelargonic acid, which is more effective against young plants than older ones, causes weeds' foliage to burn down quickly after application [56].

Regarding this, origanum (*Origanum vulgare* L.) essential oil with carvacrol as its primary component has demonstrated a significant inhibitory effect against seed germination and seedling growth of *Portulaca oleracea, Lolium multiflorum, Echinochloa crus-galli* at a range of concentrations (0.125-1 µl/ml), as well as against *Sinapsis avensis* at 2 µl/ml and Johnsongrass (S*orghum halepense* L.) [57]–[59]. *Pinus sylvestris* essential oil showed some inhibition of Ca*ssia occidentalis* (L.) Link's early root growth and *Corymbia citriodrora* essential oil had an impact on the growth of some weeds, particularly *Amaranthus viridis* seed germination (L.) [60].

Since it is difficult for weeds to develop resistance to mixtures of natural ingredients with various mechanisms of action, the development of natural herbicides based on essential oils could lessen these negative effects, particularly by combating resistant weeds. In this sense, agricultural compositions—including oregano essential oil and others from the *Lamiaceae* family, including species of *Lavandula, Mentha, Rosmarinus,* and *Salvia*—have been developed as organic pesticides [22]. Similarly, lemongrass essential oil (*Cymbopogon citratus*, Poaceae) has been used as a key ingredient in the development of a natural herbicide to control weed germination and growth [23]. Additionally, *Cymbopogon citratus* Stapf. or *Cymbopogon flexuosus* D.C. lemongrass essential oil, which contains up to 80% citral, is sold as an organic herbicide that works by interfering with the polymerization of plant microtubules [61] Because the active ingredient in lemongrass oil does not translocate, it only affects the parts of plants that are exposed to the spray solution [24]

As already mentioned for pelargonic acid, most of the existing studies about herbicidal effect of essential oils has been conducted under laboratory conditions, mainly focusing on germination inhibition, often on few weed species. Information about the efficacy of essential oils on several weed species or under field conditions is instead scarce.

Finally, it is worth pointing out that despite all the existing studies on the herbicidal effect of essential oils, there is currently no commercial herbicide based on them authorized in agriculture in the European Union.

2. AIM

The overall aim of this study is to assess the efficacy and feasibility of pelargonic acid and lemongrass essential oil as post-emergence herbicides to be included in weed management programs in field crops. In particular, the specific aim of the present study was to evaluate the efficacy of pelargonic acid, lemongrass essential oils, and their mixtures at different doses against different weed species of agronomic interest for the European cropping systems. To assess this, different trials were conducted under greenhouse (Experiments 1 and 2), and field (Experiment 4) conditions on weeds at the seedling stage (3-4 true leaves). Given the importance of environmental conditions on the herbicidal action of pelargonic acid, a further greenhouse experiment (Experiment 3) was conducted to test whether soil water content affects weed sensitivity to this organic acid.

3. CHAPTER 1 – FIRST GREENHOUSE EXPERIMENT

The aim of this experiment was to test the herbicidal efficacy of different doses of pelargonic acid on several weed species.

3.1 Materials and Methods

3.1.1 Plant Production

Seeds of *A. theophrasti* (ABUTH), *A. myosuroides* (ALOMY), *C. sumatrensis* (CONSU), *P. maculosa* (POLPE), *S. pumila* (SETPU), and berries of *S. nigrum* (SOLNI) were collected in summer and autumn in fields at the experimental farm "L. Toniolo" of the University of Padova. This farm is located at Legnaro (45°21'04" N 11°57'02" E, 8 m asl), and crop rotation includes maize, soybean, wheat, and sugar beet. Seeds of *L. rigidum* originated from plants cultivated in a greenhouse located at the same farm. Seeds were collected from at least 50 plants per each species to account for local intra-population variability. Seeds of *A. theophrasti, A. myosuroides, C. sumatrensis, L. rigidum, P. maculosa, S. pumila*, and berries of *S. nigrum* were collected by gently shaking mother plants in order to collect only fully ripened seeds and fruits which easily fell from the plants. Berries of *S. nigrum* were then squeezed on sheets of filter paper to obtain seeds. Seeds of the different species were cleaned and left to dry at room temperature (20 ◦C) for 2 weeks, then seeds were put in paper bags and stored at 4 °C until the start of the experiments.

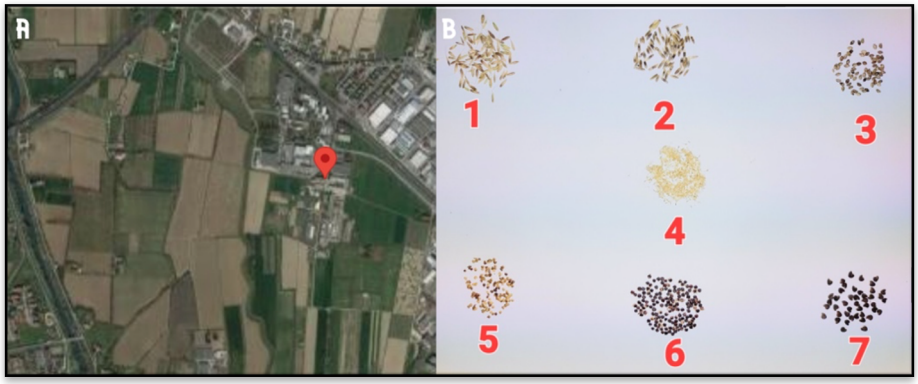

Figure 1.1 Location (A) and Seeds (B) of the 7 weed species collected (1. *L. rigidum,* 2. *A. myosuroides,* 3. *S. pumila,*4. *C. sumatrensis,* 5. *S. nigrum,* 6. *P. maculosa,* and 7.*A. theophrasti)* for herbicidal tests

Seeds of *A. theophrasti* were firstly surface sterilized by immersion in a 1% (v/v) sodium hypochlorite solution for 5 min to prevent fungal contamination during the following germination phase. Seeds of the different species were then sown in petri dishes on peat substrate moisten with 10 mL of deionized water and exposed to chilling treatment with a different length according to the specific requirements of the different species to break dormancy and promote their germination. Seeds of *A. myosuroides* and *L. rigidum* were vernalized in a fridge at 4 °C in petri dishes on peat substrate, in dark conditions

for 3 days. Seeds of *A. theophrasti, P. maculosa, S. pumila*, and *S. nigrum* required a chilling treatment with similar conditions for 7 days. Seeds of *C. sumatrensis* did not require chilling treatment and were directly incubated for germination.

After the chilling treatment, seeds were incubated in germination chambers at an alternate temperature regime of 25-15 °C and a 12 h light photoperiod, with neon tubes providing a photosynthetic photon flux density (PPFD) of 15–30 µmol m^{-2} s^{-1}. The highest temperature corresponded with the light period. The duration of germination and seedling early-growth phase varied across the different species, so Petri dishes were maintained in the germination chamber for different spans ranging from 4 days for *A. myosuroides* and *L. rigidum* to 14 days for *C. sumatrensis* and *P. maculosa*, respectively. Table 1.1 resumes the duration of chilling treatments and germination phase for the different species. For each species and replicate, 15-20 seedlings of similar growth stages were transplanted into rectangular plastic pots (160 x 160 x 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 and the soil was maintained at or near field capacity throughout the experiment. Light in the greenhouse was supplemented with metal halide lamps (400 W), 14 hours photoperiod, PPFD ~ 160 µmol m^{-2} s^{-1}. During the experiment, the minimum and maximum temperatures inside the greenhouse fluctuated from 20 to 23 ° C and from 25 to 30 ° C, respectively.

The temperature in the greenhouse fluctuated between 15 and 28 °C during the experiment.

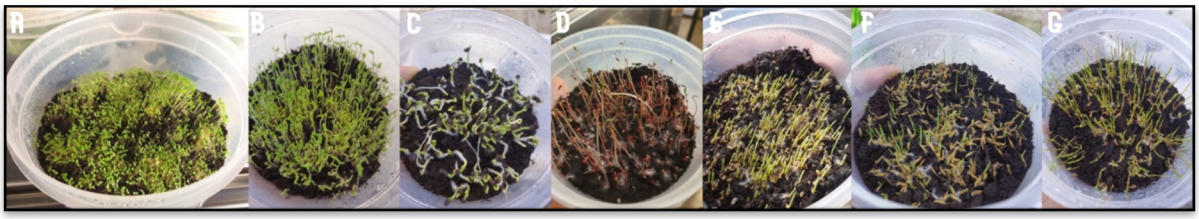

Figure 1.2 Seedlings ready for transplanting *(A) C. sumatrensis (B) S. nigrum, (C) A. theophrasti (D), P. maculosa, (E) S. pumila, (F) A. myosuroides, (G) L. rigidum)*

Species	Chilling treatment (days)	Germination (days)
A. theophrasti	7	7
A. myosuroides	3	4
C. sumatrensis	-	14
L. rigidum	3	4
P. maculosa	7	14
S. pumila	7	7
S. nigrum	7	7

Table 1.1. Duration of chilling treatment and germination phase for the different species

3.1.2 Herbicide application and experimental design

Herbicide application was done when weed plants reached the stage of 1-2 tillers or BBCH 21-22 [68] for grasses (*A. myosuroides, L. rigidum, S. pumila*) and plants at 4-6 true leaves or BBCH 14-16 for Dicots (*A. theophrasti, C. sumatrensis, P. maculosa, S. nigrum*). Herbicides were applied using a precision bench sprayer with a boom equipped with three flat fan hydraulic nozzles (TeeJet TP11001-VH, Glendale Heights, IL, USA), with a spray volume of 200 L ha^{-1} applied at a pressure of 215 kPa and speed of 0.6 m s^{-1}. Plants were treated with a range of doses of pelargonic acid (commercial Beloukha herbicide, pelargonic acid 680 g ai L^{-1}, Belchim Crop Protection Italia S.p.A, Rozzano, MI, Italy). Treatments were PEL16 (pelargonic acid 10880 g ai ha^{-1}), PEL12 (pelargonic acid 8160 g ai ha^{-1}), and PEL8 (pelargonic acid 5440 g ai ha^{-1}) corresponding to 16, 12, and 8 L ha^{-1} of the commercial Beloukha herbicide, respectively. Recommended field dose of this herbicide for crop seedbed cleaning is 16 L ha^{-1}. Untreated control replicates were also included for all species. The total number of treatments was 4 (three herbicide doses + untreated control) * 7 (weed species) = 28. Table 1.2 reports the complete list of treatments and herbicide doses. The experimental layout was a completely randomized design with three replicates, i.e., three pots of 20-15 plants each, for a total of 84 pots.

Treatment	**Dose of commercial herbicide**	**Dose of pelargonic acid**
	L ha^{-1}	**g ai ha^{-1}**
PEL16	16	10880
PEL12	12	8160
PEL8	8	5440

Table 1.2 List of treatments with the corresponding herbicide doses included in the first experiment

3.1.3 Data collection and statistical analysis

Assessment of herbicide efficacy was conducted 3 weeks after treatment (3 WAT) and examined plant survival and fresh weight reduction. Plant survival was expressed as a percentage of the plants counted before the treatment in each pot. Plants were considered dead if they did not show any active growth regardless of their colour. The fresh weight of the above-ground biomass was measured for each pot, that is each replicate, and average plant weight was estimated by dividing total pot weight by the number of alive plants before herbicide application. To assess fresh weight reduction, the average plant weight of treated replicates was then expressed as a percentage of the mean value of the untreated plants of the same species. A value of 100% for a given replicate, therefore, means that its biomass had the same fresh weight of the mean of the untreated plants of this species.

A factorial ANOVA ($p < 0.05$) was performed using JASP software (www.jasp-stats.org) to test the effect of the factors Dose, Species, and their interaction on the response variables Plant survival and Fresh weight. To identify significant differences between treatment means, Tukey's HSD test ($p < 0.05$) was then performed.

3.2 Results and Discussion

Different response to the pelargonic acid application was observed for the different weed species, even if the dynamic was similar. Symptoms of the pelargonic acid effect were visible one day after its application, with extensive leaf wilting especially for *C. sumatrensis* and *S. nigrum* plants. However, plants started soon to recover from these damages, producing new leaves and resuming their growth. Damages caused by pelargonic acid were no longer visible in many treated plants at 3WAT. Most of the treated plants survived the application of pelargonic acid, with five out of the seven weed species showing plant survival above 90% across all tested doses (Figure 1.7). Regarding the remaining two species, that is *C. sumatrensis* and *S. nigrum*, plant survival was reduced only at the highest dose of pelargonic acid with survival values of 51 ± 12.2 and 78 ± 8.20 %, respectively (Figure 1.7). As a consequence, Factorial ANOVA could not be performed to test the effect of the experimental factors Dose and Species on plant survival because variance among replicates was too low, showing most of the replicates the same values (that is 100).

Figure 1.3 Plants before treatment

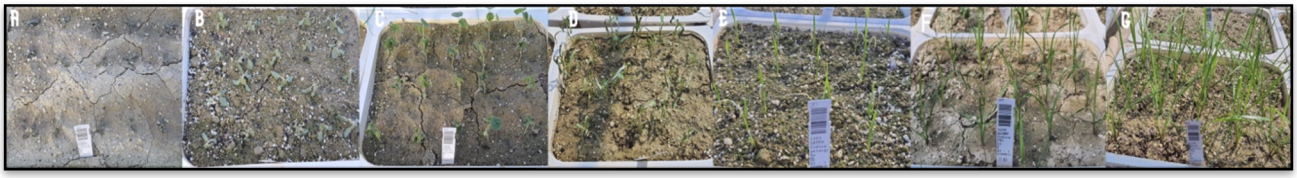

Figure 1.4 Plants after one day after treatment

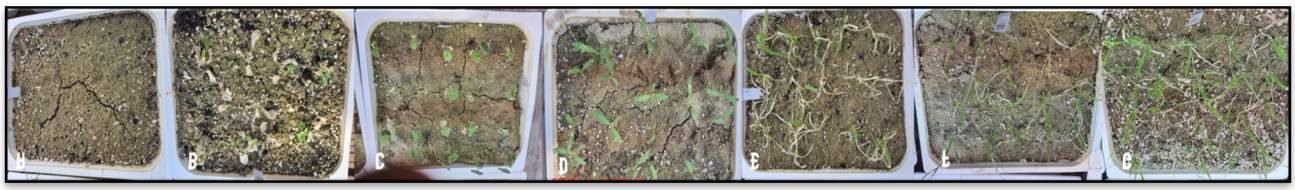

Figure 1.5 Damage scale for each species after one week of treatment

Figure 1.3,1.4,1.5. *(A) C. sumatrensis (B) S. nigrum, (C) A. theophrasti (D), P. maculosa, (E) S. pumila, (F) A. myosuroides, (G) L. rigidum)*

The fresh weight of aboveground biomass varied across the different species, anyhow the application of pelargonic acid caused a reduction of biomass measured at 3 WAT for all treated replicates and species. Factorial ANOVA identified the significant effect of the factors Dose, Species, and their interaction on biomass reduction (Table 1.3). Biomass progressively decreased across the tested doses of pelargonic acid, but the extent of this reduction varied between species (Figure 1.8). High values of biomass, close to 80% of the average of the untreated plants, were observed for *L. rigidum* and *S. pumila* even at the highest dose (10880 g ai ha^{-1}). *Abutilon theophrasti, A. myosuroides* and *P. maculosa* showed an intermediate response to the herbicide, values of biomass at the highest dose of pelargonic acid were indeed around 60% of the average of the untreated plants. Finally, a relevant reduction of biomass fresh weight was observed for *S. nigrum*, with values of 58 ± 7.8 % and 29 ± 8.2 % in comparison with the untreated plants at the intermediate and highest dose of pelargonic acid (8160 and 10880 g ai ha^{-1}) respectively, and for *C. sumatrensis* with values of 41 ± 14.3 % and 14 ± 12.2 % in comparison with the untreated plants at the intermediate and highest dose of pelargonic acid, respectively. Within the single species, significant differences were detected between the means of the first lowest doses (untreated and 5440 g ai ha^{-1}) and the two highest doses (8160 and 10880 g ai ha^{-1}) apart from *L. rigidum* and *S. pumila* where no significant differences could be detected.

The herbicidal efficacy of pelargonic acid observed in the present study is lower than those stated in previous studies conducted under similar greenhouse conditions. Kanatas et al [40] reported indeed that pelargonic acid decreased by 70-75% fresh weight in *Echinochloa crus-galli* (L.) P.Beauv. and *Sorghum halepense* (L.) [39], [40] described similar levels of efficacy on *L. rigidum* and *Avena sterilis* L. However, in those studies the evaluation of the pelargonic effect was conducted at 10-14 DAT (Days After Treatment), while in the present study, this assessment was done at 21 DAT. Since treated plants started to recover and grow back about 7 days after a pelargonic application, postponing the efficacy evaluation by 10 days, from 10 to 21 DAT, could have enabled treated plants to recover and reduce the difference with the untreated plants. It is already known that pelargonic acid has usually a temporary control efficacy against weeds, which normally begin to regrow a couple of weeks after the treatment as described by [46]. Moreover, plants were kept in the present experiment at optimal growing conditions with substrate humidity always close to field capacity. Given that pelargonic acid has no systemic effect, being under optimal growing conditions probably enabled treated plants to produce new tissues and leaves and eventually recover from the initial damage. For example, heavily damaged *S. nigrum* plants, whose shoot tip was completely destroyed by pelargonic acid application, were able to produce new stems and leaves from the axillary buds (Figure 1.6).

Figure 1.6 *Solanum nigrum* response to pelargonic acid: apical meristem was destroyed but auxiliary buds were able to produce new stems and leaves

It is therefore interesting to evaluate how environmental conditions influence plants response to the pelargonic acid application and this was the aim of the experiment described in Chapter 3.
Different level of sensitivity to pelargonic acid was observed for the different weed species, as already reported in previous greenhouse and field studies. Travlos et al [39] observed in a greenhouse experiment higher efficacy on *Galium aparin*e L. than on *A. sterilis* or *L. rigidum*. Similarly, pelargonic acid applied in field vegetables resulted in less effective control of *Cyperus esculentus* than grasses and dicots [41], [42]. Biological and morphological traits of the different species are the main driving factors determining their sensitivity to pelargonic acid. Perennial plants are less sensitive since pelargonic acid is not able to reach and damage their vegetative organs such as rhizomes or tubers. In

the present experiment, the three grass species were overall less affected by pelargonic acid than the Dicots. Grasses have narrow, elongated and erect leaves and these traits can reduce leaf surface coverage by spray droplets during herbicide application. Moreover, buds in grasses are protected by the basal leaf sheaths and not exposed on the shoot tips as in Dicots. These aspects can decrease the efficacy of contact, non-systemic herbicides as pelargonic acid. Differences were observed also in the response of the Dicots species included in the experiment: at the highest pelargonic acid dose, only a 35-40% reduction of fresh weight in comparison with control plants was obtained for *A. theophrasti* and *P. maculosa*, while efficacy was significantly higher for *S. nigrum* and *C. sumatrensis* (70-80% of reduction). This different response could be caused by several morphological traits such as leaf shape or the presence of wax and hairs on the leaf surface. Evan et al [32] observed that the obtuse leaf blade angle in *A. theophrasti* facilitates the movement of the solution sprayed on the leaf surface away from the shoot tip and towards the leaf tip, thus increasing dripping and reducing the herbicide effect. Similarly, seedlings of *P. maculosa* have narrow, convex, wax-covered leaves and this can reduce the permanence and coverage of spray solution on the leaf surface. On the contrary, large concave leaves with horizontal or acute blade angle, such as those of *S. nigrum* and *C. sumatrensis*, can increase the leaf coverage and permanence of the spray solution or even facilitate its movement towards the bud of the shoot tip, thus intensifying herbicidal effect of pelargonic acid. These differences in the specific response to pelargonic acid were larger at the lowest doses, as expected. Field application of pelargonic acid, especially at low doses, would therefore probably achieve only temporary weed control and, if repeated over time, progressively lead to a shift of weed community with the spread of the more tolerant species, such as perennials and grasses. Field experiments described in Chapter 4 were conducted to investigate this hypothesis evaluating the control efficacy of pelargonic acid on a mixed, spontaneous weed flora under real field conditions.

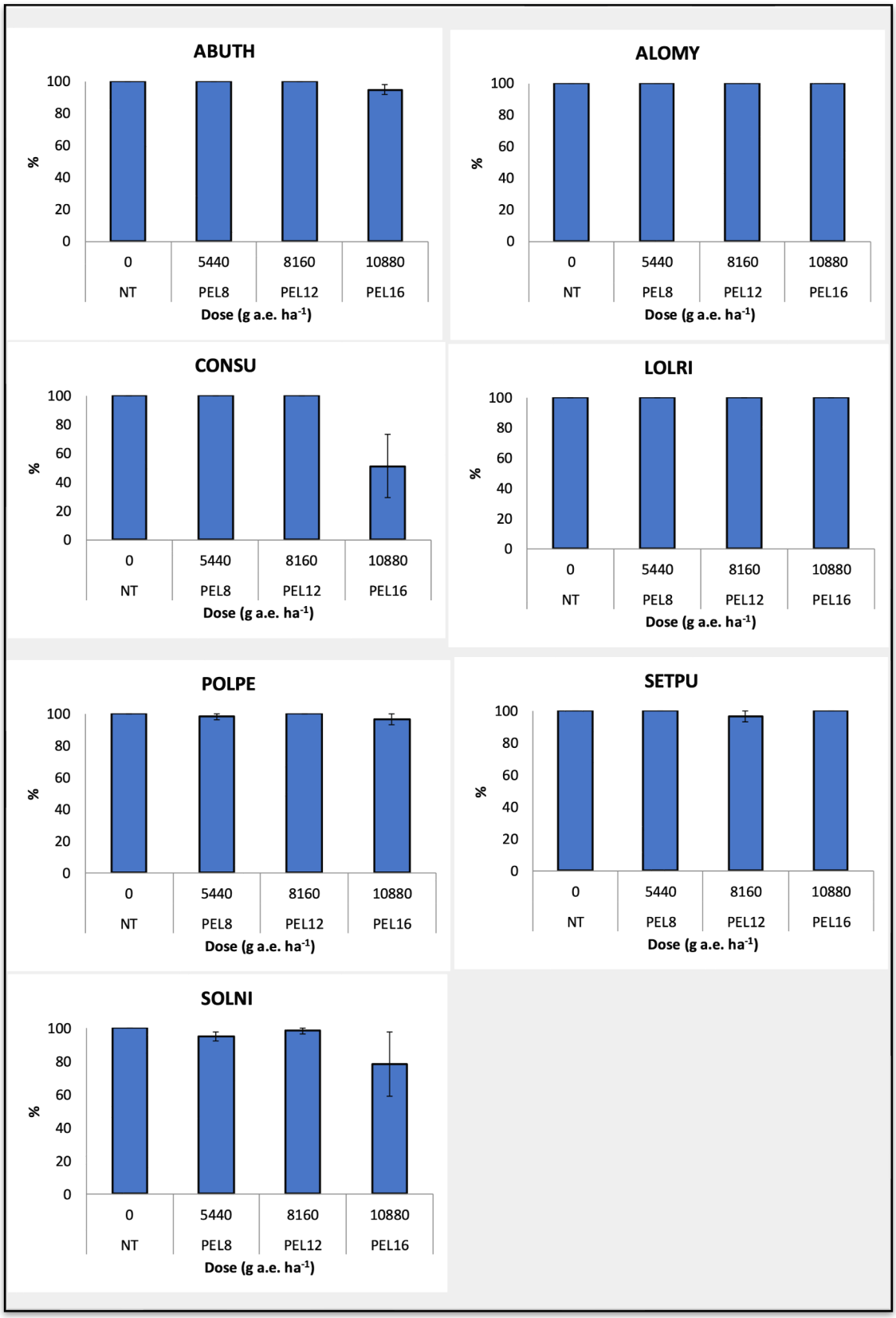

Figure 1.7 Plant survival (%) at the different doses of pelargonic acid for the different weed species. Values are the mean of three replicates, bars represent standard errors

Cases	Sum of Squares	df	Mean Square	F	*p*
Species	8780.989	6	1463.498	6.152	< .001**
Dose	29757.176	3	9919.059	41.695	< .001**
Species ✻ Dose	10695.177	18	594.177	2.498	0.005**
Residuals	13322.137	56	237.895		

Table 1.3 Factorial ANOVA to test the effect of the factors Species, Dose, and their interaction on the response variable "Biomass fresh weight". Significance level for $p < 0.05$ (** highly significant, *significant, *ns* non-significant), Type III Sum of Squares

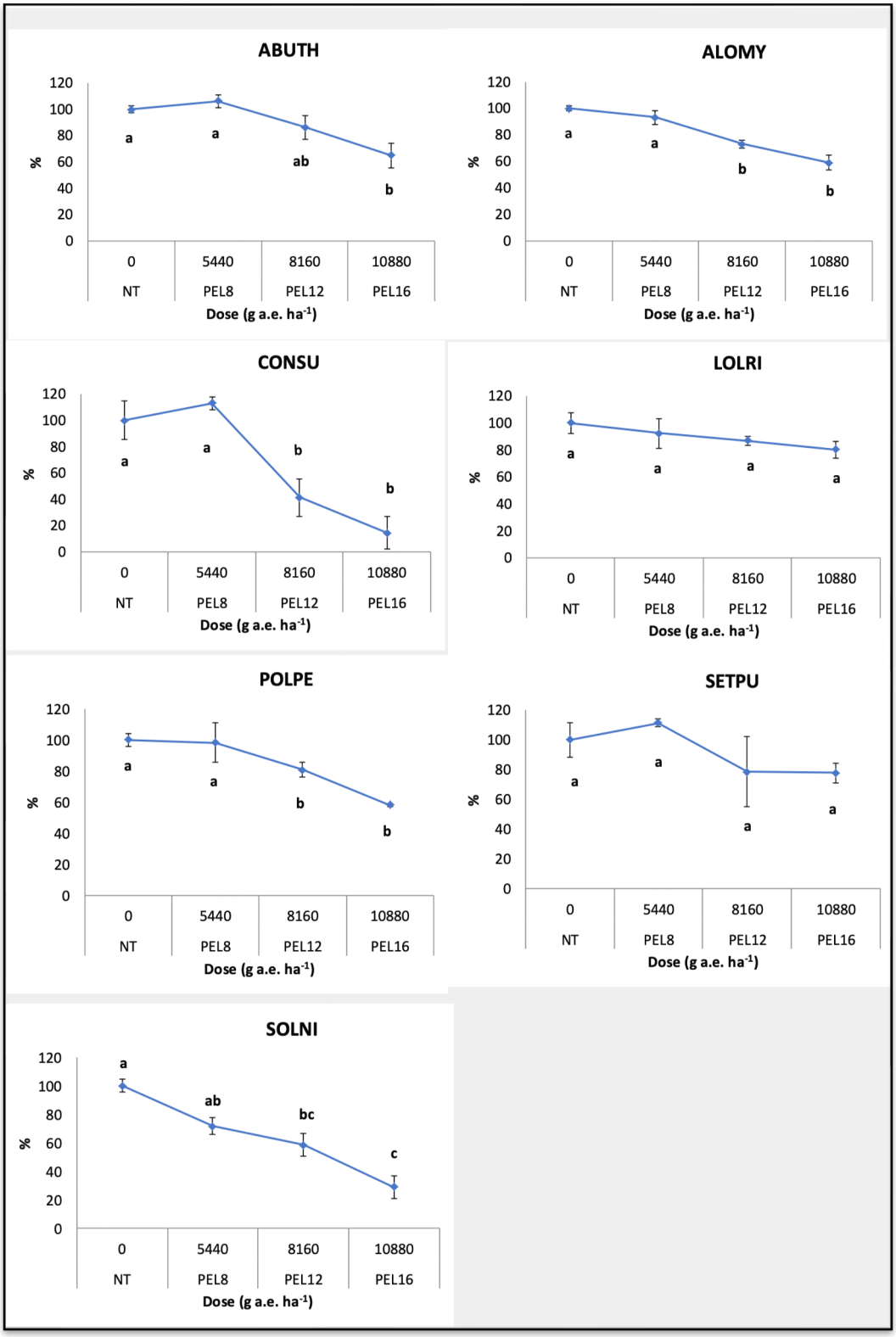

Figure 1.8 Fresh weight of plant biomass (expressed as % of the untreated plants) at the different doses of pelargonic acid for the different weed species. Values are the mean of three replicates, bars represent standard errors, and letters identify significant differences between treatments of the same species according to Tukey's HSD test ($p < 0.05$)

CHAPTER 2 – SECOND GREENHOUSE EXPERIMENT

The aim of this experiment was to test the herbicidal efficacy of different doses of pelargonic acid, alone or in combination with lemongrass essential oil on several weed species.

4.1 Materials and Methods

4.1.1 Plant Production

The list of weed species tested in this experiment was the same as in Experiment 1 (*A. theophrasti, A. myosuroides, C. sumatrensis, P. maculosa, S. pumila*, and *S. nigrum)*. The same seed batches were used and the same procedures for germination and plant production described for Experiment 1 were adopted. During the experiment, the minimum and maximum temperatures inside the greenhouse fluctuated from 20 to 25 ° C and from 27 to 35 ° C., respectively.

4.1.2 Herbicide application and experimental design

As for Experiment 1, herbicide application was done when weed plants reached the stage of 1-2 tillers or BBCH 21-22 [68] for grasses (*A. myosuroides, L. rigidum, S. pumila*) and plants at 4-6 true leaves or BBCH 14-16 for Dicots (*A. theophrasti, C. sumatrensis, P. maculosa, S. nigrum*). Herbicides were applied using a precision bench sprayer with a boom equipped with three flat fan hydraulic nozzles (TeeJet TP11001-VH, Glendale Heights, IL, USA), with a spray volume of 200 L ha^{-1} applied at a pressure of 215 kPa and speed of 0.6 m s^{-1}. Plants were treated with a range of doses of pelargonic acid (commercial Beloukha herbicide, pelargonic acid 680 g ai L^{-1}, Belchim Crop Protection Italia S.p.A, Rozzano, MI, Italy) alone or in combination with lemongrass (*Cymbopogon flexuosus* (Nees ex Steud.) W.Watson) essential oil (Lemongrass essential oil, Aromatika Bv, Soest, The Netherlands) at 5% v/v concentration. Lemon grass essential oil dose was chosen according to previous studies[43]. Treatments were PEL16 (pelargonic acid 10880 g ai ha^{-1}), PEL12 (pelargonic acid 8160 g ai ha^{-1}), PEL8 (pelargonic acid 5440 g ai ha^{-1}), LEO+PEL16 (lemon grass essential oil 5% v/v + pelargonic acid 10880 g ai ha^{-1}), LEO+PEL12 (lemon grass essential oil 5% v/v + pelargonic acid 8160 g ai ha^{-1}), LEO+PEL8 (lemon grass essential oil 5% v/v + pelargonic acid 5440 g ai ha^{-1}). These doses of pelargonic acid correspond to 16, 12, and 8 L ha^{-1} of the commercial Beloukha herbicide, respectively, whose recommended field dose for crop seedbed cleaning is 16 L ha^{-1}. An additional treatment with the lemon grass essential oil alone (LEO, lemon grass essential oil 5% v/v) was included to assess the specific herbicide efficacy of this essential oil. Untreated control replicates were also included for all species. Total number of treatments was 8 (7 herbicide doses + untreated control) * 7 (weed species) = 56. Table 2.1 reports the complete list of treatments and herbicide doses. The experimental

layout was a completely randomized design with three replicates, i.e. three pots of 20-15 plants each, for a total of 168 pots.

Treatment	Dose of commercial herbicide L ha^{-1}	Dose of pelargonic acid g ai ha^{-1}	Dose of lemongrass essential oil L ha^{-1}
PEL16	16	10880	
PEL12	12	8160	
PEL8	8	5440	
LEO			10
LEO + PEL16	16	10880	10
LEO + PEL12	12	8160	10
LEO + PEL8	8	5440	10

Table 2.1 List of treatments with the corresponding herbicide and essential oil doses included in the second experiment. Essential oil doses are calculated considering a spray volume of 200 L ha^{-1}

4.1.3 Data collection and statistical analysis

Assessment of herbicide efficacy was conducted 3 weeks after treatment (3 WAT) and examined plant survival and fresh weight reduction. Plant survival was expressed as a percentage of the plants counted before the treatment in each pot. Plants were considered dead if they did not show any active growth regardless of their colour. The fresh weight of the above-ground biomass was measured for each pot, that is each replicate, and average plant weight was estimated by dividing total pot weight by the number of alive plants before herbicide application. To assess fresh weight reduction, the average plant weight of treated replicates was then expressed as a percentage of the mean value of the untreated plants of the same species. A value of 100% for a given replicate, therefore, means that its biomass had the same fresh weight as the mean of the untreated plants of this species.

A factorial ANOVA ($p < 0.05$) was performed using JASP software (www.jasp-stats.org) to test the effect of the factors of Herbicide treatment, Species, and their interaction on the response variables of Plant survival and Fresh weight. In order to identify significant differences between treatment means, Tukey's HSD test ($p < 0.05$) was then performed.

4.2 Results and Discussion

Effects of pelargonic acid were visible one day after application, as in the first greenhouse experiment (Chapter 1), but the magnitude was higher both in terms of plant survival and biomass reduction. Factorial ANOVA detected significant effects of Herbicide treatment, Species, and their interaction on the response variables Plant survival and Fresh weight (Table 2.2, Table 2.3). However, the large majority of treated plants survived to pelargonic acid application even if species-specific response was again observed (Figure 2.1). Limited symptoms of phytotoxicity, and consequently almost no negative effect on plant survival or biomass (Figures 2.1 and 2.2), were observed on plants treated with the lemongrass essential oil alone. Similarly, the addition of lemongrass essential oil did not increase the efficacy of pelargonic acid, and no significant differences in terms of plant survival or biomass reduction at a given dose of pelargonic acid were detected between the treatment with or without the addition of lemongrass essential oil.

The majority of the treated plants survived the application of pelargonic acid and the mixture of pelargonic acid and lemongrass essential oil (Figure 2.1). *A. theophrasti, A. myosuroides, L. rigidum,* and *S. nigrum* presented no significant difference between means of herbicide treatments when compared non treated plants while the remaining three species showed different responses. Regarding *C. sumatrensis*, significant differences were observed between the highest value of plant survival (98% NT) and the two lowest ones (13% PEL 16 + LEO and 24% PEL 8). Significant differences were also detected for *P. maculosa* between the lowest value (84% PEL16) and all the other treatments, while for *S. pumila* significant differences were found only between the highest (96% NT) and the lowest value (69% PEL16).

The fresh weight of aboveground biomass differed between species. The factors Dose, Species, and their interaction were found to significantly affect biomass by Factorial ANOVA (Table 2.3). The examined pelargonic acid and mixture of pelargonic acid and lemongrass essential oil doses reduced biomass progressively, but the extent of this reduction varied between species (Figure 2.2). No significant differences between means were detected for *A. theophrasti, L. rigidum,* and *S. nigrum.* In the case of *A. myosuroides* significant differences were found between the highest value of the fresh weight of biomass (NT) and the two lowest ones (39% PEL12 and 44% PEL16). A similar response was observed for *S. pumila*, with significant differences only between the highest value (NT) and the lowest biomass values (30% PEL16). *Conyza sumatrensis* instead showed biomass decrease also at the intermediate doses, with significant differences between the two highest values of biomass (NT and LEO) and all other treatments. Finally, no differences were observed for *P. maculosa* between the untreated and the treatments with pelargonic acid, while treatment with lemongrass essential oil achieved significantly higher biomass than most of the other treatments (figure 2.2).

The application of pelargonic acid obtained a higher effect on both plant survival and biomass reduction in comparison with the first greenhouse experiment (Chapter 1). This could be caused by the higher temperatures inside the greenhouse during the second experiment, given that high temperatures are known to increase the herbicidal efficacy of organic acids [63]. Anyhow, a satisfactory level of control was not achieved for most of the tested species even at the highest pelargonic acid dose. This is in agreement with what was observed in the first experiment and the probable causes of this different result in comparison with previous studies by other Authors have been already explained in the Discussion section of Chapter 1. The differences in sensitivity to pelargonic acids, observed during the first experiment among the tested species, were confirmed in this second experiment.

The limited phytotoxic effect observed in this experiment for lemongrass essential oil disagreed with previous studies [24]. It should be underlined that different commercial products containing lemongrass essential oil were used in all those experiments and none of those products is a commercial herbicide with a standard composition and formulation. Differences in the chemical composition of the products used in the various experiments cannot be excluded, since intra-specific variability of the plants and differences in environmental growing conditions are known to affect the chemical composition of plant essential oil. He et al [64] for example reported differences in the essential oil composition of *Cymbopogon citratus* (DC.) Stapf from different regions in China. Similar variability was described for other species such as *Rosa damascena* Mill. [65] or *Petroselinum crispum* var. *tuberosum* (Mill.) Fuss [66]. Detailed analysis of the chemical composition of the specific lemongrass essential oil used in each experiment would be necessary to enable proper comparison of their herbicidal efficacy.

Moreover, the commercial product used in the present experiment is an herbal product for aromatherapy, so it did not contain any specific co-formulants or adjuvants to ensure sufficient leaf adsorption. This could have limited the phytotoxic effect of lemongrass essential oil in the present experiment and further efforts should be directed to improve the formulation of essential oil-based herbicides and find adequate adjuvants.

ANOVA - Survival

Cases	Sum of Squares	df	Mean Square	F	p
Species	38431.564	6	6405.261	35.525	< .001**
Herbicide treatment	7374.653	7	1053.522	5.843	< .001**
Species ✻ Herbicide treatment	19501.096	42	464.312	2.575	< .001**
Residuals	20194.140	112	180.305		

Table 2.2 Factorial ANOVA to test the effect of the factors of Herbicide treatment, Species, and their interaction on the response variable "Plant Survival". Significance level for $p < 0.05$ (** highly significant, *significant, *ns* non-significant), Type III Sum of Squares

ANOVA - Fresh weight

Cases	Sum of Squares	df	Mean Square	F	p
Species	136299.317	6	22716.553	22.293	< .001**
Herbicide treatment	121761.888	7	17394.555	17.070	< .001**
Species ✻ Herbicide treatment	86709.702	42	2064.517	2.026	0.002**
Residuals	114126.353	112	1018.985		

Table 2.3 Factorial ANOVA to test the effect of the factors of Herbicide treatment, Species, and their interaction on the response variable "Biomass fresh weight". The significance level for $p < 0.05$ (** highly significant, *significant, *ns* non-significant), Type III Sum of Squares

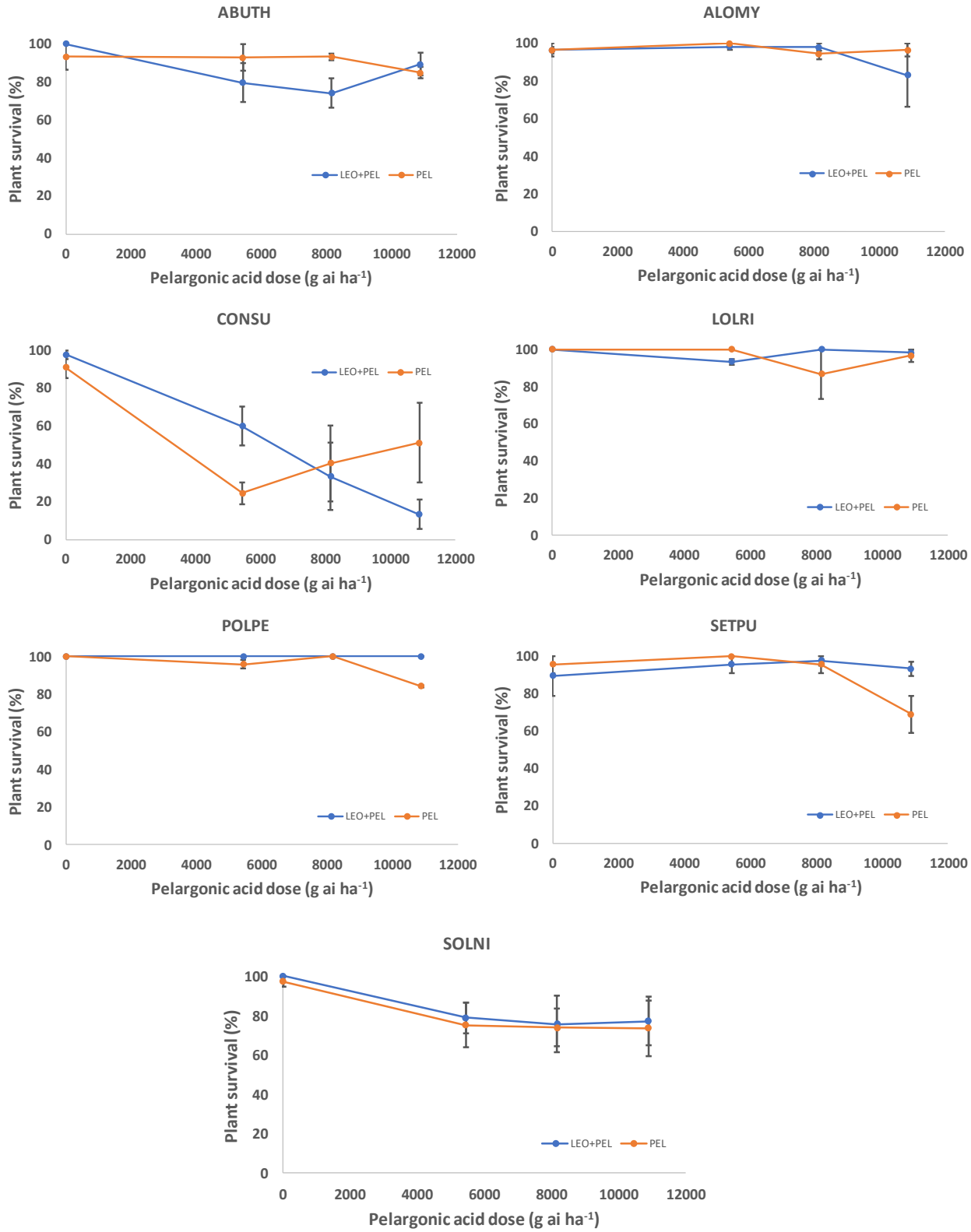

Figure 2.1 Plant survival at the different doses of pelargonic acid alone (PEL, red line) or in combination with lemon grass essential oil (LEO+PEL, blue line) for the different weed species. The dose of 0 pelargonic acid for the series LEO+PEL corresponds to the treatment with essential oil alone. Values are the mean of three replicates; bars represent standard errors.

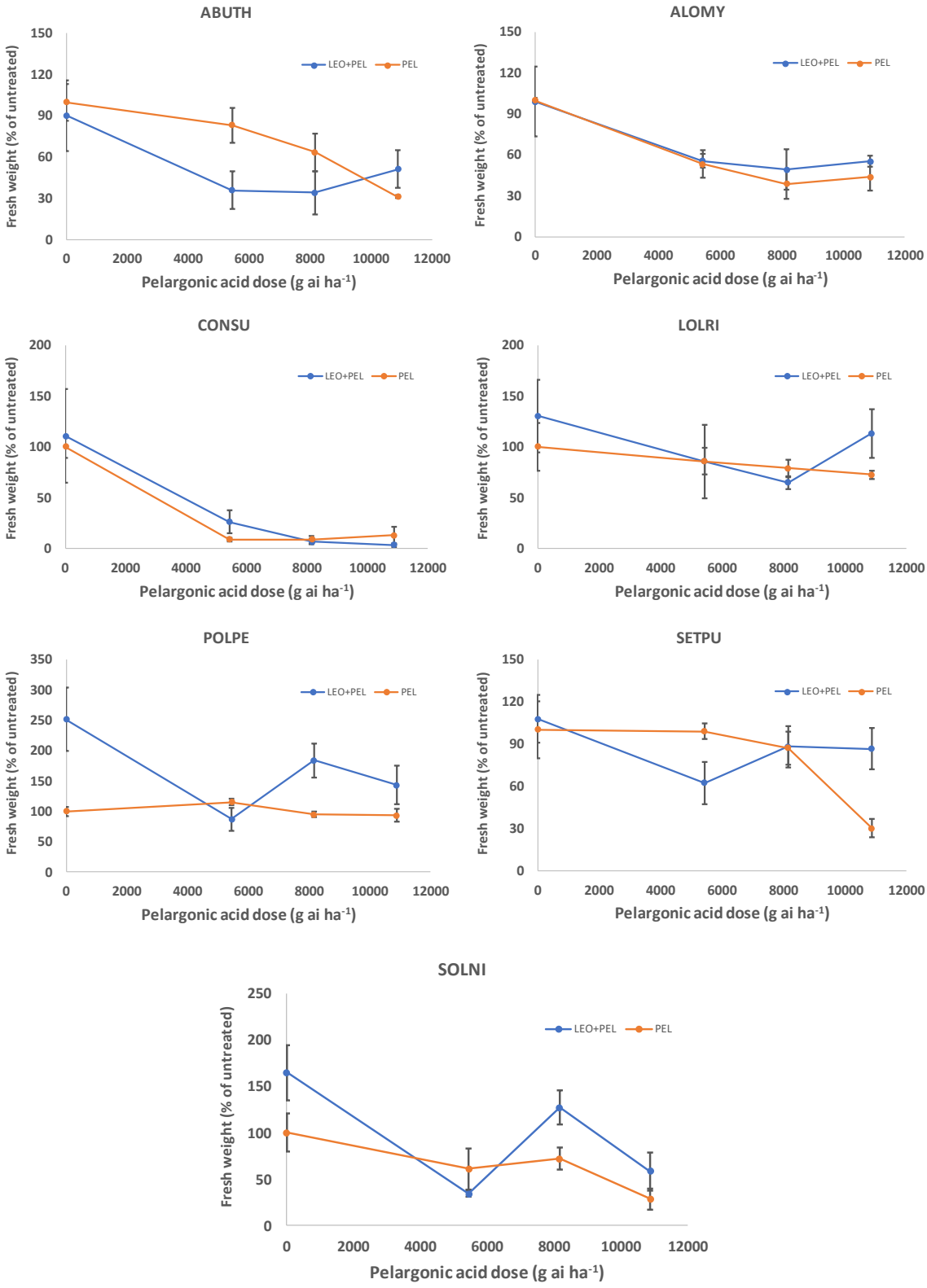

Figure 2.2 Fresh weight of biomass (expressed as % of the untreated plants) at the different doses of pelargonic acid alone (PEL, red line) or in combination with lemon grass essential oil (LEO+PEL, blue line) for the different weed species. The dose of 0 pelargonic acid for the series LEO+PEL corresponds to the treatment with essential oil alone. Values are the mean of three replicates; bars represent standard errors.

CHAPTER 3 – THIRD GREENHOUSE EXPERIMENT

The aim of this experiment was to test the influence of water availability for plants on the herbicidal efficacy of different doses of pelargonic acid and lemongrass essential oil on several weed species.

5.1 Materials and Methods

5.1.1 Plant Production

Three weed species (*C. sumatrensis, S. pumila,* and *S. nigrum*) were included in this experiment. The same seed batches were used and the same procedures for germination described for Experiment 1 were adopted. After germination 5 seedlings of similar growth stages were transplanted into plastic pots (diameter 160 mm) filled with a commercial potting mix (Ahrens Erd, HAWITA Gruppe GmbW, Vechta, Germany). Pots were transferred in the greenhouse and the soil was maintained at or near field capacity for the first days after transplant to ensure plant survival. Then for the following 2 weeks, corresponding to 1 week before and 1 week after the herbicide application, three different irrigation managements were adopted to obtain three different levels of water availability for the plants. Pots were weighed every 2-3 days to estimate water loss due to evapotranspiration that occurred from the previous measurement, then pots were irrigated according to the following treatments: 1- fully replenishment of water loss (W100), 2- 75% replenishment of water loss (W75), 3- 50% replenishment of water loss (W50). As a consequence of this methodology, differences in water content of the pots belonging to each of the three treatments progressively increased over the two weeks. After 7 days from the herbicide application, all pots were watered to field capacity and then uniform irrigation was maintained for all the pots till the end of the experiment. Light in the greenhouse was supplemented with metal halide lamps (400 W), 14 hours photoperiod, PPFD ~ 160 µmol m^{-2} s^{-1}.

5.1.2 Herbicide application and experimental design

Plants were treated with pelargonic acid (commercial Beloukha herbicide, pelargonic acid 680 g ai L^{-1}, Belchim Crop Protection Italia S.p.A, Rozzano, MI, Italy) for treatment PEL and lemongrass (*Cymbopogon flexuosus* (Nees ex Steud.) W.Watson) essential oil (Lemongrass essential oil, Aromatika Bv, Soest, The Netherlands) at 5% v/v concentration for treatment LEO. The dose of pelargonic acid adopted for this trial corresponds to the recommended field dose for crop seedbed cleaning of the commercial Beloukha herbicide, which is 16 L ha^{-1}. Lemon grass essential oil dose was chosen according to previous studies [43]. Untreated control replicates were also included for all species.
The total number of treatments was 3 (2 herbicide doses + untreated control) * 3 (weed species) * 3 (irrigation managements) = 9. Table 3.1 reports the complete list of treatments and herbicide doses.

The experimental layout was a completely randomized design with six replicates, each consisting of a pot with 5 plants, for a total of 54 pots.

Treatment	Dose of commercial herbicide L ha^{-1}	Dose of pelargonic acid g ai ha^{-1}	Dose of lemongrass essential oil L ha^{-1}
PEL16	16	10880	
LEO			10

Table 3.1 List of treatments with the corresponding herbicide and essential oil doses included in the third experiment. Essential oil doses are calculated considering a spray volume of 200 L ha^{-1}

5.1.3 Data collection and statistical analysis

Assessment of herbicide efficacy was conducted 3 weeks after treatment (3 WAT) and examined plant survival and fresh weight reduction. Plant survival was expressed as a percentage of the plants counted before the treatment in each pot. Plants were considered dead if they did not show any active growth regardless of their colour. The fresh weight of the above-ground biomass was measured for each pot and the average plant weight was estimated by dividing the total pot weight by the number of alive plants before herbicide application. To assess fresh weight reduction, the average plant weight of treated replicates was then expressed as a percentage of the mean value of the untreated plants of the same species at the irrigation management with full replenishment of water loss (W100). A value of 100% for a given replicate, therefore, means that its biomass had the same fresh weight as the mean of the untreated plants of this species at W100. Weight reduction due to herbicidal effect was compared between different irrigation managements or weed species using those relative values and not the original data.

A factorial ANOVA ($p < 0.05$) was performed using JASP software (www.jasp-stats.org) to test the effect of the factors of Herbicide treatment, irrigation management, Species, and their interactions on the response variables Plant survival, and Fresh weight. To identify significant differences between treatment means, Tukey's HSD test ($p < 0.05$) was then performed.

5.2 Results and Discussion

Effects of pelargonic acid were visible one day after application, as in the first two greenhouse experiments (Chapters 1 and 2), but the magnitude was higher both in terms of plant survival and biomass reduction for *C. sumatrensis*. However, plant survival was total (100%) for most of the treatments. Therefore, Factorial ANOVA could not be performed to test the effect of the experimental factors on plant survival because variance among replicates was too low, showing most of the replicate the same values (that is 100). On the contrary, Factorial ANOVA detected significant effects of

Herbicide treatment, Irrigation management, Species, and some of their interactions on the response variable Fresh weight (Table 3.2). Only for the interaction Species ✻ irrigation management and the third level interaction Species ✻ irrigation management ✻ Herbicide treatment no significant effects were detected. Irrigation management affected by itself biomass production, with a reduction at W50 in comparison with W100 for the three species (Figure 3.1. The biomass fresh weight of the treatment with the lowest volume of irrigation (W50 NT) was only 60, 68 and 49% of the treatment with full irrigation (W100 NT) in the case of *C. sumatrensis, S. pumila* and *S. nigrum*, respectively. However, these differences were not statistically significant even in the case of the largest observed difference between SOLNI W100 NT and SOLNI W50 NT ($p = 0.086$).

Overall, no differences were observed between plants treated with lemongrass essential oil and the untreated for the three species (Figure 3.2, 3.3, and 3.4). Similarly, no clear effect of irrigation management on herbicide efficacy was identified in the three species. On the contrary, different response to the pelargonic acid application was observed for the three species, as in the previous greenhouse experiments. *Conyza sumatrensis* was the most sensitive species (Figure 3.2), with low plant survival and low biomass fresh weight in the treated replicates across all irrigation managements (CONSU PEL W50, CONSU PEL W75, and CONSU PEL W100). *Setaria pumila* was the less sensitive species to the herbicide treatments, with only a small reduction of plant survival for treatment SETPU W75 PEL (Figure 3.3). Biomass fresh weight decreased as a consequence of pelargonic acid application, but no significant differences were detected. *Solanum nigrum* showed an intermediate level of sensitivity to pelargonic acid application, with a significant reduction of plant survival and biomass fresh weight for some treatments such as SOLNI PEL W50 and SOLNI PEL W100 (Figure 3.4).

This experiment confirmed what was observed in the previous greenhouse experiments: pelargonic acid has an herbicidal action but with temporary and species-specific effects, that is grasses are generally less sensitive while dicots with the plane, horizontal leaves are usually more affected. The lack of phytotoxic effect of the lemongrass essential oil, which is in contrast with the findings of previous studies, was also confirmed. The potential causes, such as the chemical composition of the essential oil used in this experiment or the not appropriate formulation of the product for the application of plant leaves, of this lack of effect have been already presented in the discussion of Chapter 2 so readers are recommended to refer to that section.

The different levels of pot substrate humidity due to the different irrigation managements notably affected plant growth. Water scarcity of treatment W50 lasted only 2 weeks but this was enough to reduce by 50% *S. nigrum* biomass. Plants require water to grow and thrive. When a plant is grown in a pot, it is confined to a limited volume of soil, which can dry out more quickly than soil in the ground. If a plant in a pot does not receive enough water, it can become stressed, and its growth may be

reduced. This is because the plant is not able to take up enough water and nutrients from the soil to support its normal growth and development. Water limitation can also cause the plant's leaves to wilt and its roots to become damaged, which can further reduce its growth [67], [68]. Water stress has a negative impact on several characteristics of plant physiology, including photosynthetic activity. Plant development and productivity are significantly lowered if the stress is extended [69]. According to a global meta-analysis, weed germination, growth, and seed production are all inhibited by water stress, and the quantitative response is greater as water stress levels rise [70]. Anyhow, no significant increase in herbicide efficacy was observed for the treatments under water scarcity. Plants under water limitation conditions were probably more exposed to the consequences of water loss due to herbicide damage on leaf cuticles; however, they at the same time were probably more protected from herbicide action. Water stress conditions may lead to modifications of leaf traits, such as increased leaf hairiness, increased deposition of wax on leaf cuticle, and reduction of stomatal opening, meant to limit water transpiration but that can also hurdle leaf penetration by herbicides. Weed development and growth are impacted by changes in soil water potential, which may also affect the effectiveness of herbicides [71], [72]. Foliar herbicides are applied to the leaves of plants and are typically most effective when the plants are actively growing, and the leaves are wet. High humidity can help to increase the effectiveness of foliar herbicides by keeping the leaves of the plants moist and ensuring that the herbicide is able to penetrate the leaves more easily. However, extremely high humidity can also reduce the efficacy of foliar herbicides by washing the herbicide off the leaves before it has a chance to be absorbed [73]–[75]. Several studies indeed reported higher control efficacy under conditions of high air or soil humidity for different herbicides such as vinegar [70], mesotrione [76] This result leads to interesting practical information to ensure the high herbicidal efficacy of pelargonic acid under field conditions. Firstly, field application should be performed under conditions of high relative humidity and limited solar radiation, i.e., early in the morning or late in the evening, that ensures prolonged persistence of spray droplet on the leaf surface, induce stomatal opening and consequently on the whole increase herbicide penetration and absorption. On the contrary, the application of pelargonic acid on plants under water stress conditions may reduce control efficacy.

ANOVA – Fresh weight

Cases	Sum of Squares	df	Mean Square	F	p
Species	45661.211	2	22830.605	37.777	< .001**
IM	11813.241	2	5906.621	9.774	< .001**
HT	90952.157	2	45476.079	75.248	< .001**
Species ✻ IM	2350.414	4	587.604	0.972	0.425ns
Species ✻ HT	21222.463	4	5305.616	8.779	< .001**
IM ✻ HT	13536.714	4	3384.178	5.600	< .001**
Species ✻ IM ✻ HT	3669.363	8	458.670	0.759	0.639ns
Residuals	81586.651	135	604.346		

Table 3.2 Factorial ANOVA to test the effect of the factors Herbicide treatment (HT), irrigation management (IM), Species, and their interactions on the response variable "Biomass fresh weight". Significance level for $p < 0.05$ (** highly significant, *significant, *ns* non-significant), Type III Sum of Squares

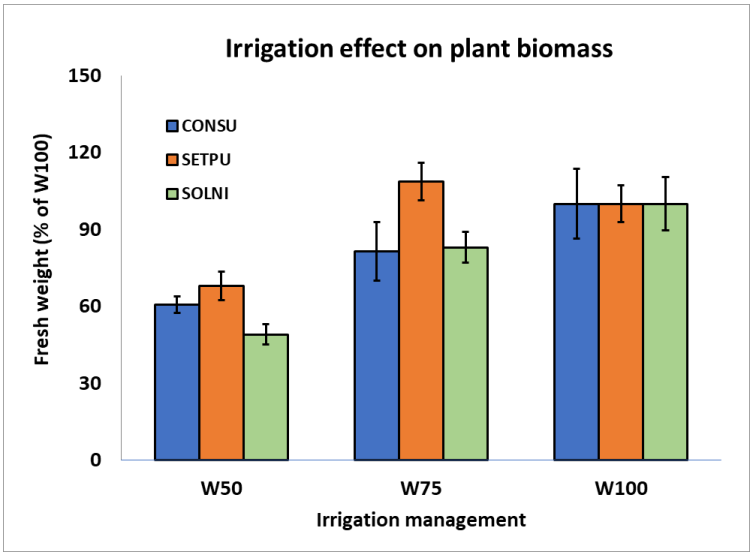

Figure 3.1 Biomass fresh weight of the untreated plants at the different irrigation managements for the different weed species (CONSU, *C. sumatrensis*, blue bar; SETPU, *S. pumila*, red bar; SOLNI, *S. nigrum*, green bar). Values are the mean of six replicates; bars represent standard errors.

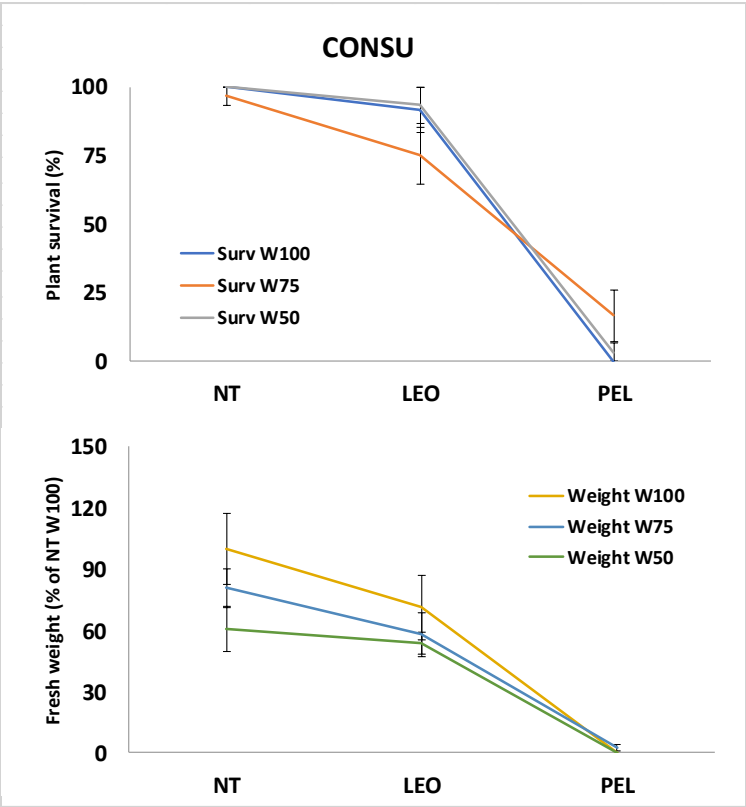

Figure 3.2 Plant survival (above) and biomass fresh weight (below) observed at the different combinations of Herbicide treatment (NT, untreated; PEL, pelargonic acid; LEO, lemongrass essential oil) and irrigation managements (W100, red line; W75, blue line; W50, green line) for *C. sumatrensis*. Values are the mean of six replicates; bars represent standard errors.

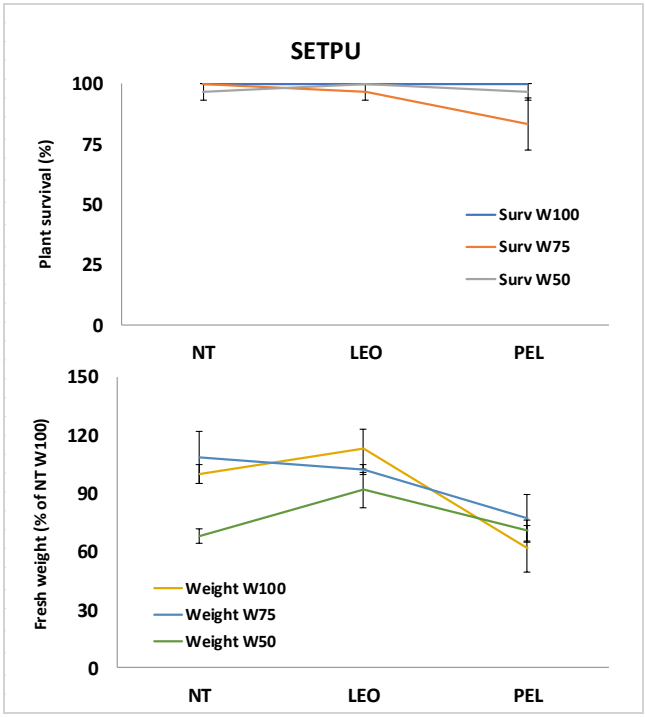

Figure 3.3 Plant survival (above) and biomass fresh weight (below) observed at the different combinations of Herbicide treatment (NT, untreated; PEL, pelargonic acid; LEO, lemongrass essential oil) and irrigation managements (W100, red line; W75, blue line; W50, green line) for S. pumila Values are the mean of six replicates; bars represent standard errors.

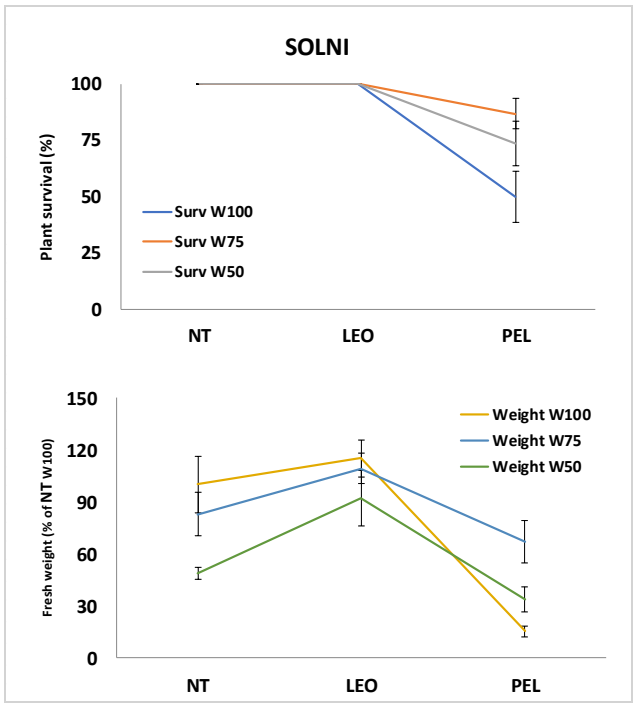

Figure 3.4 Plant survival (above) and biomass fresh weight (below) observed at the different combinations of Herbicide treatment (NT, untreated; PEL, pelargonic acid; LEO, lemongrass essential oil) and irrigation managements (W100, red line; W75, blue line; W50, green line) for S. nigrum Values are the mean of six replicates; bars represent standard errors.

6. CHAPTER 4 – FIELD EXPERIMENT

The aim of this experiment was to test the herbicidal efficacy of different doses of pelargonic acid on a natural mixed weed flora under field conditions.

6.1Materials and Methods

6.1.1 Field experiment management

A field experiment was conducted twice in spring-summer 2022 and then repeated in the following autumn to simulate the condition of pelargonic acid application for seedbed cleaning or stale seedbed technique. The experiment was set up at the experimental farm "L. Toniolo" of the University of Padova. This farm is located at Legnaro (45°21'04" N 11°57'02" E, 8 m asl) and has silt-loamy soil. Three doses of pelargonic acid (commercial Beloukha herbicide, pelargonic acid 680 g ai L^{-1}, Belchim Crop Protection Italia S.p.A, Rozzano, MI, Italy) were tested: PEL16 (pelargonic acid 10880 g ai ha^{-1}), PEL12 (pelargonic acid 8160 g ai ha^{-1}), and PEL8 (pelargonic acid 5440 g ai ha^{-1}) corresponding to 16, 12, and 8 L ha^{-1} of the commercial Beloukha herbicide, respectively. Recommended field dose of this herbicide for crop seedbed cleaning is 16 L ha^{-1}. These doses correspond to those tested in the first and second greenhouse experiments. Untreated control plots were also included. Randomized block design with 3 replicates, each consisting of a 10 m^2 plot, per treatment was adopted for both runs of a field experiment. Weather data were collected throughout the experiment from the local weather station. Tillage was performed for seedbed preparation for crop sowing and then the field was irrigated to promote weed seed germination.

6.1.2 Data collection and statistical analysis

Weed emergence and growth were monitored and weed assessment was done just before herbicide application. Weeds were identified and counted in two 30 * 30 cm quadrats per replicate. These assessments were conducted on the 25th of May, 8th of July, and 2nd of November for the first, second, and third repetition of the experiment, respectively. Pelargonic acid was applied on weeds at the initial growth stages (from 2-3 true leaves to 2 tillers), that is on the 30th of May, 12th of July, and 3rd of November for the first, second, and third repetition of the experiment, respectively. Pelargonic acid was distributed using a back-pack sprayer (MOD. 40007 Fox Sprayers; nozzle 8261036, light blue, RS 110-10.) with a spray volume of 350 L ha^{-1}. A second weed assessment was conducted 2 weeks after the herbicide application, that is 14th June 26th July, and `17th November for the first and second repetition of the experiment, to evaluate the herbicidal efficacy of pelargonic acid at different doses. Weed biomass was collected in 4 quadrats (30*30 cm) per replicate and fresh weight was measured. Biomass of the main weed species was also recorded.

A factorial ANOVA ($p < 0.05$) was first performed using JASP software (www.jasp-stats.org) to test the effect of the factor repetition and block, as random factors, and pelargonic dose, as an experimental factor, on weed biomass expressed as original data. This determined whether data from the three repetitions could be pooled and analyzed together. Otherwise, factorial ANOVA will be performed for each repetition as an individual experiment with a Completely Randomized Block Design.

6.2 Results and Discussion

6.2.1 Weather conditions

Weather conditions obviously varied between the three trials, but all trial periods had warmer and drier conditions than the average (Figure 4.1). In particular, daily air mean temperature remained around or above 20 °C throughout the spring trial with less than 20 mm of rainfall, while weather is usually milder and wetter in this season. Weather trends during the second trial were typical of summer conditions, with daily air mean temperature around 25 °C and about 40 mm of rain. Weather was instead again unusually warmer and dry during the first part of the third trial, with daily air mean temperature around 15 °C and no precipitations until the first days of November. Temperatures then decreased, with daily air mean temperature fluctuating around 10 °C till the end of the trial. Total precipitation during the third trial was lower than 40 mm.

Given the dry conditions that occurred during all the trials, sprinkler irrigation for a total of 25 mm was performed at the beginning of each trial to promote weed germination and seedling establishment.

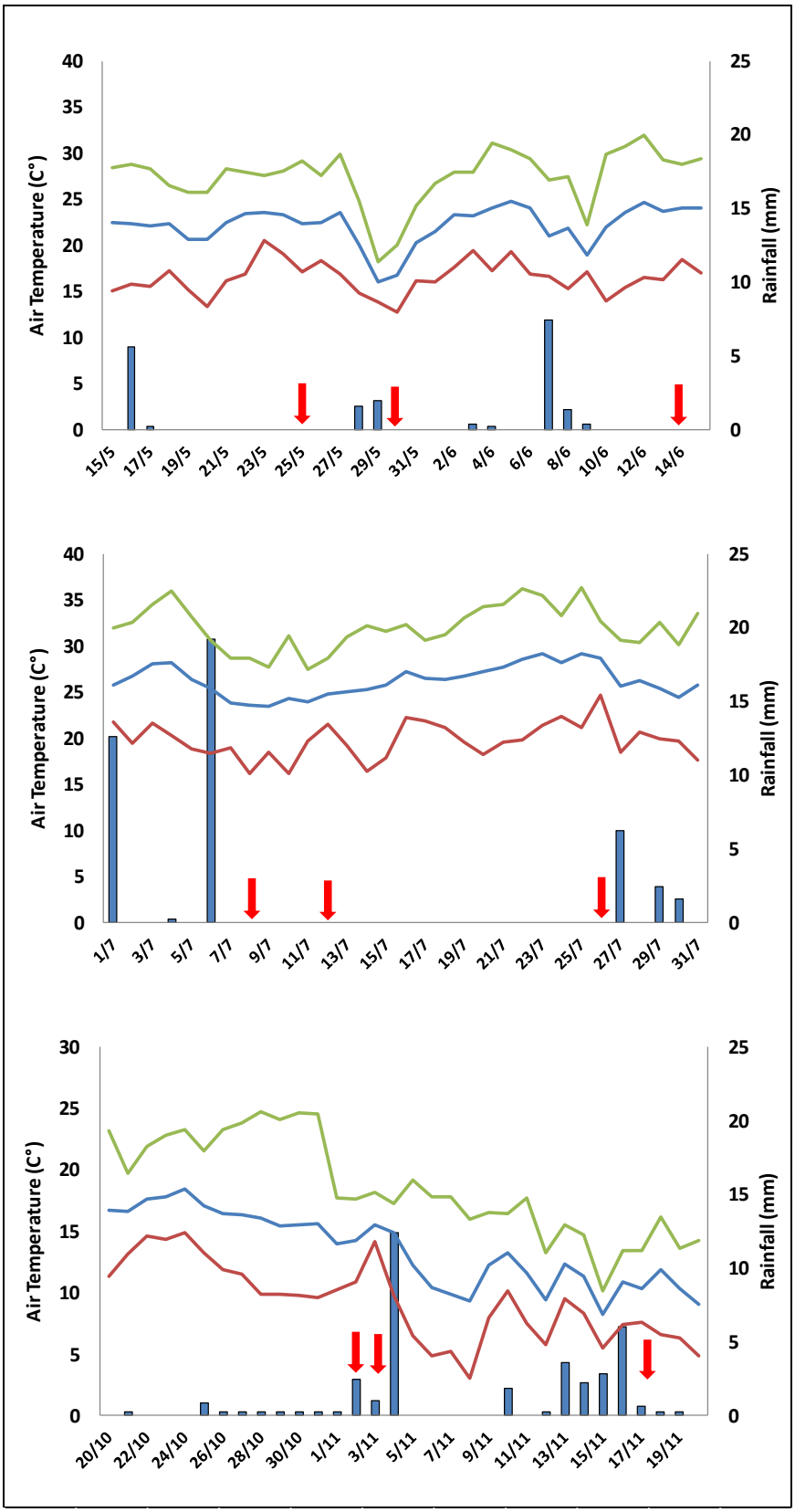

Figure 4.1 Weather conditions during the field trials (spring trial upper graph, summer trial middle graph, autumn trial lower graph). Daily air temperature (Tmax green line, Tmean blue line, Tmin red line) and rainfall (blue bar) are reported. Red arrows indicate the moments of the first weed assessment, pelargonic acid application, and second weed assessment, respectively.

6.2.2 Weed botanical composition and density

Weed flora varied across the three experiments, but also between the block of the same experiment, in terms of botanical composition and density. In the first field experiment in spring 2022, weed density ranged across the different plots between 300 and 1000 plant m^{-2} (Table 4.1). The weed community was dominated by grasses, *Digitaria sanguinalis* (L.) Scop. 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 ranged between 90 and 600 plant m^{-2} (Table 4.2). The three types of grass (*D. sanguinalis, E. crus-galli* and *S. pumila*) were still very abundant but *P. oleracea* was the dominant species this time thanks to its perfect adaptation to high summer temperatures and dry conditions. In the third experiment in autumn 2022 weed density ranged between 250 and 700 plant m^{-2} (Table 4.3). 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 abundant.

		Treatment			
		NT	**PEL8**	**PEL12**	**PEL16**
Block 1		**plant m^{-2}**			
Chenopodium album	D	16.7	11.1	11.1	5.6
Cirsium arvense	D				38.9
Digitaria sanguinalis	M	711.1	500.0	738.9	900.0
Echinochloa crus-galli	M	88.9	27.8	83.3	27.8
Portulaca oleracea	D	11.1	16.7	33.3	44.4
Setaria pumila	M	27.8	27.8	11.1	11.1
Solanum nigrum	D	5.6			
Sonchus asper	D		5.6		
Veronica persica	D			5.6	
TOTAL		**861.1**	**588.89**	**883.3**	**1027.8**

		Treatment			
		NT	**PEL8**	**PEL12**	**PEL16**
Block 2		**plant m^{-2}**			
Acalypha virginica	D			5.6	
Chenopodium album	D		5.6		
Digitaria sanguinalis	M	505.6	283.3	183.3	238.9
Echinochloa crus-galli	M	5.6	22.2	16.7	22.2
Polygonum aviculare	D	11.1			
Portulaca oleracea	D	27.8	55.6	44.4	77.8
Setaria pumila	M	5.6	5.6	33.3	5.6
Solanum nigrum	D			5.6	
Taraxacum officinale	D			5.6	
TOTAL		**555.6**	**372.2**	**294.4**	**344.4**

		Treatment			
		NT	**PEL8**	**PEL12**	**PEL16**
Block 3		**plant m^{-2}**			
Amaranthus retroflexus	D	5.6			
Anagallis arvensis	D		5.6	5.6	
Chenopodium album	D	27.8		22.2	27.8
Convolvulus arvensis	D	5.6		11.1	11.1
Digitaria sanguinalis	M	505.6	461.1	416.7	338.9
Echinochloa crus-galli	M	38.9	16.7	33.3	38.9
Euphorbia helioscopia	D				5.6
Oxalis acetosella	D				5.6
Portulaca oleracea	D	38.9	83.3		44.4
Setaria pumila	M	16.7	11.1		11.1
Solanum nigrum	D				5.6
Sonchus asper	D	5.6			5.6
Sorghum halepense (seed)	M	5.6	11.1	5.6	
Sorghum halepense (rhizome)	M		11.1	5.6	
TOTAL		**650.0**	**600.0**	**500.0**	**494.4**

Table 4.1 Botanical composition and density (as plant m^{-2}) in the different treatment plots and blocks in the first field trial (spring 2022). M and D stand for Monocots and Dicots, respectively.

		Treatment			
		NT	**PEL8**	**PEL12**	**PEL16**
Block 1			**plant m^{-2}**		
Chenopodium album	D		11.11	11.1	
Digitaria sanguinalis	M	55.6	155.6	244.4	77.8
Echinochloa crus-galli	M	33.3			33.3
Portulaca oleracea	D	22.2	66.7	188.9	22.2
Setaria pumila	M		11.1		
Sorghum halepense (seed)	M	11.1			
TOTAL		**122.2**	**244.4**	**444.4**	**133.3**
		Treatment			
Block 2		**NT**	**PEL8**	**PEL12**	**PEL16**
Digitaria sanguinalis	M	211.1	66.7	44.4	155.6
Portulaca oleracea	D	77.8	33.3	44.4	22.2
Setaria pumila	M	55.6			
TOTAL		**344.4**	**100.0**	**88.9**	**177.8**
		Treatment			
Block 3		**NT**	**PEL8**	**PEL12**	**PEL16**
Amaranthus retroflexus	D		22.2		
Chenopodium album	D			22.2	22.2
Digitaria sanguinalis	M		244.4	44.4	222.2
Echinochloa crus-galli	M	44.4	33.3		
Portulaca oleracea	D	344.4	11.1	444.4	55.6
Setaria pumila	M		44.4	44.4	44.4
Sorghum halepense (rhizome)	M	22.2			
TOTAL		**611.1**	**355.6**	**555.6**	**344.4**

Table 4.2 Botanical composition and density (as plant m^{-2}) in the different treatment plots and blocks in the second field trial (summer 2022). M and D stand for Monocots and Dicots, respectively.

		Treatment			
		NT	**PEL8**	**PEL12**	**PEL16**
Block 1				**plant m^{-2}**	
Amaranthus retroflexus	D	26.7		5.6	
Capsella bursa-pastoris	D	106.7	11.1	44.4	55.6
Cerastium holosteoides	D	13.3	5.6	22.2	
Chenopodium album	D			11.1	
Digitaria sanguinalis	M	33.3	27.8	22.2	5.6
Echinochloa crus-galli	M	453.3	211.1	250.0	422.2
Lamium purpureum	D	60.0	5.6	38.9	11.1
Plantago lanceolata	D				5.6
Portulaca oleracea	D	53.3		33.3	11.1
Sonchus asper	D		5.6	11.1	5.6
Sorghum halepense (seed)	M	6.7	11.1	5.6	
Stellaria media	D	6.7	16.7	16.7	
TOTAL		**760.0**	**294.4**	**461.1**	**516.7**

		Treatment			
		NT	**PEL8**	**PEL12**	**PEL16**
Block 2				**plant m^{-2}**	
Amaranthus retroflexus	D			6.7	
Capsella bursa-pastoris	D	38.9	38.9	46.7	13.3
Cardamine hirsuta	D		5.6	6.7	13.3
Cerastium holosteoides	D	5.6			6.7
Chenopodium album	D	16.7			13.3
Digitaria sanguinalis	M		22.2	33.3	26.7
Echinochloa crus-galli	M	238.9	194.4	160.0	366.7
Lamium purpureum	D		16.7	46.7	6.7
Picris hieracioides	D	5.6	5.6		
Poa annua	M	11.1			
Portulaca oleracea	D		22.2	13.3	
Sonchus asper	D	5.6	22.2		
Sorghum halepense (rhizome)	M			20.0	6.7
Sorghum halepense (seed)	M	77.8			20.0
Stellaria media	D			13.3	
Taraxacum officinale	D	11.1			
TOTAL		**411.1**	**327.8**	**346.7**	**473.3**

		Treatment			
		NT	**PEL8**	**PEL12**	**PEL16**
Block 3			**plant m^{-2}**		
Amaranthus retroflexus	D		5.6		11.1
Capsella bursa-pastoris	D	33.3	55.6	5.6	33.3
Cardamine hirsuta	D	11.1			
Cerastium holosteoides	D			11.1	
Chenopodium album	D	38.9	5.6	38.9	33.3
Convolvulus arvensis	D		5.6		50.0
Crepis vesicaria	D			11.1	
Digitaria sanguinalis	M	5.6	5.6		11.1
Echinochloa crus-galli	M	238.9	127.8	100.0	177.8
Lamium purpureum	D		5.6	16.7	
Lolium multiflorum	M			5.6	
Papaver rhoeas	D	5.6			
Picris hieracioides	D	11.1			5.6
Poa annua	M	5.6	5.6		5.6
Portulaca oleracea	D			11.1	
Setaria pumila	M				5.6
Solanum nigrum	D				5.6
Sonchus asper	D	5.6			
Sorghum halepense (rhizome)	M		5.6		11.1
Sorghum halepense (seed)	M		5.6	22.2	16.7
Stellaria media	D	105.6	22.2	77.8	16.7
Veronica persica	D	5.6			5.6
TOTAL		**466.7**	**250.0**	**300.0**	**388.9**

Table 4.3 Botanical composition and density (as plant m^{-2}) in the different treatment plots and blocks in the third field trial (autumn 2022). M and D stand for Monocots and Dicots, respectively.

6.2.3 Effect of pelargonic acid on weeds

As already observed in the greenhouse trials, phytotoxic effects of pelargonic acid were visible short after its application, but many weed plants recovered from those symptoms so total control was not achieved in any plots (Figure 4.2). A gradient of sensitivity to pelargonic acid was observed across the different weed species in all three trials, even if the experimental design did not enable to draw of appropriate statistical inferences. Among grass weeds, *D. sanguinalis* seemed more sensitive to pelargonic acid than *S. pumila* or *E. crus-galli* since its plants turned brownish and stopped their growth after the herbicide application while the other grasses showed little symptoms (Figure 4.3). Poor herbicidal effect of pelargonic acid was observed also against *P. oleracea* with limited and temporary phytotoxic symptoms such as small circular lesions on leaves (Figure 4.4) so treated plants usually recovered and showed no biomass reduction in comparison with the untreated a few days after the treatment.

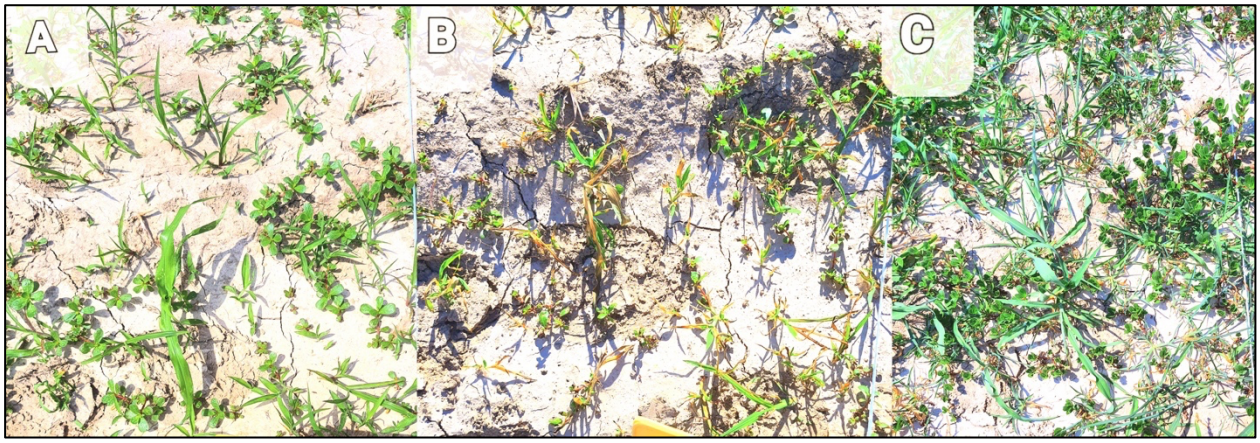

Figure 4.2 Plant's response to pelargonic acid A. plants before treatment B. plants one day after treatment, C. plants 10 Days after treatment

Figure 4.3 Plants of *D. sanguinalis* with more intense symptoms (brownish or necrotic leaves) after the application of pelargonic acid in comparison with the nearby plants of *S. pumila* or *E. crus-galli*

Figure 4.4 Plants of *P. oleracea* with light symptoms (small circular necrotic lesions) after the application of pelargonic acid in comparison with the nearby plants of *S. pumila* or *D. sanguinalis*

The first factorial ANOVA ($p < 0.05$) identified a significant effect of the factor repetition (Table 4.4), the results of the single trials were therefore analyzed separately as a Completely Randomized Block Design.

Cases	Sum of Squares	df	Mean Square	F	p
P Dose	$3.001\times10^{+6}$	3	$1.000\times10^{+6}$	8.874	0.002**
Rep	$1.769\times10^{+7}$	2	$8.844\times10^{+6}$	78.464	<0.001**
P Dose ∗ Rep	$4.051\times10^{+6}$	6	675118.538	5.990	0.004**
Block	$2.725\times10^{+6}$	2	$1.363\times10^{+6}$	12.090	0.001**
P Dose ∗ Block	$1.683\times10^{+6}$	6	280446.447	2.488	0.084^{ns}
Rep ∗ Block	$4.270\times10^{+6}$	4	$1.068\times10^{+6}$	9.471	0.001**
Residuals	$1.353\times10^{+6}$	12	112709.605		

Table 4.4 Factorial ANOVA to test the effect of the factors pelargonic dose (P Dose), repetition (Rep) and block and their interaction on the response variable "Weed Biomass". Significance level for $p < 0.05$ (** highly significant, *significant, *ns* non-significant), Type III Sum of Squares

A significant effect ($F = 5.791$, $p = 0.033$) of the pelargonic acid dose (P Dose) on weed biomass was detected for the first trial; however, significant differences from the untreated (NT) were detected only for the treatment with the highest dose of pelargonic acid (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 indeed around 2000-2500 g m^{-2}, while it was slightly above 600 g m^{-2} for treatment PEL 16 (Figure 4.5). Grasses were the dominant group of weeds, accounting for more than 70% of total biomass across all treatments. No significant effect ($F = 2.809$, $p = 0.108$) of the pelargonic acid dose (P Dose) on weed biomass was detected for the second trial, with the value of the fresh weight of weed biomass ranging from around 500 g m^{-2} for PEL8 to almost 1300 g m^{-2} for PEL12, respectively. It is worth mentioning that plots of the treatment with the intermediate of pelargonic acid (PEL12), which had the highest value of weed biomass, were colonized by a large number of *P. oleracea* plants (Table 4.2). The density of this weed was particularly high in Block 2 and 3 plots, with above 180 and 400 plant m^{-2}, respectively. No significant effect ($F = 2.228$, $p = 0.186$) of the pelargonic acid dose (P Dose) on weed biomass was detected for the third trial; however, values of fresh weight of weed biomass of treatments with pelargonic acid (PEL8, PEL12, and PEL16) were less than a half (approximately 55-65 g m^{-2}) than the untreated (approximately 160 g m^{-2}). It is interesting to underline that weed biomass in the autumn trial was on the overall a 10-fold lower than in the spring and summer trials.

The weed control level achieved with the application of pelargonic acid varied among the trials, anyhow it was on the overall only partial, confirming what was observed in the greenhouse experiments (Chapter 1-3) but also what was already reported in previous field experiments conducted on spontaneous weed flora [48], [52], [77]. The botanical composition of weed flora could be a relevant factor in determining the level of weed control achievable with a pelargonic acid application, given that remarkable inter-specific differences in the sensitivity to this herbicide have been largely described [41], [42], [77]. Pannacci et al [77] reported large variations in the sensitivity to pelargonic acid, expressed as ED_{50} value, between the most (*Kickxia spuria* (L.) Dumort., ED_{50} = 2600 g ai ha^{-1}, *E. crus-galli*, ED_{50} = 3400 g ai ha^{-1}) and the least sensitive species (*P. oleracea*, ED_{50} > 18700 g ai ha^{-1}, *Lolium multiflorum* Lam., ED_{50} > 21800 g ai ha^{-1}) in their field studies. Contrasting control levels are therefore expectable in the case of weed communities dominated by sensitive or tolerant species and this was observed also in the present experiment. The dominant weed species in the spring trial was *D. sanguinalis* that seemed even more sensitive to pelargonic acid than *E. crus-galli* and relevant weed biomass reduction was obtained with the application of the highest herbicide dose. On the contrary, *P. oleracea* was the dominant species in many plots of the summer trial, and poor control level was observed in those areas. Finally, *E. crus-galli* was the dominant species in the autumn trial, and on overall large weed biomass reduction was achieved with the application of pelargonic acid.

Weather conditions during field trials could have been another important factor affecting the efficacy of pelargonic acid in different ways. Dry, hot conditions such as those occurring in the spring and summer trials could have promoted drought-tolerance traits on weed leaves, such as increased deposition of wax in the cuticle, increased leaf hairiness, and limited stomatal opening. Those traits also hinder herbicide leaf penetration and adsorption, leading to lower herbicide sensitivity. Besides, dry, hot weather conditions at the moment of field application can further decrease herbicide efficacy by lessening the persistence of spray droplets on the leaf surface and at the same time reducing stomatal opening. This can limit herbicide penetration, adsorption, and consequently its efficacy, as already reported for vinegar-based herbicide with the same mode of action of pelargonic acid [70]. Thus, it can be supposed that the combination of weather effect on weed sensitivity and herbicide leaf penetration had reduced the control efficacy of pelargonic acid in the spring and summer trials, leading to unsatisfactory control levels, particularly in the case of the summer trial due to the massive presence of the *P. oleracea* that is highly tolerant to pelargonic acid.

To conclude, it should be assumed that weed control level obtained with field application of pelargonic acid can significantly vary on the base of the botanical composition of weed communities and environmental conditions. Reducing field doses of pelargonic acid would increase the variability and uncertainty of weed control level and it does not seem a widely recommendable practice.

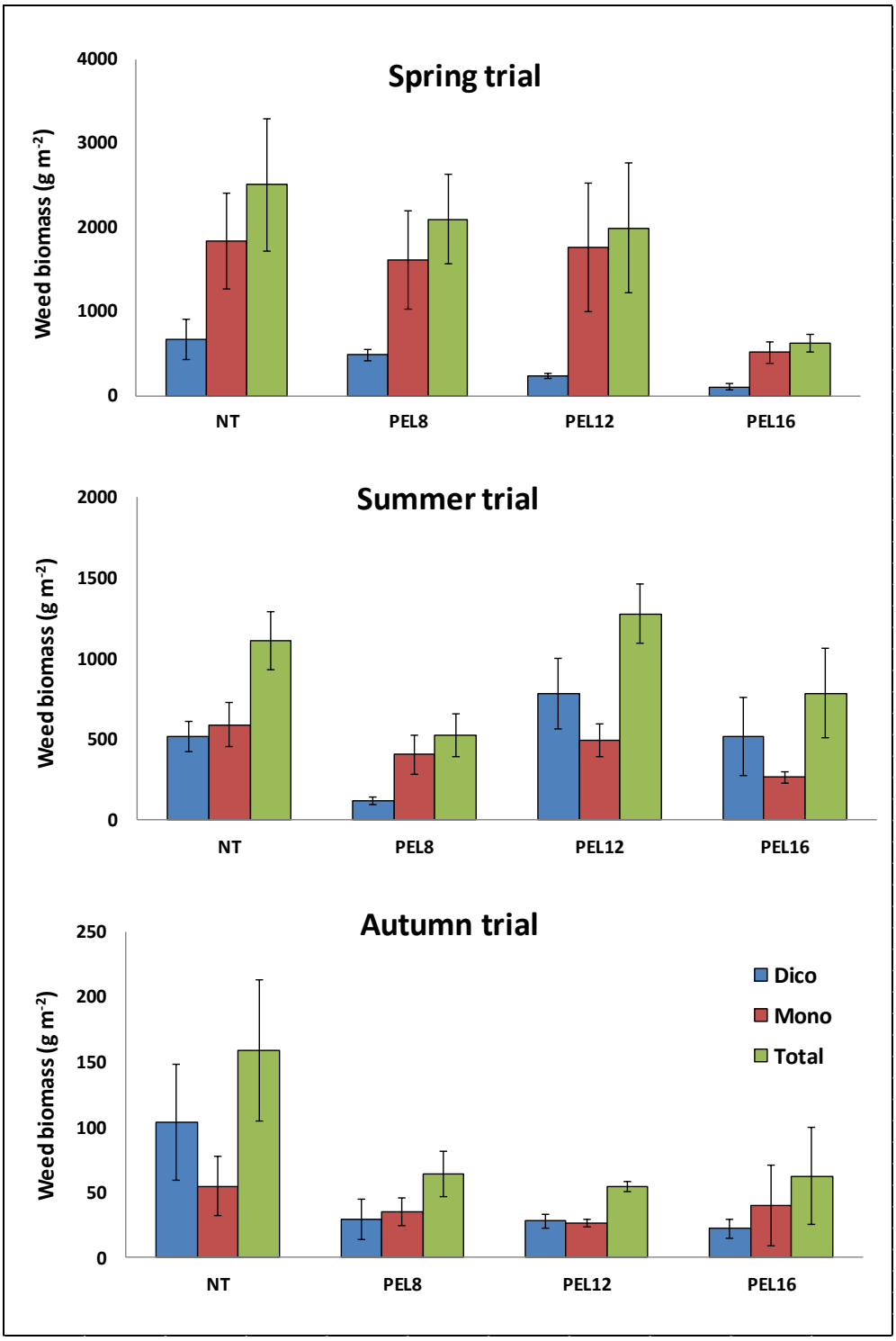

Figure 4.5 Weed biomass measured for the different treatments in the three trials. Biomass of Monocots species (blue bar), Dicots species (red bar), and Total biomass (green bar) are reported. Values are the mean of three replicates, bars represent standard error.

7. CONCLUSION

Synthetic chemical herbicides have been key tools in weed management strategies for decades, providing important economic and operational benefits [78] but a significant reduction in their use is expectable in the coming years for many different factors spanning from the evolution of herbicide resistance to the lack of discovery of new modes of action or the more and more restrictive regulations in herbicide registration and use [19]. Great interest has therefore arisen to identify non-synthetic alternatives for weed control and remarkable research efforts have been directed to evaluate natural products, such as organic acids or plant essential oils, as potential herbicides. Despite all these studies, few natural products have reached the final stage of being available on the market for professional users in the EU. Pelargonic acid is probably among the most successful cases, with several commercial products registered for use on different crops and non-agricultural areas. However, its high cost is currently limiting the widespread adoption, so it is interesting to test the efficacy of reduced doses for specific uses as in the case of stale seedbed or seedbed cleaning. Moreover, although good herbicidal efficacy has been obtained with pelargonic acid in pot or greenhouse experiments [23], [39], [40], contrasting and often erratic results with large inter-specific differences of sensitivity have been reported when used under field conditions [41], [46], [48], [52].

One of the main aims of the present study was therefore to assess the efficacy of different doses of pelargonic acid on several weed species with contrasting morphological traits. The different greenhouse and field experiments produced consistent findings in agreement with previous studies conducted with similar methodologies, that is that pelargonic acid achieved only partial and temporary control with large species-specific variability. Grass weeds, in particular *A. myosuroides* and *L. rigidum*, were more tolerant to pelargonic acid, with no mortality and limited biomass reduction even at the highest application doses. The large difference in sensitivity was observed also among dicots weeds, with *P. oleracea* and *A. theophrasti* being more tolerant than *C. sumatrensis* and *S. nigrum*, on the base of specific leaf traits as leaf angle, leaf hairiness, or wax layer on the cuticle. Reducing the doses of pelargonic acid caused a decrease in control level with amplification of inter-specific variability in both greenhouse and field trials, therefore it does not seem a feasible practice in the case of broadcast field application. In order to reduce the dose of pelargonic acid per hectare and consequently the corresponding cost, tactics successfully tested with other herbicides, such as band application along crop rows [79] or patch-spraying [80], seem more promising and practicable since they allow to maintain a high herbicide dose in the sprayed areas. Environmental conditions have been proven to affect the herbicidal efficacy of pelargonic acid in different simultaneous ways. Hot and dry conditions can indeed promote weeds leaf traits, such as hairiness or wax deposit on the cuticle,

that reduce sensitivity to pelargonic acid. Moreover, those environmental conditions reduce the persistence of spray droplets on the leaf surface, limiting herbicide penetration inside weed leaves and consequently hindering its efficacy. 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, therefore, relevant to maximize pelargonic acid efficacy. To conclude, it should be assumed that weed control level obtained with field application of pelargonic acid can significantly vary on the basis of the botanical composition of weed communities and environmental conditions; anyhow, full and persistent weed control is hardly achievable with the sole application of this product. Pelargonic acid can therefore be a valuable tool for specific uses, such as stale seedbed technique, within multi-tactics weed management strategies, while it does not seem reliable as a stand-alone weed control tactic.

No clear herbicidal or phytotoxic activity was observed for lemongrass essential oil in this study, in disagreement with the findings of previous studies [39]. Differences in the chemical composition of the products used in the various experiments cannot be excluded, given that none of those products was a commercial herbicide with a standard composition and formulation. Since intra-specific variability of the plants and differences in environmental growing conditions can alter the chemical composition of plant essential oil, chemical analysis of the specific lemongrass essential oil used in each experiment would be necessary to enable proper comparison. Moreover, the commercial product used in the present experiment did not contain any specific co-formulants or adjuvants to ensure sufficient leaf penetration. This could have limited the phytotoxic effect of lemongrass essential oil in the present experiment and further efforts should be directed to improve the formulation of essential oil-based herbicides and find adequate adjuvants. Studies have been conducted in the past years to test encapsulation with natural polymers of different origins or with specific nanoparticles to improve the shelf-life and field efficacy of essential oils or other natural chemicals [81], [82]. Anyhow, despite all those efforts no commercial herbicide based on essential oils have been developed till full marketability in Europe. Similarly, herbicides based on lemongrass essential oil are still undergoing the first steps of technological development, so no commercial product could be reasonably expected from a few years perspective.

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