The herbicidal potential of Pelargonic Acid to control *Cirsium arvense* (L.) Scop. in relation to the timing of application and the application volume

Die Wirkung von Pelargonsäure zur Kontrolle von Cirsium arvense (L.) Scop. in Abhängigkeit von Applikationszeitpunkt und -volumen

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Abstract

Pelargonic Acid (PA) is a naturally occurring fatty acid that is used as a bio-based herbicide to control both annual and perennial weeds. In this particular study, we investigated the effects of influencing factors on the efficacy of PA on *Cirsium arvense* (L.) Scop. under semi-field and greenhouse conditions. For this purpose, we examined the effects of different timings, application volumes, and adjuvants of PA to control *C. arvense*. Two application volumes (10.9 kg PA at 200 L and 400 L water/ha), and five plant growth stages were tested in a semi-field experiment. In a greenhouse experiment, Paraffin oil, as an adjuvant, was added to both PA application volumes to evaluate the possible increase in PA herbicidal efficacy.

Our results showed that 10.9 kg PA in the volume of 400 L water/ha improved the PA efficacy. Paraffin oil, as an adjuvant, can enhance PA efficacy. The most effective times to control using PA were early-elongation and the seven-to-ten leaf stages for *C. arvense*.

We conclude that 10.9 kg PA with an application volume of 400 L/ha and added adjuvant is a mixture with a herbicidal potential when applied at an earlier stage of *C. arvense.*

Keywords: bio-based herbicide, *Cirsium arvense*, pelargonic acid, perennial weed

Zusammenfassung

Die natürlich vorkommende Fettsäure Pelargonsäure (PA) wird als biobasiertes Herbizid zur Kontrolle einjähriger und ausdauernder Unkräuter und Ungräser eingesetzt. In der vorliegenden Studie werden die Einflussfaktoren Applikationszeitpunkt, Konzentration und Einsatz von Additiven auf die Wirkung von PA zur Kontrolle von *Cirsium arvense* (L.) Scop. getestet. Die Wirkung der PA-Konzentration (10.9 kg PA in 200 l Wasser und 10.9 kg PA in 400 l Wasser/ha) wurden in fünf phänologischen Entwicklungsstadien im Halbfreiland untersucht. Der Zusatz von Paraffin-Öl zu den zwei PA-Konzentrationen wurde im Gewächshaus geprüft.

Unsere Ergebnisse bestätigen die höhere Wirkung der Konzentration 10.9 kg PA in 400 l Wasser/ha zur *C. arvense* Kontrolle. Der Zusatz von Paraffin-Öl erhöhte die Wirkung der PA-Konzentration 10.9 kg PA in 400 l/ha Wasser zusätzlich. Die effektivste Kontrolle durch den Einsatz von PA konnte in den frühen Entwicklungsstadien der Pflanzen erzielt werden.

Wir schlussfolgern, dass Pelargonsäure in der Konzentration 10.9 kg PA in 400 l Wasser/ha und der Zugabe des Additivs ein herbizides Potential hat, wenn es in frühen Entwicklungsstadien von *C. arvense* appliziert wird.

Stichwörter: Biobasiertes Herbizid, *Cirsium arvense*, mehrjähriges Unkraut, Pelargonsäure

Introduction

Cirsium arvense known as creeping thistle is a perennial weed with a creeping root system that is difficult to control in all kinds of farming systems with reduced or no reliance on herbicides (BICKSLER & MASIUNAS, 2009). In recent years, there has been a growing interest in implementing more environmental-friendly practices towards weed management. Bio-based herbicides are alternatives for weed control (ABOUZIENA & HAGAAG, 2016). Since 2015, following the authorization of the active ingredient pelargonic acid, one bio-based herbicide (Beloukha®) is registered within the EU for defoliation (CORDEAU et al., 2016). Pelargonic Acid (PA) is a non-selective and broad-spectrum contact herbicide. Its herbicidal activity is a rapid membrane dysfunction resulting from an intracellular pH-reduction that causes loss of membrane integrity and, finally, cell death. The phytotoxic effects and necrotic lesions are visible within a short time after treatment (TRAVLOS et al., 2020). Yet, it has so far not been tested on controlling perennials.
Previous studies on herbicidal effects of PA recommend further investigation on the improvement of PA efficacy (MUÑOZ et al., 2020; TRAVLOS et al., 2020). Various factors such as growth stage of weeds at the time of herbicide application, carrier volume and, adjuvants affect herbicide efficacy (KNOCHE, 1994; KIELOCH & DOMARADZKI, 2011). Depending on the type of herbicide, increasing carrier volume leads to improved herbicide efficacy (KNOCHE, 1994; BOLAT et al., 2018). Adding adjuvant, e.g., paraffin oil, is commonly used to improve the herbicide performance (BURROUGHS et al., 1999). According to MCWHORTER et al. (1992), droplets of herbicide applied with paraffin oil spread 100 times greater than when the herbicide is applied alone. As PA is an emulsifiable contact herbicide with a hydrophobic characteristic (KEGLEY et al., 2010), a potential exists for paraffin oil to enhance the PA performance by reducing surface tension and spreading droplets on the leaf surface.
The specific objectives of this study are to determine whether (1) the efficacy of PA on *C. arvense* enhances by increasing the application volume, (2) the growth stage of *C. arvense* at application time affects PA efficacy and (3) paraffin oil increases the PA efficacy on this weed.

Material and methods

Experimental setup

This study contains two experiments that were carried out at the University of Rostock. In December 2019, creeping roots of *C. arvense* were collected from Rostock university field and planted and kept in culture by propagation in pots at the greenhouse. For both experiments, roots of >3 mm in diameter were cut into pieces of 5 cm length and were planted directly at 5 cm soil depth, one piece each pot. Pot size in the first experiment was 12 L and in the second 4 L. The selection of various pot sizes was due to the final plant size, plant growth, and experiment duration. The soil mixture was ½ field soil, ¼ potting soil, and ¼ compost. In this research, Beloukha® (680 g/L of pelargonic acid) was used as PA and Promanal HP® (830 g/L of paraffin oil) used as Paraffin oil. Promanal HP® is registered as plant protection product in Germany (Register of Plant Protection Products, 2021). We used Promanal HP® due to the high content of paraffin oil per liter.

Experiment 1

In spring 2020, a factorial pot experiment with four replicates was set up under semi-field conditions to examine the effects of application volume and growth stage on PA efficacy. Roots of *C. arvense* were planted at five different dates with ten-day intervals to have five growth stages at the application date. Treatments were applied using a plot spraying device with a pressure of 2.1 bar and speed of 4 kilometres per hour. Flat jet nozzles size 02 and 04, respectively, were used for 200 L and 400 L application volume. The treatments are given in Table 1.

Experiment 2

To test the improvement of PA efficacy on the best growth stage by increasing the application volume and adding an adjuvant, the second experiment was designed as a completely randomized pot experiment with six replicates. This experiment was conducted in November 2020 and repeated in February 2021 under greenhouse conditions. Plants were at the seven-to-ten leaf stage at the application date. Treatments were applied using a stationary application system with a speed of 0.675 km/h at a pressure of 1.8 bar. The treatments are given in Table 1.

Assessments

Above and below ground dry biomass were measured. Percent reduction of dry weight compared to the untreated control plants was defined as the efficacy of the treatments, and the calculation was as follow (JAVID & TANVEER, 2013):

$$Herbicide\ Efficacy(\%) = \frac{dry\ biomass\ of\ untreated\ control - dry\ biomass\ of\ treated\ plant}{dry\ biomass\ of\ untreated\ control} \times 100$$

In experiment 2, the growth parameters such as shoot height were measured at 1, 3, 7, 14 and 21 days after treatment (DAT).

Table 1 Treatments in Experiments 1 and 2

Tabelle 1 *Versuchsglieder in Experiment 1 und 2*

Experiment 1	**Experiment 2**
Growth stage	**Adjuvant**
Early-development = 17 < BBCH	PR = 6.2 kg/ha Paraffin oil
Seven-to-ten-leaf = 17 ≤ BBCH < 21	PA2 = 10.9 kg/ha Pelargonic Acid in 200 L Water
Side-shoot-formation = 21 ≤ BBCH < 30	PA4 = 10.9 kg/ha Pelargonic Acid in 400 L Water
Early-elongation = 30 ≤ BBCH < 38	PA2_PR = PA2 + PR
Late-elongation = 38 ≤ BBCH ≤ 45	PA4_PR = PA4 + PR
	Ctrl = Untreated Control
Application volume	
Untreated control	
PA200 = 10.9 kg PA in 200 L/ha water	
PA400 = 10.9 kg PA in 400 L/ha water	

Statistical analysis

Linear mixed models were used in experiment 1 analysis with two factors (application volume, growth stage). In this analysis, two factors (Growth-stage with five levels and Application volume with two levels) and their interaction were fixed effects. Replicates were random effects. To analyze Experiment 2, LMMs were fitted, considering herbicide treatments as fixed effects and replicates of each treatment and repetition of the whole experiment as random effects. We performed all statistical analysis in R version 4.0.4 (R CORE TEAM, 2021). R-package lme4 was used to run the Linear Mixed Model analysis (LMMs).

Results and Discussion

Experiment 1

Linear Mixed Model analysis on the efficacy of PA with different application volumes showed that the fixed effect of application volume on both shoot and root biomass was significant (Tab. 2).

The highest efficacy on the root biomass (72%) and shoot biomass (65%) was obtained from PA400 at the early-elongation stage. For the late-elongation and seven-to-ten-leaf stages, the PA400 efficacy on shoot biomass was respectively 56% and 58%. It was 61% on root biomass for both growth stages. There was a significant difference between growth stages for PA400 efficacy on shoot and root biomass (p<0.05). The PA200 efficacy on roots was 17% to 46% (early-elongation stage) and 14% to 47% on shoots (early-elongation stage). However, the growth stages were not significantly different from each other in the PA200 efficacy on root biomass (Tab. 2).

Table 2 Linear Mixed Models': Efficacy of PA400 and PA200 on the root and shoot biomass of five growth stages of *Cirsium arvense.*

Tabelle 2 *Lineare Gemischte Modelle: Wirksamkeit der Versuchsglieder PA400 und PA200 auf die Wurzel- und Sprossbiomasse von fünf Wachstumsstadien von* Cirsium arvense*.*

		Efficacy on root biomass (%)			**Efficacy shoot biomass (%)**	
	Growth stage	**Mean**	**Pr(>|t|)**		**Mean**	**Pr(>|t|)**
Application volume (PA200)	Late-elongation	36.8	9.55e-05 ***		15.6	9.82e-03 **
	Early-elongation	42.5	6.19e-01		46.8	6.50e-04 ***
	Side-shoot-formation	25.6	3.34e-01		16.7	9.38e-01
	Seven-to-ten-leaf	46.4	4.06e-01		43.4	1.75e-03 **
	Early-development	17.4	1.006e-01		14.1	8.49-01
Application volume (PA400)	Late-elongation	61.5	3.87e-02*		56.1	1.26e-05 ***
	Early-elongation	71.8	3.70e-01		64.8	2.87e-01
	Side-shoot-formation	29.6	9.30e-03 **		22.5	4.26e-02 *
	Seven-to-ten-leaf	61.1	9.73e-01		58.5	7.76e-01
	Early-development	15.0	3.56e-04 ***		17.9	1.57e-04 ***

Significance codes: *P < 0.05, **P < 0.01 and ***P < 0.001.
For both application volumes, the p values for each growth stage were compared to the growth stage Late-Elongation (intercept of LMMs)

Results of Experiment 1 showed that increasing the water volume improved the PA efficacy on *C. arvense*. In the seven-to-ten leaf stage, PA with higher application volume caused approximately 15% more reduction on both shoot and root biomass. It enhanced the PA efficacy up to 30% and 40% on root and shoot biomass, respectively, for early- and late-elongation stages.

According to our research, early-elongation stage exhibited the most constant and highest efficacy of herbicides. This means that *C. arvense* is vulnerable at early-elongation stage. This confirms findings of other studies on the *C. arvense*. HODGSON (1968) found that the carbohydrate root reserves in *C. arvense* declined from early spring to a minimum in June before the appearance of flower buds. Other studies by BAKKER (1960), SAGAR & RAWSON (1964) and WILSON et al. (2006) showed the same results. Another report mentioned that mowing in the time before the opening of flower buds, compared to other times could more effectively suppress *C. arvense* (GUSTAVSSON, 1997).

In our research, the seven-to-ten leaf stage occurred before the stem elongation. Applications at this growth stage showed a high efficacy on both root and shoot biomass for both application volumes. These results were in line with previous studies on above-ground disturbance of *C. arvense*. According to VERWIJST et al. (2018), applying above-ground disturbance at the minimum regenerative capacity of *C. arvense* reduces the carbohydrate reserve of the roots. It prevents root extension and leads to an above-ground biomass reduction. The eight-to-ten-leaf stage is the growth stage in which this plant is most vulnerable to above-ground disturbance (GUSTAVSSON, 1997). TAVAZIVA et al. (2019) reported the high efficacy of herbicide on *C. arvense* at the four-to-ten leaf stage.

Experiment 2

According to the Linear Mixed Model, the two repetitions of the experiment 2 had the same trend in all evaluated traits and were not significantly different from each other (Fig. 1 and 2).

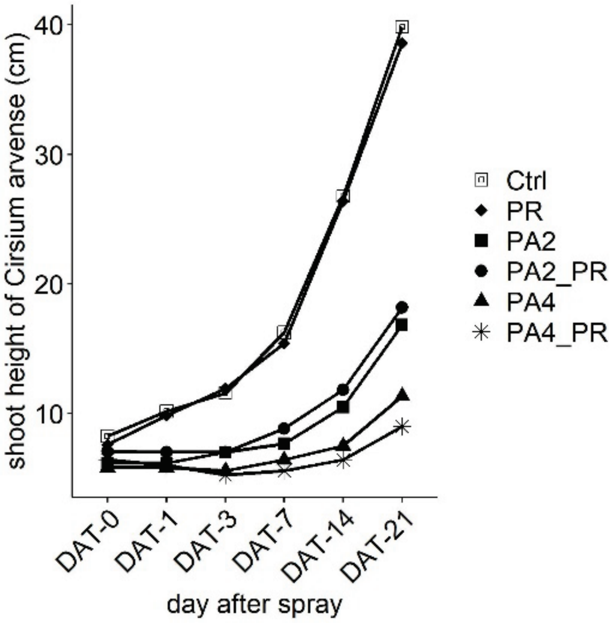

Figure 1 Linear Mixed Model: Effects of Pelargonic acid (PA) with different application volume after adding Paraffin oil as adjuvant on shoot height of *Cirsium arvense*. Treatments: see Table 1. DAT-0 = the day before application of the treatments.

Abbildung 1 *Lineare Gemischte Modelle: Wirkungen von Pelargonsäure (PA) mit unterschiedlichem Applikationsvolumen nach Zugabe von Paraffinöl als Additiv auf die Sprosshöhe von* Cirsium arvense*. Versuchsglieder: siehe Tabelle 1. DAT-0 = der Tag vor der Anwendung den Versuchsglieder.*

The day before application (DAT-0), the plant's height in all treatments was 6 to 8 cm. After applying the treatments, in the PA and PA_PR treatments the *C. arvense* growth stopped (Fig. 1). *C. arvense* plant height declined or did not change until DAT-7. The difference between the untreated control and PR with PA4 and PA4_PR treatments was significant at DAT-3 ($P<0.05$) and continued to be the same till DAT-21. The regrowth started at DAT-7. Plants began to regrow from the underground parts or aboveground broken parts. The regrowth was significantly slower for PA4_PR compared to all the others ($p<0.001$). At DAT-21, the height for untreated control and PR were 40 cm while it was around 9 cm for PA4_PR ($p < 0.001$).

The results on the efficacy of PA with paraffin oil (Fig. 2) showed that the highest efficacy on shoot and root biomass caused by PA4_PR was 79% and 80%, respectively. These efficacies were significantly higher than the PR and PA2_PR. Efficacy on root biomass was 10 to 20% higher than PA2 (69%) and PA2_PR (61%). PA4 showed 78% efficacy on *C. arvense* shoot and root biomass. Both were significantly higher than PR and PR2_PR treatments.

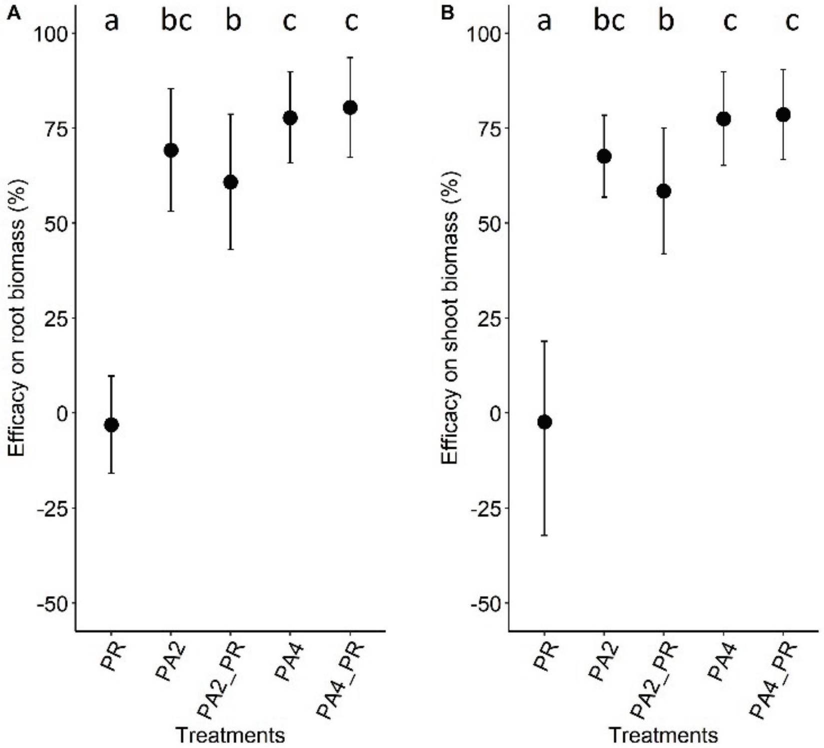

Figure 2 Efficacy of PA with different application volume after adding Paraffin oil as adjuvant. **A**: Efficacy on root biomass, **B**: Efficacy on shoot biomass. Treatments: see Table 1. Linear Mixed Model analysis. Different letters denote significant differences (p<0.05) according to the pairwise comparison of means by Tukey contrast.

Abbildung 2 *Wirksamkeit von PA bei unterschiedlichem Applikationsvolumen nach Zugabe von Paraffinöl als Adjuvants. A: Wirksamkeit auf Wurzelbiomasse, B: Wirksamkeit auf Sprossbiomasse. Versuchsglieder: siehe Tabelle 1. Lineare gemischte Modellanalyse. Unterschiedliche Buchstaben kennzeichnen signifikante Unterschiede (p<0,05) (Tukey-Test).*

Overall, PA resulted in better *C. arvense* control when applied with paraffin oil. According to previous studies, adding an adjuvant to PA could improve its efficacy in reducing the biomass of weed species. COLEMAN & PENNER (2008) reported the increase of PA efficacy after adding organic acids on *C. arvense* and some other weed species. In another research on *Lolium rigidum* Gaud., *Avena sterilis* L. and *Galium aparine* L, pelargonic acid formulations including maleic hydrazide and manuka oil as adjuvants showed higher efficacy compared to the formulation without adjuvant (TRAVLOS et al., 2020).

Increasing the application volume to 400 L and adding 6.2 kg/ha Paraffin oil caused 10% to 20% more reduction in root and shoot biomass of *C. arvense* compared to PA with lower application volume and without adjuvant.

We conclude that PA with an application volume of 400 L/ha and added adjuvant is a mixture with a herbicidal potential on *C. arvense*. However, our recommendation is to test it in field conditions. According to our results, there are two growth stages of *C. arvense* at which the efficacy of PA is promising: (1) The early-elongation stage before the appearance of flower buds. At this stage, the plant uses the assimilates to develop aboveground parts, and less assimilates are stored in the roots. In the case of herbicide application at this stage, compensating the damage by the plant leads to reduced root extension and overall biomass reduction. (2) The seven-to-ten leaf stage at which the plant is at its minimum regenerative capacity. Application of herbicide at this stage will postpone the plant development and reduces both above- and below-ground biomass.

Beloukha® is registered for grapevine to destroy suckers and control weeds, and for desiccation of potatoes (German Register of Plant Protection Products, 2021). Bio-based herbicides like PA could in addition assist in agro-ecological management by terminating the shoots of perennial weeds without ploughing.

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