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Containing perennial weeds with the bioherbicide Pelargonic Acid through direct control and support of cover crop management

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Table of Contents

Summary 3

Zusammenfassung 5

List of Figures 7

List of Tables 9

Abbreviations 10

Chapter 1: General Introduction 11

1.1 Perennial weeds 11

1.1.1 Biological attributes of *Cirsium arvense* 11

1.1.2 Biological attributes of *Sonchus arvensis* 12

1.1.3 Economic importance of *C. arvense* and *S. arvensis* 14

1.1.4 Control methods 14

1.1.4.1 Cultural methods 15

1.1.4.2 Mechanical methods 15

1.1.4.3 Chemical control 17

1.1.4.4 Biological solutions for weed control 18

1.1.4.4.1 Biological weed control 18

1.1.4.4.2 Bio-herbicides 18

1.1.4.4.3 Pelargonic acid as bio-herbicide 19

1.2 Cover crop management 20

1.3 Objectives of the study 21

1.4 Chapter outline 21

1.5 References 22

Chapter 2: The herbicidal potential of Pelargonic Acid to control *Cirsium arvense* (L.) Scop. in relation to the timing of application and the application volume 31

2.1 Abstract 31

2.2 Introduction 31

2.3 Materials and Methods 32

2.4 Results and Discussion 33

2.5 Acknowledgements 35

2.6 References 35

Chapter 3: The effect of two-year application of pelargonic acid on the growth of *Cirsium arvense* (L.) Scop. and *Sonchus arvensis* L. 37

3.1 Abstract 37

3.2 Introduction 37

3.3 Materials and Methods 39

3.4 Results 43

3.5 Discussion 49
3.6 Conclusions 51
3.7 Acknowledgements 52
3.8 Supplementary tables and figures..52
3.9 References 54
Chapter 4: Estimating the Reduction in Cover Crop Vitality Followed by Pelargonic Acid Application Using Drone Imagery..59
4.1 Abstract 59
4.2 Introduction 59
4.3 Materials and Methods 61
4.4 Results..65
4.5 Discussion 70
4.6 Conclusions 72
4.7 Acknowledgements 73
4.8 Appendix A 73
4.9 References 74
Chapter 5: General Discussion..77
5.1 The influence of application timing, volume, and the addition of adjuvants on the herbicidal potential of PA 77
5.2 Effects of PA application over two consecutive years on the same spot 78
5.3 Application of PA on cover crops as a desiccant 79
5.4 UAV Imaging for herbicide efficacy monitoring..80
5.5 Benefits and drawbacks of PA as a bio-herbicide..80
5.6 The limitations of containing perennial weeds..82
5.7 Further research..83
5.8 References 83
Acknowledgements..87

Summary

Perennial weeds such as *Cirsium arvense* (L.) Scop. and *Sonchus arvensis* L. pose major challenges in weed management for both conventional and organic agricultural systems. Due to the negative impacts on the environment and human health, reducing the use of synthetic herbicides has become essential. Consequently, regulatory restrictions are implemented by governments in many countries, including those within the European Union. Considering the central role of herbicides like glyphosate in controlling perennial weeds, identifying and developing ecologically sustainable alternatives, such as bio-herbicides derived from natural compounds, has become a priority in weed management strategies. Pelargonic acid (PA), a naturally occurring fatty acid, has gained attention as a potential bio-herbicide for controlling weeds in arable cropping systems. The primary aim of this thesis was to evaluate strategies to enhance PA efficacy and explore its potential in managing both perennial weeds and cover crops. Given the limited information available on bio-herbicides for cover crop termination, another objective of this study was to fill this knowledge gap by evaluating the herbicidal efficacy of PA for cover crop desiccation. In this context, the study focused on leveraging PA's benefits, addressing its limitations, and assessing its effectiveness in suppressing perennial weeds and serving as a desiccation tool for terminating cover crops.

Furthermore, the variability in herbicide efficacy, short-term efficacy, weed regrowth, and requirements for multiple applications or higher dosages were considered the main challenges in PA application as a bio-herbicide. Therefore, the following sub-objectives were defined. The first one was to investigate whether considering the following technical aspects: (1) carrier volume increase, (2) adding adjuvant, and (3) application at a specific growth stage *C. arvense*, affects PA efficacy. The second aim was to evaluate the effectiveness of twice PA application over two consecutive years to the same pots—where each large pot contained a patch of *C. arvense* and *S. arvensis*, grown from a single ramet, considering their initial ramet sizes. Therefore, the study aimed to assess if the initial ramet size affects the PA efficacy and the regrowth of these two species following PA application and whether there are differences between these species concerning PA efficacy and regrowth patterns.

The experimental setups of the current thesis include greenhouse, semi-field, and field trials. To address the first sub-objective, PA efficacy on *C. arvense* was assessed under semi-field and greenhouse conditions in 2020 and 2021. The semi-field experiment evaluated various PA application volumes—specifically 16 L/ha in 200 L and 400 L carrier volume—across five phenological growth stages of *C. arvense*. The greenhouse experiment examined the effect of paraffin oil as an adjuvant to enhance PA's herbicidal efficacy. Results indicated that applying 16 L/ha of PA in 400 L of carrier volume significantly improved its efficacy, and adding paraffin oil enhanced this effect further. The highest control was achieved during the early elongation and seven-to-ten leaf stages of *C. arvense*.

To evaluate the long-term efficacy of PA on perennial weeds, a semi-field experiment was conducted from spring 2020 to autumn 2021. PA was tested on *C. arvense* and *S. arvensis* with initial ramet sizes of 5 cm, 10 cm, and 15 cm, and its effects were compared to those of glyphosate. Results showed that PA applied twice over consecutive growing seasons reduced plant coverage, above and belowground biomass, and flower production, especially for plants with smaller initial ramet sizes (5 cm). PA was more effective on *C. arvense* than *S. arvensis* but did not prevent regrowth, while glyphosate demonstrated superior efficacy.

To address the cover crop management, the study compared PA with two other synthetic herbicides for cover crop desiccation. This experiment was conducted in 2019 and repeated in 2021 in northeast Germany, employing a completely randomized block design. Various herbicide treatments were tested, including PA at 16, 8, and 5 L/ha, glyphosate, and pyraflufen. Assessment methods included visual estimates of crop vitality and drone-based vegetation indices (RGB and NIR). PA, especially at 16 L/ha or as a double application of 8 L/ha, effectively desiccated cover crops within a week, though its efficacy decreased over time due to cover crop regrowth. Glyphosate showed the most consistent effectiveness, resulting in the lowest crop vitality by the end of the experiment. Drone-based vegetation indices,

particularly RGB indices like EXG, provided detailed and accurate assessments, demonstrating their potential for cost-effective crop and weed infestation monitoring.

In summary, the efficacy of PA as a contact herbicide was improved when all relevant technical factors, such as carrier volume, weed growth stage, and adjuvant, were considered simultaneously. This means that when these factors were combined, efficacy increased significantly. This indicates that optimizing the PA application requires considering coverage, water solubility, timing of application relative to weather and weed growth stages, and the biological characteristics of the target weed species. PA can be used alongside other tools to lower perennial weed infestations. It also has shown potential for cover crop desiccation. Although, it is still not registered for broader management in arable crops. Currently, PA is approved in Europe as a plant desiccant for potatoes, for killing suckers and weed control in non-cultivated areas. Given the urge to reduce the use of synthetic herbicides in farming, further research into the technical aspects of the PA application as well as economic features are crucial to support its use in integrated weed and crop management.

Zusammenfassung

Mehrjährige Wurzelunkräuter wie *Cirsium arvense* (L.) Scop. und *Sonchus arvensis* L. stellen sowohl in konventionellen als auch in biologischen Landwirtschaftssystemen große Herausforderungen bei der Unkrautbekämpfung dar. Aufgrund der negativen Auswirkungen auf die Umwelt und die menschliche Gesundheit ist die Reduktion chemisch-synthetischer Herbizide unumgänglich. Infolgedessen werden von den Regierungen in vielen Ländern, auch innerhalb der Europäischen Union, gesetzliche Beschränkungen eingeführt. Angesichts der zentralen Rolle von Herbiziden wie Glyphosat bei der Kontrolle mehrjähriger Wurzelunkräuter ist die Identifizierung und Entwicklung ökologisch nachhaltiger Alternativen, wie etwa aus natürlichen Verbindungen gewonnener Bioherbizide, zu einer Priorität bei der Unkrautbekämpfungsstrategie geworden. Pelargonsäure (PA), eine natürlich vorkommende Fettsäure, hat als potenzielles Bioherbizid zur Unkrautbekämpfung in landwirtschaftlichen Anbausystemen an Aufmerksamkeit gewonnen. Das Hauptziel der vorliegenden Dissertation war die Bewertung von Strategien zur Verbesserung der Wirksamkeit von PA sowie die Erforschung ihres Potenzials bei der Kontrolle von mehrjährigen Wurzelunkräutern und der Sikkation von Zwischenfrüchten. Angesichts der begrenzten verfügbaren Informationen zu Bioherbiziden für die Sikkation von Zwischenfrüchten bestand ein weiteres Ziel dieser Studie darin, diese Wissenslücke zu schließen, indem die herbizide Wirksamkeit von PA für die Sikkation von Zwischenfrüchten bewertet wurde. In diesem Zusammenhang konzentrierte sich diese Dissertation darauf, die positiven Eigenschaften der PA zu nutzen, ihre Grenzen zu berücksichtigen und ihre Wirksamkeit bei der Unterdrückung von mehrjährigen Wurzelunkräutern sowie als Sikkationsmittel für die Sikkation von Zwischenfrüchten zu bewerten.

Darüber hinaus wurden die Variabilität der Herbizidwirksamkeit, die kurzfristige Effizienz, der Wiederaustrieb von Unkraut sowie die Notwendigkeit mehrmaliger Applikationen oder höherer Dosierungen als die größten Herausforderungen bei der Anwendung von PA als Bioherbizid identifiziert. Folglich wurden die folgenden Teilziele festgelegt. Erstens sollte untersucht werden, ob die Berücksichtigung der folgenden technischen Aspekte – (1) Erhöhung des Trägervolumens (Wasseraufwandmenge), (2) Zugabe von Additiven und (3) Anwendung in einem bestimmten Wachstumsstadium von *C. arvense* – die Wirksamkeit von PA beeinflusst. Das zweite Ziel bestand darin, die Wirksamkeit einer zweimaligen PA-Applikation in zwei aufeinanderfolgenden Jahren in denselben Töpfen zu bewerten, wobei jeder Topf ein Nest von *C. arvense* und *S. arvensis* enthielt, das aus einem einzelnen "Ramet" (Klonindividuum) gewachsen war. Dabei wurde die Anfangsgröße der Rameten berücksichtigt. Ziel der Studie war es daher, zu untersuchen, ob die Anfangsgröße der Rameten die PA-Wirksamkeit sowie den Wiederaustrieb dieser beiden mehrjährigen Wurzelunkräuter nach der PA-Applikation beeinflusst und ob Unterschiede zwischen den beiden Pflanzenarten hinsichtlich der PA-Wirksamkeit und des Wiederaustriebs bestehen.

Die experimentellen Ansätze der vorliegenden Dissertation umfassen Gewächshaus-, Halbfreiland- und Feldversuche. Zur Erreichung des ersten Teilziels wurde die Wirksamkeit von PA auf *C. arvense* unter Halbfreiland- und Gewächshausbedingungen in den Jahren 2020 und 2021 untersucht. Im Halbfreilandversuch wurden verschiedene PA- Applikationsvolumen - insbesondere 16 l/ha in 200 l und 400 l Trägervolumen - über fünf phänologische Wachstumsstadien von *C. arvense* bewertet. Im Gewächshausexperiment wurde die Wirkung von Paraffinöl als Additiv zur Verbesserung der herbiziden Wirksamkeit von PA untersucht. Die Ergebnisse zeigten, dass die Anwendung von 16 l/ha PA in 400 l Trägervolumen (Wasser) die Wirksamkeit signifikant verbesserte und dass die Zugabe von Paraffinöl diesen Effekt weiter verstärkte. Die höchste Kontrolle wurde im frühen Längenwachstum des Haupttriebs und im Stadium der sieben bis zehn Blätter von *C. arvense* erzielt.

Um die langfristige Wirksamkeit von PA gegen mehrjährige Wurzelunkräuter zu bewerten, wurde von Frühjahr 2020 bis Herbst 2021 ein Halbfreilandversuch durchgeführt. PA wurde an *C. arvense* und *S. arvensis* mit einer anfänglichen Rametgröße von 5 cm, 10 cm und 15 cm appliziert, und seine Wirkung wurde mit der von Glyphosat verglichen. Die Ergebnisse zeigten, dass die zweimalige Applikation von PA in aufeinanderfolgenden Wachstumssaisons zu einer Reduktion der Pflanzenbedeckung, der ober- und unterirdischen Biomasse sowie der Blütenproduktion führte, insbesondere bei Pflanzen mit einer

kleineren Anfangsgröße der Rameten (5 cm). PA war gegen *C. arvense* wirksamer als gegen *S. arvensis*, verhinderte jedoch nicht das Wiederaustreiben. Im Gegensatz dazu zeigte Glyphosat eine höhere Wirksamkeit.

Um das Management von Zwischenfrüchten zu untersuchen, wurde PA in der Studie mit zwei anderen synthetischen Herbiziden zur Sikkation von Zwischenfrüchten verglichen. Der Versuch wurde in den Jahren 2019 und 2021 im Nordosten Deutschlands in einem vollständig randomisierten Blockdesign durchgeführt. Es wurden verschiedene Herbizidbehandlungen getestet, darunter PA in Mengen von 16, 8 und 5 l/ha, Glyphosat und Pyraflufen. Zu den Bewertungsmethoden gehörten visuelle Schätzungen der Vitalität der Pflanzen und drohnengestützte Vegetationsindizes (RGB und NIR). PA, insbesondere in einer Aufwandmenge von 16 l/ha oder als Doppelapplikation von 8 l/ha, führte innerhalb einer Woche zur Sikkation der Zwischenfrüchte; jedoch nahm seine Wirksamkeit im Verlauf aufgrund des Wiederaustriebs der Zwischenfrüchte ab. Glyphosat zeigte die beständigste Wirksamkeit und führte am Ende des Versuchs zur geringsten Vitalität der Zwischenfrüchte. Drohnengestützte Vegetationsindizes, insbesondere RGB-Indizes wie EXG, lieferten detaillierte und genaue Bewertungen, was ihr Potenzial für eine kosteneffiziente Überwachung von Kulturpflanzen und Unkrautbefall unterstreicht.

Zusammenfassend konnte die Wirksamkeit von PA als Kontaktherbizid verbessert werden, wenn alle relevanten technischen Faktoren, wie Trägervolumen, Unkrautwachstumsstadium und Additiv, simultan berücksichtigt wurden. Dies zeigt, dass die Wirksamkeit durch die Kombination dieser Faktoren signifikant gesteigert werden kann. Daraus lässt sich schließen, dass bei der Optimierung der PA-Applikation Aspekte wie die Abdeckung, die Wasserlöslichkeit, der Applikationszeitpunkt in Abhängigkeit von Witterungsbedingungen und Wachstumsstadien der Unkräuter sowie die biologischen Eigenschaften der Zielunkrautarten berücksichtigt werden müssen. Die Applikation von PA kann in Kombination mit anderen Maßnahmen eingesetzt werden, um den Befall von mehrjährigen Wurzelunkräutern zu reduzieren. Es hat sich zudem als potenziell geeignet für die Sikkation von Zwischenfrüchten erwiesen. Allerdings ist PA derzeit noch nicht für eine breitere Anwendung in der Landwirtschaft zugelassen. Aktuell ist PA in Europa als Sikkationsmittel für Kartoffeln, zur Entfernung von Stockaustrieben (z.B. im Weinbau) sowie zur Unkrautbekämpfung auf nicht landwirtschaftlich genutzten Flächen zugelassen. Angesichts der zunehmenden Forderung, den Einsatz synthetischer Herbizide in der Landwirtschaft zu reduzieren, sind weitere Forschungen sowohl zu den technischen Aspekten der PA-Applikation als auch zu den ökonomischen Aspekten entscheidend, um den Einsatz von PA im integrierten Unkraut- und Pflanzenmanagement zu fördern.

List of Figures

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. 34

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. 35

Figure 3. Experimental set up, assessments, and activities over the two years of the experiment. 40

Figure 4. Air temperature (°C), soil temperatures at 5 cm and 20 cm belowground level, and precipitation (mm) during the experimental period in 2020 and 2021. Information for both years was obtained from the research weather station of the University of Rostock's Department of Hydrology and Applied Meteorology 42

Figure 5. PA treatment efficacy (%) [degree of necrotization] compared with the untreated control and GLY treatment at 1st, 7th, 14th, and 21st_DAT after application on *C. arvense* and *S. arvensis* in the first year and repeated application in the second year of experiment. PA: Pelargonic acid; GLY: glyphosate; UC: untreated control. 44

Figure 6. Results of the LMM analysis followed by Tukey's HSD on shoot density per m^2 at the end of the experiment. Significance codes obtained from the LMM analysis for main effects shown in this graph are as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Groups with different letters are significantly different at $p < 0.05$ level based on Tukey's HSD test. PA: Pelargonic acid; GLY: glyphosate; UC: untreated control; Cirsium: *Cirsium arvense*; Sonchus: *Sonchus arvensis*. 5 cm, 10 cm and 15 cm represent the initial size of ramets. 45

Figure 7. Results of the LMM analysis followed by Tukey's HSD on soil surface coverage (%) at the end of the experiment. Significance codes obtained from the LMM analysis for main effects shown in this graph are as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Groups with different letters are significantly different at $p < 0.05$ level based on Tukey's HSD test. PA: Pelargonic acid; GLY: glyphosate; UC: untreated control; Cirsium: *Cirsium arvense*; Sonchus: *Sonchus arvensis*. 5 cm, 10 cm and 15 cm represent the initial size of ramets. 46

Figure 8. Results of the LMM analysis followed by Tukey's HSD on the number of produced flowers at the end of the experiment. Significance codes obtained from the LMM analysis for main effects shown in this graph are as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Groups with different letters are significantly different at $p < 0.05$ level based on Tukey's HSD test. PA: Pelargonic acid; GLY: glyphosate; UC: untreated control; Cirsium: *Cirsium arvense*; Sonchus: *Sonchus arvensis*. 5 cm, 10 cm and 15 cm represent the initial size of ramets. 46

Figure 9. Aboveground and belowground biomass of *C. arvense* and *S. arvensis* after regrowth. Different letters show significant differences between treatments at $p < 0.05$ using Tukey's HSD test separately for aboveground biomass and belowground biomass. PA: Pelargonic acid; GLY: glyphosate; UC: untreated control; 5 cm, 10 cm and 15 cm represent the initial size of ramets. 47

Figure 10. Monthly measured plant heights (cm) of regrown *C. arvense* and *S. arvensis* after herbicide applications during the experimental period. Different letters show significant differences between treatments considering the interactions of the main factors together and with years at $p < 0.05$ using Tukey's HSD test. PA: Pelargonic acid; GLY: glyphosate; UC: untreated control. 48

Figure 11. Study area of Rostock, northeast Germany. 62

Figure 12. Air temperature (°C) and precipitation during the growing season (mm) in (a) 2019 and (b) 2021. Information for both years was obtained from the research weather station of the Department of Hydrology and Applied Meteorology of the University of Rostock. 62

Figure 13. Visually estimated crop vitality after application of herbicides on the cover crop in both years. Small letters show significant differences between treatments within each day in 2019 and capital letters show significant differences between treatments within each day in 2021 at $p<0.05$ using the Kruskal-Wallis test. Error bars indicate the standard deviation for each treatment within each assessment day. 65

Figure 14. (a) Estimated crop vitality obtained by visual observation in 2019; (b), (c), (d), (e): calculated VIs relative to the untreated control obtained by a drone in 2019. 66

Figure 15. (a) Estimated crop vitality obtained by visual observations in 2021; (b), (c), (d), (e): calculated VIs relative to the untreated control obtained by a drone in 2021. 66

Figure 16. Changes in plant vitality due to different herbicides during the experiment in 2021, expressed by RGB. 67

Figure 17. Changes in plant vitality due to different herbicides during the experiment in 2021, expressed by NDVI. 67

Figure 18. Relationship between VIs (relative values compared with before application) and visually estimated vitality in 2019. Linear and polynomial regression analysis ($p<0.05$). 68

Figure 19. Relationship between VIs (relative values compared with before application) and visually estimated vitality in 2021. Linear and polynomial regression analysis ($p<0.05$). 68

Figure 20. Relationship between VIs (absolute values) in 2019. Linear and polynomial regression analysis ($p<0.05$). 69

Figure 21. Relationship between VIs (absolute values) in 2021. Linear regression analysis ($p<0.05$). 70

Supplementary Figure 1. Results of the LMM analysis followed by Tukey's HSD on herbicide efficacy at 21st_DAT in both years. Significance codes obtained from the LMM analysis for main effects shown in this graph are as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Within each year, groups with different letters are significantly different at $p < 0.05$ level based on Tukey's HSD. PA: Pelargonic acid; GLY: glyphosate; UC: untreated control; Cirsium: *Cirsium arvense*; Sonchus: *Sonchus arvensis*. 5 cm, 10 cm and 15 cm represent the initial size of ramets. 54

List of Tables

Table 1. Treatments in Experiments 1 and 2. 32

Table 2. Linear Mixed Models': Efficacy of PA400 and PA200 on the root and shoot biomass of five growth stages of *Cirsium arvense* 33

Table 3. Overview of factorial experiment design with plant species, herbicide treatments, and initial ramet size as factors 40

Table 4. Herbicide treatments used in the experiments in 2019 and 2021 63

Table 5. Sunlight and cloud conditions during the drone flights, collected by the authors 63

Supplementary Table 1. Results of the LMM analysis followed by Tukey's HSD on herbicide efficacy at all days after applications (DATs) in both years 52

Supplementary Table 2. Results of the LMM analysis followed by Tukey's HSD on the aboveground biomass at the end of experiment 53

Supplementary Table 3. Results of the LMM analysis followed by Tukey's HSD on the belowground biomass at the end of experiment 53

Supplementary Table 4. Results of the LMM analysis followed by Tukey's HSD on monthly measured plant heights over 2020 and 2021 53

Supplementary Table 5. Results of a comparison of treatments using absolute values of vegetation indices at different DAT in 2019 73

Supplementary Table 6. Results of a comparison of treatments using absolute values of vegetation indices at different DAT in 2021 73

Abbreviations

Abb.	abbreviation for treatment names
BRDF	bi-directional reflectance distribution function
Ctrl	control treatment
DAT	days after treatment
e.g.	exempli gratia
et al.	et alia
EWRS	European Weed Research Society
EXG	excess green index
GLY	glyphosate
i.e.	id est
KTBL	Kuratorium für Technik und Bauwesen in der Landwirtschaft e. V.
LCI	leaf chlorophyll index
LMM	Linear mixed model
NIR	near infrared
NDVI	normalised difference vegetation index
p	p value: as the probability in statistic
PA	pelargonic acid
PA_2T	2 times application of 8 l/ha pelargonic acid with one week interval
PA2	16 l/ha pelargonic acid in 200 l/ha water
PA4	16 l/ha pelargonic acid in 400 l/ha water
PA2_PR	16 l/ha pelargonic acid in 200 l/ha water mixed with paraffin oil
PA4_PR	16 l/ha pelargonic acid in 400 l/ha water mixed with paraffin oil
PA200	16 l/ha pelargonic acid in 200 l/ha water
PA400	16 l/ha pelargonic acid in 400 l/ha water
PA_R	5 l/ha pelargonic acid
PR	paraffin oil
Pr	p value: as conditional probability in statistic
PYR	pyraflufen
PYR + PA_R	pyraflufen + 5 l/ha pelargonic acid
RGB	red, green, and blue
UC	untreated control
VARI	visible atmospherically resistant index
VI	vegetation index
VIs	vegetation indices
VRA	variable rate application
UAV	unmanned aerial vehicle

Chapter 1: General Introduction

1.1 Perennial weeds

Perennial weeds are species that live for several years with vegetative reproduction ability (Hartzler and Buhler, 2007; Tørresen et al., 2017) and are considered as important species in all cropping systems from a global point of view (Håkansson, 2003; Hatcher, 2017). Due to the ability to develop vegetative organs, they can withstand unfavorable conditions such as winter and water-deficient growing phases (Håkansson, 1982). While various classifications for perennial weeds can be found in scientific literature, such as those outlined by Håkansson (1982) and Korsmo (1930, 1954), this study specifically adopts the classification presented in Hatcher (2017). According to Hatcher (2017), they are classified as simple and creeping herbaceous perennials. The usual mode of reproduction for simple herbaceous perennials is through seeds, except when they are subjected to cut, but creeping herbaceous perennials propagate through both seeds and vegetative organs (Hatcher, 2017). Their vegetative reproduction modes are diverse, including creeping stems (stolons and runners) (Hatcher, 2017) and creeping root system (thickened plagiotropic roots) with buds capable of generating new shoots (Håkansson, 1982; Hatcher, 2017). The root system structure is referred to as a "clonal fragment" (Bittebiere et al., 2020). This structure can be broken into smaller pieces by various soil mechanical practices, such as tillage (Håkansson, 1982; Håkansson, 2003). These smaller pieces are known as "ramets" (Bittebiere et al., 2020; Tørresen and Gerowitt, 2022). A ramet is an individual plant unit that has the potential to survive independently and generate new plants (Hatcher, 2017; Bittebiere et al., 2020). Ramets derived from the same clonal fragment are genetically identical to the original mother plant (Bittebiere et al., 2020; Tørresen and Gerowitt, 2022).

Perennial weeds pose substantial challenges to agricultural productivity particularly in organic agriculture (Melander et al., 2012). They are capable of enduring, continuously expanding, and competing with crops for essential resources (Ramesh et al., 2017). Within the category of perennials with creeping root systems, *C. arvense* L. Scop. and *S. arvensis* L. are considered the most troublesome weeds in temperate regions (Lemna and Messersmith, 1990; Tiley, 2010; Liew et al., 2013). These species are widely present in various types of agricultural crops (Tørresen et al., 2010; Brandsæter et al., 2017). For effectively controlling these perennial weeds, it is crucial to understand their biology (Favrelière et al., 2020).

1.1.1 Biological attributes of *Cirsium arvense*

C. arvense, known as Canada thistle or creeping thistle, belongs to the Asteraceae family (Moore, 1975). Apart from rapidly spreading by horizontal creeping roots, *C. arvense* also produces numerous flowers and seeds (Moore, 1975; Donald, 1994). This species is dioecious, meaning that female flowers grow on separate plants from male flowers (Moore, 1975; Tiley, 2010). It is distributed globally and has been identified in various regions, including Canada, the USA, Europe, Asia (Tiley, 2010), Africa, New Zealand, and Australia (Moore, 1975; Amor and Harris, 1975; Bourdôt et al., 2015). The habitat of the species is characterized by moderate temperatures and a rainfall range of 400-750 mm (Detmers, 1927). This species exhibits adaptability to a wide variety of soils (Reed, 1970); however, silt loams offer an optimal environment for *C. arvense* growth (Hodgson, 1968). Well-drained fertile soil facilitates robust growth, whereas poorly aerated soils or water-saturated soils can limit the growth (Hodgson, 1968; Tiley, 2010). Areas with higher humidity are favorable for *C. arvense* (Moore, 1975; Håkansson 2003). This species is commonly located near roads, railroad slopes, lawns, gardens, unused areas and disturbed fields (Moore, 1975; Liew et al., 2013).

The plants persist continuously through their root system in wintertime (Liew et al., 2013). While the aboveground shoots are destroyed by severe frost, the roots, which store plenty of nutrient reserve (e.g., carbohydrates), endure the winter conditions, leading to the formation of numerous aboveground shoots and the establishment of new, independent plants in the growing season each year (Moore, 1975; Magnusson et al., 1987). In one season, the roots can extend up to 6 to 12 meters long (Hayden, 1934;

Hamdoun, 1972). Approximately 54% of the roots are situated at depths ranging from 7 to 22 cm beneath the soil surface, while 30% extend between 22 and 37 cm and 16% between 37 to 52 cm; however, root penetration can reach depths of 2-3 meters (Hodgson, 1968). The amount of root extension is contingent upon soil texture, fertility, moisture, and vegetative cover (Hayden, 1934). Individual roots have a lifespan of approximately 2 years (Fykse, 1977), after which the new roots that originate from the existing ones replace them (Moore, 1975; Fykse, 1977). The root fragments, aged between 6 weeks and 2 years, give rise to visible aboveground shoots (Moore, 1975). The quantity of accessible nutrient reserves in the underground parts of *C. arvense* changes over the course of the year (McAllister and Haderlie, 1985). Seely (1952) discovered that after cultivation, the nutrient reserve in the roots remains fairly stable for about 30 days but then starts to increase. The lowest point in nutrient reserve in the root system occurs in early June, coinciding with the onset of flowering (Welton et al., 1929; Weigel, 2024). Bakker (1960) noted a decline in water-soluble sugars in the roots from spring until June, with a consistent level during flowering (June-August), followed by a rapid increase to a high level in September and October. Hodgson (1968) found similar patterns in his research in Montana. It was observed that 5 out of 36 root fragments, with a thickness of 3-6 mm and a length of 8 mm, were capable of producing shoots (Moore, 1975). Additionally, all root fragments with a length of 10 to 13 mm could potentially contribute to shoot production (Hamdoun, 1972; Moore, 1975; Nadeau and Vanden Born, 1989).

Detmers (1927) reported that a single robust shoot of *C. arvense* has the potential to produce 100 heads in a season, while Bakker (1960) discovered a range of 32 to 69 heads per shoot under favorable growth conditions. In this species, the flowers require insects for pollination (Moor, 1975; Tiley, 2010; Leathwick and Bourdôt, 2012). The quantity of seeds generated is contingent on the effectiveness of the pollination process (Bakker, 1960). Hayden (1934) noted that substantial quantities of seeds were produced when male and female plants were within 33 meters of each other. However, only 2-3 seeds per head were formed when they were separated by distances ranging from 160 to 200 meters. Hodgson (1968) observed significant seed production when the male and female plants were growing within 16.5 meters. A single plant exhibits the potential to generate up to 5300 seeds, but an average production of 1530 seeds is more commonly observed (Moore, 1975). Seedlings can establish themselves in disturbed soil but will not survive in conditions of strong competition and low light intensity (Hodgson, 1968; Bakker, 1960). If the seedling survives, it initially forms a tap-fibrous root, which thickens within a few months and gives rise to lateral roots spreading horizontally (Tiley, 2010). After reaching a length of 6-12 cm, the horizontal roots develop, and aerial shoots emerge from the buds on them (Moore, 1975; Tiley, 2010). One seedling has the potential to generate a large patch of aboveground shoots, and the clone's expansion can persist continuously, with clusters of shoots, gaining independence when the root system is fragmented (Tiley, 2010). The introduction of a single plant can lead to a large infestation, but no seed production occurs in such instances (Moore, 1975). For seed production to happen, both male and female plants are necessary (Moore, 1975; Tiley, 2010), implying the need for multiple introductions in an area (Moore, 1975).

1.1.2 Biological attributes of *Sonchus arvensis*

S. arvensis, known as perennial sow thistle, is a member of the Asteraceae (Compositae) family (Lemna and Messersmith, 1990; Zollinger and Kells, 1991) and described in the literature as a robust, deep-rooted perennial herb (Lemna and Messersmith, 1990). Similar to *C. arvense*, this species can be propagated both vegetative and by seeds (Zollinger, 1989; Anbari et al., 2016). Geographically, *S. arvensis* is present across Europe, western Asia, and Iceland (Lemna and Messersmith, 1990). Although distributed throughout Europe, it is most common in northwestern Europe and temperate zones of the world (Liew et al., 2013; Brandsæter et al., 2020), occurring less frequently in central Europe, and it is considered rare in southern Europe (Lemna and Messersmith, 1990). It can be found in Canada, North America, South America, New Zealand, and Australia (Peschken et al., 1983; Lemna and Messersmith, 1990). *S. arvensis* is compatible with various soil types but tends to occur more frequently in loam soils, with a preference for moist fine-textured soils over dry, coarse-textured sand (Zollinger and Kells, 1993). It is sensitive to soil compaction (Anbari, 2015). Another research indicated that the infestation of this species in uncompact cultivated moist soils was higher compared to artificially compact moist soils (Njoes, 1982; Lemna and Messersmith, 1990). Due to soil compaction caused by prolonged use of minimal cultivation, the creeping roots of *S. arvensis* are restricted to spreading near the soil surface (Mokshin, 1978). Typically, this species thrives in temperate regions with higher precipitation levels and sunny conditions (Lemna and Messersmith, 1990). *S. arvensis* is prevalent along roadsides, riverbanks, lakeshores, and in waste areas and cultivated fields (Surat et al., 2008; Liew et al., 2013; Anbari, 2015). The plants are susceptible to light frosts, and frost conditions have the potential to kill leaves and stems (Lemna and Messersmith, 1990).

The creeping roots of *S. arvensis* play a crucial role in the rapid colonization of new areas and ensuring persistence despite cultivation and disturbances (Surat et al., 2008; Anbari, 2015). These roots are located at soil depths of 5 to 12 cm (Arny, 1932), originating from branched, shortened spindle-shaped primary roots (Korsmo, 1954) and are typically 0.25 to 0.5 cm in diameter (hardly reaching 1 cm) (Arny, 1932). New root branches may emerge from either existing roots or adventitious roots forming in the subterranean parts of aerial stems (Lemna and Messersmith, 1990). Vertical roots can penetrate into depths of 2 meters (Arny, 1932). The buds, which survive the winter on either vertical or spreading roots or on the basal segments of aerial stems, can give rise to new shoots (Håkansson 1982). Additionally, the weed can also persist through the winter in the form of seeds (Lemna and Messersmith, 1990).

When the soil temperature increases sufficiently, new shoots and roots begin to grow in an established patch of *S. arvensis* in late April (Håkansson, 1969; Anbari, 2015). According to Håkansson (1969), most shoots emerge from the top 10 cm of the soil, although it is plausible for some shoots to emerge from a deeper part. The roots close to the soil surface produce small purplish leaves about a week after the initial growth (Lemna and Messersmith, 1990), and adventitious root development begins 3 to 4 weeks later (Håkansson, 1969). At the 5-7 leaf stage, the initial thickening of new roots begins (Håkansson and Wallgren, 1972; Taab, et al., 2018). By 3 months after the initial growth, creeping roots have a diameter of 4 mm and can exceed 200 cm in length (Lemna and Messersmith, 1990; Anbari, 2015). By the middle of summer, the thickening of new roots stops, but new shoots continue to develop from roots of 2-3 mm in diameter until the end of summer (Lemna and Messersmith, 1990). A root fragment of 2.5 cm or smaller can produce plants if well-developed buds are present, and root fragments with a length of 1 cm can generate a flowering plant in less than a year (Håkansson and Wallgren, 1972).

The amount of nutrient reserve in the underground storage tissue varies throughout the growing period (Arny, 1932; Guncan, 1973). When the aboveground shoots of *S. arvensis* are in the 5-7 leaf stage, the minimum belowground dry matter occurs (Håkansson, 1969; Håkansson and Wallgren, 1972), which is the period before the new roots thicken. This condition represents the stage with the lowest regenerative capacity following soil disturbance (Håkansson and Wallgren, 1972). During stem elongation, when the flowers are developing, another period of reduced regenerative ability occurs in *S. arvensis*. The new shoot emergence does not occur at the end of summer, and vegetative growth is inhibited in autumn (Håkansson, 1969). Previous studies have suggested that *S. arvensis* has an innate dormancy, restricting the formation of new shoots and roots from both new and old roots by the beginning of autumn, even when roots are fragmented (Håkansson, 1982; Håkansson and Wallgren, 1972a). The dormancy period may develop due to decreasing temperatures, the senescence of top growth, or decreasing day length (Lemna and Messersmith, 1990).

One *S. arvensis* plant has the ability to create a patch by propagation through its creeping root system (Anbari, 2015), as explained before. Isolated patches cannot produce seeds, likely due to self-incompatibility (Lemna and Messersmith, 1990). The exact maximum lifespan of roots is uncertain but is at least 2 years (Håkansson, 1969). Vertical roots seldom survive beyond their second year (Lemna and Messersmith, 1990). The formation of flowering stems starts at the 12-15 leaf stage (Håkansson, 1969). Typically, in the first days of July, flowering begins and continues until the end of the growing season in late summer. Seeds will mature approximately 10 days after the onset of flowering (Anbari, 2015). Seeds do not germinate until the soil reaches a suitable temperature, and the majority of seedlings typically emerge in the middle to late part of May (Lemna and Messersmith, 1990). *S. arvensis* flowers are self-incompatible and rely on insect pollination (Derscheid and Schultz, 1960; Tavaziva, 2012). *S. arvensis* can produce between 150 and 240 fertile flowers, but the quantity of produced achenes differs significantly among heads, plants, and locations because of various factors, including environmental conditions and the presence of appropriate pollinators (Lemna and Messersmith, 1990). *S. arvensis* usually bears an average of 30 achenes per head, with the potential to produce up to 50,000 achenes per 0.9 m^2 (Lemna and Messersmith, 1990). Depending on the environmental conditions each year, in wild populations around 20 to 40 or 60 to 80 achenes per head can be produced (Derscheid and Schultz, 1960).

1.1.3 Economic importance of *C. arvense* and *S. arvensis*

The creeping thistle infestation is observed in various crops like wheat (Hodgson, 1968; Hodgson, 1977; Mamolos and Kalburtji, 2001), maize (Líška et al., 2007), legume crops (Golubinova and Ilieva, 2015), potatoes (Týr, 2008), and Forage crops (Gabruck et al., 2013) as well as in pastures and ranges (Bourdôt et al., 2011), where it competes with the crops for essential resources such as light, water, and nutrients (Moore, 1975; Tavaziva, 2012). This competition decreases crop yield (Moore, 1975; Favrelière et al., 2020). The range of crop yield decrease caused by *C. arvense* can be from 30% to 50% when there are 15 to 20 shoots per m^2, respectively (O'Sullivan et al., 1982, 1985; Favrelière et al., 2020; Lacroix et al., 2021). Previous studies indicate that as the density of *C. arvense* extends further than ten shoots per m^2, the yield loss continues to rise, potentially reaching up to 70% in certain circumstances (Tiley, 2010). The creeping thistle has been observed to decrease wheat density, subsequently influencing wheat yield (Donald and Khan, 1996). Allelopathic effects could be the cause of yield loss in some crops (Evans, 1984; Pilipavičius and Romaneckas, 2014; Golubinova and Ilieva, 2015; Favrelière et al., 2020). Studies by Hodgson (1968, 1977) showed that in the USA, the creeping thistle had a significant negative impact on spring wheat yield.

Perennial sow-thistle infestations lead to economic losses due to decreased crop yield (Zollinger, 1989; Tavaziva, 2012), higher costs for cultivation and herbicide application (Lemna and Messersmith, 1990; Tavaziva, 2012), and a decline in the value of the affected land (Lemna and Messersmith, 1990). *S. arvensis* is frequently found in numerous cereal and oilseed crops in the northern area of Canada and the USA (Peschken et al., 1983). It is also a significant problem in countries like Hungary, Norway, Poland, east Europe and Russia (Lemna and Messersmith, 1990). In one instance, a patch of this plant reduced oat yield by 69% (Zollinger, 1989). Another study showed that having 70 shoots of this plant per m^2 decreased oat yield by 25% (Friesen and Shebeski, 1960). *S. arvensis* densities of 3 to 15 plants per m^2 caused a 4.5 to 21% reduction in spring wheat yield (Shashkov et al., 1977; Zollinger, 1989).

1.1.4 Control methods

Due to the importance of creeping thistle and perennial sow thistle, numerous studies have investigated control methods (Lemna and Messersmith, 1990; Håkansson, 2003; Vanhala et al., 2006; Knudson, 2009; Juneau, 2013; Anbari, 2015; Favrelière et al., 2020). The challenges in controlling them arise from their perennial nature and their capacity to propagate through both spreading roots and seeds (Moore, 1975; Lemna and Messersmith, 1990; Donald, 1994; Leathwick and Bourdôt, 2012). The response of both species to control measures is influenced by various factors such as climate conditions, habitat, soil type, clonal structure, and the timing and the growth stage at which the control measures are applied (Zollinger and Kells, 1991; Tworkoski, 1992; Donald, 1994; Krueger-Mangold et al., 2002; Wilson and Michiels, 2003; Knudson, 2009; Anbari, 2015). Since the early 1900s, various management strategies have been developed (Hodgson, 1968; Knudson, 2009) and can be categorized into three major groups: cultural and preventive methods, physical or mechanical methods, and chemical methods (Vidme, 1961; Vanhala et al., 2006; Knudson, 2009; Chhokar et al., 2012; Juneau, 2013; Favrelière et al., 2020). Examples of preventive methods include using uncontaminated seeds (Chhokar et al., 2012) and fertilizers (Håkansson, 2003; Knudson, 2009; Chhokar et al., 2012). Cultural methods encompass practices such as crop rotation and planting competitive crops (Håkansson, 2003; Knudson, 2009). Tillage, mowing, and mulching are also exemplars of mechanical methods, (Håkansson, 2003; Vanhala et al., 2006; Anbari, 2015). Moreover, the utilization of bioherbicide is another defoliation strategy (Ringselle et al., 2020) that has been increasingly investigated in recent years (Cordeau et al., 2016).

Effective management of *C. arvense* and *S. arvensis* typically demands contributions from all three management categories (Vanhala et al 2006; Melander et al., 2012). To balance the cropping system and minimize the risk of perennials proliferating uncontrollably, preventive and cultural methods are essential. However, cropping systems often need to accommodate various interests, leading to situations that may favor perennials. In such cases, direct control methods become necessary to manage the weeds (Melander et al., 2012). Some of these measures will be described in detail in the following sections.

1.1.4.1 Cultural methods

Crop rotation

Crop selection and rotation are key factors in an effective weed management strategy (Cook et al., 2018; Ofosu et al., 2023). Before the introduction of chemical weed control methods, rotations played a crucial role in establishing good farming practices (Cook et al., 2018), reducing pest and disease occurrence while enhancing soil fertility (Baral, 2012; Cook et al., 2018). According to Cook et al. (2018), to manage perennial weeds such as creeping thistle and sow thistle in potato fields, it is advisable to suppress them in previous crops in the rotation cycle, as there are limited effective weed control methods available for potatoes against these particular weeds. As noted by Vanhala et al. (2006), a red clover-timothy ley rotation with spring barley reduced *S. arvensis* biomass but not density.

Competitive crops (main and subsidiary crops)

Numerous studies have investigated the use of competitive plant species to control creeping thistle (Moor, 1975; Knudson, 2009; Sciegienka, 2009; Tiley, 2010; Favrelière et al., 2020) and sow thistle (Sjursen et al., 2012; Anbari, 2015). The photosynthetic capacity and development of weeds become restricted in competition for light, which can be achieved by various means such as sowing crops that provide substantial soil cover, planting tall crops, and reducing row space (Favrelière et al., 2020). Competition for water and nutrients is influenced by root system characteristics (Dunbabin, 2007; Favrelière et al., 2020). *C. arvense* and *S. arvensis* have deep root systems, reaching depths of several meters, affording them a competitive advantage relative to numerous cultivated species (Hayden, 1934; Moore, 1975; Lemna and Messersmith, 1990; Eckersten et al., 2010; Anbari, 2015). Since *C. arvense* roots can penetrate deep into the soil, competing with it requires a deep-rooted crop like alfalfa, which allows the crop to absorb nutrients and water from the same depth, thereby increasing its ability to compete more effectively. (Donald, 1990; Nadeau and Vanden Born, 1989; Favrelière et al., 2020). Competitive “smother” crops have been suggested in various studies as a control method for creeping thistle, as it was observed that this weed could not tolerate shade (Donald, 1990). Another research testing artificial shade reported biomass reduction in *S. arvensis* (Putri et al., 2018). Significant reductions in *C. arvense* have been observed with the use of cover crops, such as sudangrass (Bicksler et al., 2012; Wedryk and Cardina, 2012). Preparing the soil early in summer and establishing a crop with a dense autumn canopy, such as a fast-growing or tall cash crop or cover crop, is an effective strategy to suppress late-autumn sprouting of *C. arvense* (Bicksler et al., 2012; Wedryk and Cardina, 2012; Tørresen and Gerowitt, 2022).

1.1.4.2 Mechanical methods

Tillage (plowing, harrowing and stubble cultivation)

Since the introduction of tools like the hoe, harrow, and plow, tillage has remained the most important technique for weed management (Ringselle et al., 2020). Several factors influence the response of weeds to tillage, including the weed regenerative traits, the employed tillage method (such as depth and extent of injury), environmental conditions and soil conditions (such as composition, temperature, and moisture levels) (Håkansson, 2003). Generally, the use of a moldboard plow relocates weed propagules, such as seeds, to deeper soil layers, thereby reducing their germination and emergence (Gruber and Claupein, 2008). In the case of perennial weeds, besides relocation, tillage treatments fragment subterranean plant structures, promoting the emergence of new shoots (Liew et al., 2012). This process aims to exhaust the creeping roots and diminish their nutrient reserves, thereby inhibiting plant growth (Håkansson, 2003; Liew et al., 2012). The control method can be conducted at or just before the compensation point, when nutrient reserves in creeping roots are at their lowest level, there is no immediate new supply from photosynthesis and therefore these weeds are at their most vulnerable stage and their resprouting is reduced (Gustavsson, 1997; Nkurunziza and Streibig, 2011; Weigel, 2024). Several studies have focused on the influence of tillage on controlling *C. arvense* and *S. arvensis* with regard to the aforementioned aspects (Graglia et al., 2006; Lukashyk et al., 2008; Brandsæter et al., 2010; Brandsæter et al., 2011; Thomsen et al., 2011; Melander et al., 2012; Brandsæter et al., 2017).

By definition, according to KTBL (2020), stubble cultivation entails shallow tillage that brings sprouted weeds and dispersed seeds to the surface and buries them, along with the stubble from unused land or residue of the previous crop. Earlier research suggests that the most effective way to manage perennial weeds is through stubble cultivation using shallow plowing prior to autumn harrowing (Brandsæter et al., 2012; Brandsæter et al., 2017). However, comparable control to shallow plowing followed by harrowing can be obtained through rotary tillage for stubble cultivation (Brandsæter et al., 2012). In the management of *C. arvense*, shallow plowing, coupled with a second round in late autumn, has proven to be effective (Gruber and Claupein, 2009; Brandsæter et al., 2012). Additionally, Melander et al. (2012) concluded that intensive post-harvest cultivation combined with deep inverting tillage effectively controls perennial weeds on sandy soils, though the degree of efficacy can vary among weed species.

Mowing and hoeing

Mowing and hoeing have the potential to effectively control *S. arvensis* and *C. arvense* (Vanhala et al., 2002; Anbari et al 2016; Melander et al., 2012). Frequent defoliation caused by mowing reduces root assimilates, eventually depleting food reserves in the roots through repeated sprouting (Graglia et al. 2006; Lukashyk et al. 2008; Brandsæter et al. 2012; Anbari et al., 2016). Mowing is more sustainable than tillage operations, as it causes less erosion and nutrient leaching and consumes less energy (Brandsæter et al., 2012).

Mowing of *C. arvense* at various growth stages, particularly during the eight-to-ten-leaf stage, has been evaluated in several studies, which found significant decreases in *C. arvense* aboveground biomass, biomass per shoot (Tavaziva et al., 2019), shoot density (Verwijst et al., 2017; Tavaziva et al., 2019), and consequently seed production (Verwijst et al., 2018). *S. arvensis* is also quite vulnerable to defoliation and burial if the control measures are applied before the six-to-seven-leaf stage when it can be eradicated with only a few repetitions (Håkansson, 1969; Liew, 2013). Additionally, the timing of mowing may play a crucial role in managing both species due to the plant stage, the root reserve and dormancy in the root (Detmers, 1927; Donald, 1990; Teasdale et al., 2007; Andersson et al., 2013). According to the literature, repeated mowing as a single treatment often fails to control *C. arvense* effectively (Teasdale et al., 2007; Favrelière et al., 2020).

Repeated hoeing can also stimulate regrowth of aerial parts, though its effectiveness in controlling *C. arvense* is limited (Favrelière et al., 2020). Inter-row hoeing, when done repeatedly, may offer some degree of control over *S. arvensis* and *C. arvense* (Graglia et al., 2006; Vanhala et al., 2006; Melander et al., 2012; Brandsæter et al., 2020). However, tools like weed harrows and finger weeders generally fail to be effective (Melander et al., 2012 In barley cultivation with a row spacing of 24 cm, five to six consecutive hoeing were performed, resulting in a reduction of *C. arvense* biomass; however, this reduction was not statistically significant (Graglia et al., 2006). While the destruction of aerial parts only offers limited control of *C. arvense*, it can be considered as part of a broader control strategy that combines multiple practices (Favrelière et al., 2020).

Other defoliation alternatives

Beyond mowing and cutting, there are several other methods to eliminate aboveground part of perennial weeds without directly affecting belowground creeping roots (Ringselle et al., 2020; Peerzada and Chauhan, 2018). These include mechanical destructive treatments (e.g., air-propelled grit) (Ringselle et al., 2020) and thermal control methods such as hot foam, flaming and hot water (Ringselle et al., 2020; Antonopoulos et al., 2023). These alternatives are less researched compared to mowing, particularly with regard to *C. arvense* and *S. arvensis* (Knudson, 2009; Pergher et al., 2019; Martelloni et al., 2020: Antonopoulos et al., 2023). Flaming has had limited success in controlling creeping thistle, with effectiveness depending on the season and soil moisture (Knudson, 2009). The results of one study indicated that applying steam twice per growing season across the experimental plots was the most effective method for controlling weeds such as *S. arvensis* and *C. arvense* in onion fields (Vasinauskienė et al., 2019). The significant disadvantages of these methods are the regrowth of perennial weeds, and the high energy and time requirements (Pergher et al., 2019; Ringselle et al., 2020; Martelloni et al., 2020; Peerzada and Chauhan, 2018). However, they could be used selectively, between rows, or during the period between crops (Pergher et al., 2019; Ringselle et al., 2020).

1.1.4.3 Chemical control

Starting from the 1940s, synthetic herbicides, including systemic ones, were introduced for weed control (Håkansson, 2003). For many years, herbicides were applied to control both *C. arvense* and *S. arvensis* (Brandsæter et al., 2017); however, their efficacies varied from low to highly effective (Moor, 1975; Lemna and Messersmith, 1990; Juneau, 2013; Knudson, 2009; Fogelfors and Lundkvist, 2008).

Traditional herbicides for treating *C. arvense* and *S. arvensis* encompass a broad range of various active ingredients. Among the commonly utilized ones are picloram, clopyralid, chlorsulfuron, mixture of triclopyr and clopyralid, 2,4-D, and glyphosate (Devine and Born, 1985; Lemna and Messersmith, 1990; Fogelfors and Lundkvist, 2008; Knudson, 2009; Juneau, 2013). The effectiveness of herbicides on *C. arvense* and *S. arvensis* varies, influenced by factors such as timing, frequency, application method, year, growth stage, and even the specific plant ecotypes (Vidme, 1961; Lemna and Messersmith, 1990; Knudson, 2009; Fogelfors and Lundkvist, 2008; Cripps et al., 2019). Frank and Tworkoski (1994) found substantial variation in how different clones of *C. arvense* responded to various herbicide treatments. They suggested that the diverse effects of chlorsulfuron, glyphosate, and clopyralid on these clones might be attributed to genetic differences, potentially explaining why herbicides failed to consistently suppress these plants. According to previous research, *S. arvensis* showed great tolerance to many broadleaf herbicides (Lemna and Messersmith, 1990; Fogelfors and Lundkvist, 2008). Among the abovementioned traditional herbicides, glyphosate has been effective as a pre-harvest treatment on *S. arvensis* in Britain; however, application in the fall was reported to be less effective on *S. arvensis* (Lemna and Messersmith, 1990).

Glyphosate, introduced in 1974, is a non-selective herbicide known for its unique mode of action (Beckie et al., 2020; Kanatas et al., 2021). It is extensively utilized in agriculture for controlling weeds (Duke and Powles, 2008; Benbrook, 2016). Glyphosate's success is attributed to several factors: its effectiveness as a broad-spectrum herbicide, its excellent translocation properties, and its unique mechanism of inhibiting the EPSPS enzyme, with no competing analogs and low cost (Duke and Powles, 2008; Kanatas et al., 2021). The increasing use of glyphosate highlights concerns related to potential risks. Growing dependence on this broad-spectrum herbicide has led to the global spread of tolerant and resistant weeds (Powles, 2008; Heap, 2014; Owen et al., 2014; Benbrook, 2016). Farmers often increase glyphosate application rates and spray more frequently to manage these tolerant weeds (Benbrook, 2016). Furthermore, due to environmental and health concerns about glyphosate addressed by some studies in recent years (Van Bruggen et al., 2018; Paganelli et al., 2010), European governments decided to impose restrictions on glyphosate use (Antier et al., 2020). In November 2022, the European Weed Research Society (EWRS) evaluated the available alternatives to glyphosate and identified four essential uses of glyphosate in EU cropping systems: managing perennial weeds, weed control in conservation agriculture, vegetation management in tree and vine crops, and managing herbicide resistance (Neve et al., 2024). Additionally, according to the EU Farm to Fork strategy, pesticide utilization should be decreased by 50% by 2030 (Wesseler, 2022). Therefore, identifying and developing effective alternatives is crucial, particularly given glyphosate's central role in managing perennial weeds and supporting conservation agriculture. Without viable substitutes, achieving the EU's pesticide reduction goals will be challenging (Neve et al., 2024).

1.1.4.4 Biological solutions for weed control

There are various products, tools and strategies as biological solutions for weed problems under the term “biocontrol”. According to Amichot et al. (2024), these biological solutions are classified into three categories:

1. Macroorganisms: Includes insects, nematodes, or mites, which can be either native or non-native species.
2. Microorganisms: Encompasses viruses, bacteria, oomycetes, or fungi.
3. Natural active substances: Refers to natural substances derived from plants, animals, microbes, or minerals, either naturally sourced or synthetically produced to mimic the natural versions.

In the EU, thirteen natural products have been authorized for use. Nine are formulated with bacterial microorganisms, three with fungal microorganisms, and only one contains a natural plant extract as its active ingredient (Torres-Pagán et al., 2024).

1.1.4.4.1 Biological weed control

Biological weed control is a technique used to reduce the population density, germination, and growth of weeds to economically manageable levels by employing living organisms (natural enemies or biotic agents) (Kremer, 2005). Therefore, this method of weed control belongs to the aforementioned categories 1 and 2 (Amichot et al., 2024).

The biological control of *C. arvense* has been studied in numerous research efforts, whereas investigations into *S. arvensis* in this regard have been less extensive (Berestetskiy et al., 2008). Controlling the creeping thistle using organisms started in the 1950s due to concerns over herbicide use, initially in North America and later in New Zealand (Tiley, 2010). Reviews by various researchers (e.g., Nuzzo, 1997; Cripps, et al., 2011; Müller and Nentwig, 2011) indicated that existing biological control methods for *C. arvense* are not sufficiently effective when applied as a single treatment. In another investigation, efforts were made to introduce insects as biological control agents for *Sonchus* spp. in Canada, but these efforts failed because the agents could not establish themselves (Lemna and Messermith, 1990; Anbari, 2015). Nuzzo (1997) suggested combining biocontrol agents with other herbicides for better results. It should be mentioned that the presence of a root damaging agent is required since the most current available agents target the aerial parts of the plant (Ang et al., 1995; Tiley, 2010). Due to the presence of a creeping root system with nutrient reserves, it is necessary to deplete these reserves to weaken the perennial weeds and achieve effective control (Håkansson, 2003). A root-feeding agent could damage the creeping roots and deplete these reserves (Ang et al., 1995).

1.1.4.4.2 Bio-herbicides

Some literature define bio-herbicides as plant pathogens, phytotoxins derived from pathogens or other microorganisms, insects, or plant extracts that are applied to control weeds (Hoagland, 1996; Hoagland et al., 2007; Hasan et al., 2021). In the current study, natural active substances or biochemicals are considered as bio-herbicides and the definition of bio-herbicide is according to the aforementioned category 3 of biological solutions by Amichot et al. (2024).

Bio-herbicides offer benefits over synthetic chemicals due to their short lifespan and the absence of toxic residues in the environment (Saxena and Pandey, 2001). Recently, bio-herbicides have been considered as an important component of weed control, serving as an alternative rather than a complete replacement for chemical herbicides (Hasan et al., 2021). Many plant pathogens, particularly necrotrophic and hemibiotrophic fungi, produce phytotoxins that cause disease, making them potential sources of these valuable metabolites (Berestetskiy et al., 2008; Evidente et al., 2011). Microbial phytotoxins or their synthetic equivalents could be developed into new herbicidal compounds (Evidente and Motta, 2001; Berestetskiy et al., 2008). Metabolites derived from plants can also be an alternative for developing bio-herbicides in sustainable agriculture. These compounds have shown good potential for weed control (Hasan et al., 2021; Acheuk et al., 2022). In nature, certain plants produce allelochemicals such as alcohols, fatty acids, phenolics, flavonoids, terpenoids, and steroids that suppress the growth and development of weeds (Hasan et al., 2021). Natural volatile compounds, known as essential oils, obtained from various plant parts, including leaves, seeds, and flowers, have shown phytotoxic effects (Acheuk et al., 2022). The development of natural herbicides based on organic acids or essential oils represents a significant advancement in reducing adverse environmental impacts. Their low persistence in the environment and reduced likelihood of inducing herbicide resistance are attributed to the diverse modes of action of these natural products (Muñoz et al., 2022; Travlos et al., 2020).

Several microbial phytotoxins have shown potential as new agrochemicals for controlling *S. arvensis*. Results of a study using fungal pathogen metabolites demonstrated the high toxicity of a phytotoxin produced by *Phoma exigua* var. *exigua* on *S. arvensis* leaves (Anbari, 2015). Despite the discovery of many metabolites with bio-herbicidal potential, most of them are not aggressive enough to overcome weed defense mechanisms and provide effective weed control (Hoagland, 1996; Cripps, et al., 2011; Müller and Nentwig, 2011).

1.1.4.4.3 Pelargonic acid as bio-herbicide

Pelargonic acid (PA), also known as n-nonanoic acid (chemical formula: CH3(CH2)7CO2H), is a saturated fatty acid found in various fruits and vegetables such as oranges and potatoes, as well as in dairy products like milk (Ciriminna et al., 2019; Muñoz et al., 2022). It appears as a clear, oily liquid with a faint yellow color at room temperature, characterized by a fatty odor, a hint of coconut flavor and low solubility in water (Ciriminna et al., 2019). Widely recognized as a safe substance with respect to toxicity parameters, PA is approved in multiple countries for various applications. (Johnson et al., 2011; Ciriminna et al., 2019). It functions as a flavoring agent in food and is frequently employed in commercial solutions for peeling fruits and vegetables. Additionally, it acts as a sanitizing or antimicrobial agent in food products at concentrations of up to 1% (Ciriminna et al., 2019; White et al., 2021). The US Environmental Protection Agency approved the first pesticide products containing ammonium salt of PA as a bio-herbicide in 1992 (Campos Cuevas, 2023). This was the first generation of natural herbicide products utilizing PA as the active ingredient (Ciriminna et al., 2019), which later have been developed and authorized as blossom thinner (Fallahi, 1997; Ciriminna et al., 2019), sucker killer in grapevine production (Barić et al., 2018) and tobacco (Short et al., 2020), as desiccant in potato (Coleman and Penner, 2006; Kardasz et al., 2019), dry bean, seed crops such as alfalfa, clovers and cotton (Coleman and Penner, 2006). PA is widely employed globally for managing annual and perennial broadleaf and grass weeds, as well as controlling mosses and other cryptogams in walkways, roads, railways, parks, urban and residential areas, golf courses, gardens, and indoor environments (Ciriminna et al., 2019). In the EU, according to the list of products published by the German Federal Office of Consumer Protection and Food Safety (BVL) in July 2024, PA is primarily approved for use as a herbicide in both professional and non-professional settings. Its applications include managing vine shoots and suckers in vineyards, desiccating potato haulm, hop trimming, kill of root suckers in stone fruit production, killing stolon in strawberry production and weed control in non-cultivated areas such as paths, places with woody plants, and open areas. It is also approved for use on ornamental shrubs, decorative lawns, and home gardens. Additionally, it is used to control weeds in ornamental shrubs and turf.

PA functions as a non-selective, contact and burn-down herbicide, exerting its effect through cuticle destabilization, similar to other short-chain fatty acids, resulting in rapid dehydration of plant tissues (Coleman and Penner, 2006). To explain more precisely, PA induces two sequential processes during its phytotoxic action: (i) it causes cellular membrane leakage by intercalating with the acid, and (ii) it triggers light-driven peroxidative activity through singlet oxygen, resulting in necrosis of plant tissues (Loddo et al., 2023). Obvious damage is perceived between 15 to 60 minutes following the application (Kanatas et al., 2022) and the effect becomes more apparent within a few hours of application (Muñoz et al., 2020; Travlos et al., 2020; Kanatas et al., 2022). However, since PA functions as a contact herbicide and does not translocate within plants, only the plant parts directly exposed to spray droplets are affected and exhibit necrosis (Kanatas et al., 2022; Pannacci et al., 2022; Loddo et al., 2023). To my knowledge, the use of PA as a bio-herbicide has not yet been tested on *C. arvense* and *S. arvensis* and will be addressed in the upcoming chapters of this study.

1.2 Cover crop management

As mentioned in section 1.1.4.1, competitive plant species such as subsidiary crops are utilized as control methods for perennial weeds e.g., creeping thistle and sow thistle (Teasdale et al., 2007; Sjursen et al., 2012; Favrelière et al., 2020; Neve et al., 2024). The term "subsidiary crops" refers to crops that are primarily grown for the various agroecological advantages they offer, rather than for economic yield (Reimer et al., 2019; Lizarazo et al., 2020). Therefore, subsidiary crops encompass a wide range of crop types, each serving different objectives, one of which is cover crops (Reimer et al., 2019). Cover crops are extensively studied for their role in preventing soil erosion by improving soil health and minimizing nutrient loss (Vukicevich et al., 2016; Wick et al., 2017; Banik et al., 2020; Tataridas et al., 2022). They are beneficial in both conventional and organic farming (Masilionyte et al., 2017; Tataridas et al., 2022). In perennial weed management, cover crops are one of the important non-chemical strategic methods (Neve et al., 2024). Cover crops occupy an ecological niche comparable to that of weeds (Liebman and Davis, 2000; Bàrberi, 2002) and therefore, they can compete with them for light, water, space, and nutrients (Cook et al., 2018). Additionally, they may release allelopathic substances that suppress weed development (Teasdale et al., 2007; Almoussawi et al., 2020). During fallow times, utilization of cover cropping can effectively suppress perennial weeds (Blackshaw et al., 2001; Teasdale et al., 2007). Blackshaw et al. (2001) observed that yellow sweet clover successfully controlled *S. arvensis*. Additionally, a combination of cultivation and competitive cover crops in cereal-based rotations has proven effective in managing significant perennial weeds, such as *S. arvensis* and *C. arvense* (Håkansson, 2003). Cover cropping is likely to be most effective when competition occurs at the perennial weeds' compensation point (Teasdale et al., 2007).

In farming systems with reduced or no tillage, or with limited herbicide use, cover cropping plays a crucial role in controlling perennial weeds (Teasdale et al., 2007). Managing cover crops involves balancing their benefits and drawbacks (Lu et al., 2000). Key decisions, especially when using them for weed control, include selecting the cover crop species, determining the sowing and termination times, choosing the termination method, and deciding on the intensity of tillage (Lu et al., 2000; Salonen and Ketoja, 2019). According to Salonen and Ketoja (2019), in northern latitudes where there is a limited timespan for effective management, the integration of cover crops with other weed control methods and consideration of an appropriate termination time are essential. It is important to effectively terminate the cover crop (Kornecki and Kichler, 2022) to prevent it from becoming a problem for subsequent crops and to avoid yield loss (Whalen et al., 2020). Cover crops can be terminated through frost in wintertime, and by mowing, tillage, or herbicide application (Legleiter et al., 2012; Whalen et al., 2020; Kornecki and Kichler, 2022). Non-selective herbicides like glyphosate are commonly used for termination due to their effectiveness at any plant growth stage (Balkcom et al., 2020; Pittman et al., 2020). Winter cover crops are often terminated in early to late spring with a non-selective herbicide, which also controls the weeds (Rosario-Lebron et al., 2019). The timing of termination influences weed populations, cover crop biomass, and soil residue (Rosario-Lebron et al., 2019; Balkcom et al., 2020). Early termination prevents nitrogen depletion and speeds up residue decomposition, benefiting the next crop (Rosario-Lebron et al., 2019), while late termination enhances weed suppression through greater biomass but can deplete soil moisture, affecting the next crop (Rosario-Lebron et al., 2019; Pittman et al., 2020).

To mitigate the abovementioned risks when weed suppression is the target, herbicide application can be utilized due to its rapid effect when the termination window is short and should be for instance closer to the next crop's sowing time (Balkcom et al., 2020; Kornecki and Balkcom, 2020). However, concerns over the environmental and health impacts of synthetic herbicides, particularly glyphosate, have led European governments, such as Germany, to consider severe restrictions (Antier et al., 2020). In response, for cover crop termination, alternative options like bio-herbicides can be explored due to their rapid degradation and perceived advantages over synthetic chemicals (Hasan et al., 2021; Amichot et al., 2024).

1.3 Objectives of the study

The main objective of this thesis was to evaluate the herbicidal effect of PA on *C. arvense* and *S. arvensis*, focusing on various application parameters. The efficacy of PA as a bio-herbicide for perennial weed management, considering their creeping roots, was investigated.

From one perspective, cover crops suppress weeds and could therefore be used as an alternative method to the utilization of synthetic herbicides, such as glyphosate, for controlling perennial weeds. However, herbicide application is also a common method for terminating cover crops. Integrating cover crop management into weed control strategies is essential for maximizing the suppression of perennial weeds and preventing cover crops from becoming problematic in subsequent crops, thereby contributing to sustainable agricultural practices. In this regard, the potential of pelargonic acid (PA) as a bioherbicide for cover crop termination was investigated as a secondary aim.

The specific objectives include:

i. Application parameters:
  - Evaluating the impact of increasing the application volume on the efficacy of PA
  - Assessing how the growth stage of *C. arvense* at the time of application influences the herbicidal effectiveness of PA.
  - Investigating the potential enhancement of PA efficacy on *C. arvense* by adding paraffin oil as an adjuvant.

ii. Long-term efficacy and regrowth:
  - Investigating the long-term impact of repeated PA applications over two consecutive years on the same pots containing *C. arvense* and *S. arvensis*—where each large pot had a patch of one species from a single ramet—while considering the initial size of the ramets.
  - Comparing the herbicidal efficacy of PA with untreated control and glyphosate (GLY) to comprehensively analyze PA’s effectiveness in perennial weed management.

iii. Species-specific responses:
  - Determining whether the initial ramet size influences PA efficacy and regrowth of *C. arvense* and *S. arvensis*.
  - Evaluating the differences in PA efficacy and regrowth between these perennial weeds, focusing on the hypothesis that *S. arvensis* might be more susceptible to PA than *C. arvense* due to seasonal variations in sprouting abilities.

iv. Herbicide mixtures:

  Exploring the efficacy of reduced PA dosage in combination with pyraflufen (PYR), a post-emergence contact herbicide used to control broadleaf weed species, to determine if a reduced dosage of PA can still achieve effective control.

v. Vegetation Indices (VIs) for assessing herbicide effectiveness in cover crop termination:

  Evaluating the credibility and effectiveness of using RGB- and NIR-based vegetation indices, derived from drone imagery, to measure herbicide effects on cover crop desiccation at different assessment times post-application.

1.4 Chapter outline

In Chapter 2, the effects of different timings, application volumes, and adjuvant addition on PA efficacy utilized for controlling *C. arvense* were investigated. Two application volumes and five plant growth stages were tested in a semi-field experiment and a greenhouse experiment where paraffin oil was added as an adjuvant to both PA application volumes to evaluate the potential increase in PA herbicidal efficacy.

In Chapter 3, the feasibility of repeated PA applications over two consecutive years on the same pots of *C. arvense* and *S. arvensis* was examined. Each large pot contained a patch of either *C. arvense* or *S. arvensis* from a single ramet, and the discussion considers the ramets' initial size. PA's herbicidal impact on the entire life cycle of these perennial weed species and their regrowth, which might not be captured in shorter-term studies, were assessed. Additionally, the efficacy of PA as a bio-herbicide treatment was compared to that of glyphosate. The study specifically focused on evaluating the influence of the initial ramet size on PA efficacy after two applications over consecutive years to determine whether this factor affects the efficacy and regrowth, and whether these two perennial species also differ from each other in this regard.

In Chapter 4, the herbicidal potential of PA on cover crops compared with other chemical herbicides was presented to narrow the knowledge gap in cover crop termination using bio-herbicides and providing alternatives for synthetic herbicides. Additionally, the research verified the credibility of RGB- and NIR-based vegetation indices derived from drone images to measure the effect of herbicides on cover crop desiccation at different assessment times.

1.5 References

Acheuk, F., Basiouni, S., Shehata, A. A., Dick, K., Hajri, H., Lasram, S., Yilmaz, M., Emekci, M., Tsiamis, G., Spona-Friedl, M., May-Simera, H., Eisenreich, W., and Ntougias, S. (2022). Status and Prospects of Botanical Biopesticides in Europe and Mediterranean Countries. Biomolecules, 12(2), 311. https://doi.org/10.3390/biom12020311.

Almoussawi, A., Lenoir, J., Spicher, F., Dupont, F., Chabrerie, O., Closset-Kopp, D., Brasseur, B., Kobaissi, A., Dubois, F., and Decocq, G. (2020). Direct seeding associated with a mixture of winter cover crops decreases weed abundance while increasing cash-crop yields. Soil and Tillage Research, 200, 104622. https://doi.org/10.1016/j.still.2020.104622.

Amichot, M., Bertrand, C., Chauvel, B., Corio-Costet, M.-F., Martin-Laurent, F., Le Perchec, S., and Mamy, L. (2024). Natural products for biocontrol: review of their fate in the environment and impacts on biodiversity. Environmental Science and Pollution Research. 1-36. https://doi.org/10.1007/s11356-024-33256-3

Amor, R. L., and Harris, R. V. (1975). Seedling establishment and vegetative spread of Cirsium arvense (L.) Scop, in Victoria, Australia. Weed Research, 15(6), 407-411. https://doi.org/10.1111/j.1365-3180.1975.tb01338.x

Andersson, L., Boström, U., Forkman, J., Hakman, I., Liew, J., and Magnuski, E. (2013). Sprouting capacity from intact root systems of Cirsium arvense and Sonchus arvensis decrease in autumn. Weed Research, 53(3), 183-191. https://doi.org/10.1111/wre.12013

Antier, C., Kudsk, P., Reboud, X., Ulber, L., Baret, P. V., and Messéan, A. (2020). Glyphosate use in the European agricultural sector and a framework for its further monitoring. Sustainability, 12(14), 5682. https://doi.org/10.3390/su12145682

Anbari, S. (2015). Population dynamics of the perennial weed species Sonchus arvensis L. [Doctoral dissertation]. Swedish University of Agricultural Sciences, Department of Crop Production Ecology. https://res.slu.se/id/publ/68299

Anbari, S., Lundkvist, A., Forkman, J., and Verwijst, T. (2016). Population dynamics and nitrogen allocation of Sonchus arvensis L. in relation to initial root size. Acta Agriculturae Scandinavica, Section B–Soil and Plant Science, 66(1), 75-84.

Ang, B. N., Kok, L. T., Holtzman, G. I., and Wolf, D. D. (1995). Canada thistle [Cirsium arvense (L) Scop.] response to density of Cassida rubiginosa Müller (Coleoptera: Chrysomelidae) and plant competition. Biological control, 5(1), 31-38.

Antonopoulos, N., Kanatas, P., Gazoulis, I., Tataridas, A., Ntovakos, D., Ntaoulis, V. N., Zavra, S. M., and Travlos, I. (2023). Hot foam: Evaluation of a new, non-chemical weed control option in perennial crops. Smart Agricultural Technology, 3, 100063. https://doi.org/10.1016/j.atech.2022.100063

Arny, A. C. (1932).Variations in the organic reserves in underground parts of five perennial weeds from late April to November. Technical Bulletin - Agricultural Experiment Station, University of Minnesota, 84, 28 pp.

Bakker, D. (1960). A comparative life-history study of Cirsium arvense (L.) Scop. and Tussilago farfara L., the most troublesome weeds in the newly reclaimed polders of the former Zuiderzee. The Biology of Weeds: A Symposium of the British Ecological Society, Blackwell Scientific Publications, Oxford, pp. 205-222.

Balkcom, K., Schomberg, H., and Lee, R. D. (2020). Chapter 5: Cover crop management. In J. Bergtold and M. Sailus (Eds.), Conservation tillage systems in the Southeast: Production, profitability and stewardship (pp. 56-76). Sustainable Agriculture Research and Education (SARE), Washington DC, USA.

Banik, C., Bartel, C. A., Laird, D. A., Moore, K. J., and Lenssen, A. W. (2020). Perennial cover crop influences on soil C and N and maize productivity. Nutrient Cycling in Agroecosystems, 116(2), 135-150. https://doi.org/10.1007/s10705-019-10030-3

Baral, K. R. (2012). Weed management in organic farming through conservation agriculture practice. Journal of Agriculture and Environment, 13, 60–66. https://doi.org/10.3126/aej.v13i0.7589

Bàrberi, P. (2002). Weed management in organic agriculture: are we addressing the right issues? Weed research, 42(3), 177-193. https://doi.org/10.1046/j.1365-3180.2002.00277.x

Barić, K., Šćepanović, M., Pintar, A., Šoštarčić, V., Lakić, J., and Ostojić, Z. (2018). Application of pelargonic acid for trunk sucker growth control on grapevine. Fragmenta Phytomedica, 32(2), 12-22. https://www.cabidigitallibrary.org/doi/full/10.5555/20203167715 (Accessed on June 7, 2024).

Beckie, H.J., Flower, K.C. and Ashworth, M.B. (2020). Farming without glyphosate? Plants. 9(1): 96. https://doi.org/10.3390/plants9010096

Benbrook, C. M. (2016). Trends in glyphosate herbicide use in the United States and globally. Environmental Sciences Europe, 28, 1-15. https://doi.org/10.1186/s12302-016-0070-0

Berestetskiy, A., Dmitriev, A., Mitina, G., Lisker, I., Andolfi, A., and Evidente, A. (2008). Nonenolides and cytochalasins with phytotoxic activity against Cirsium arvense and Sonchus arvensis: A structure–activity relationships study. Phytochemistry, 69(4), 953-960. https://doi.org/10.1016/j.phytochem.2007.11.003

Bicksler, A. J., Masiunas, J. B., and Davis, A. (2012). Canada thistle (Cirsium arvense) suppression by sudangrass interference and defoliation. Weed science, 60(2), 260-266. https://doi.org/10.1614/WS-D-11-00145.1

Bittebiere, A. K., Benot, M. L., and Mony, C. (2020). Clonality as a key but overlooked driver of biotic interactions in plants. Perspectives in Plant Ecology, Evolution and Systematics, 43, 125510. https://doi.org/10.1016/j.ppees.2020.125510

Blackshaw, R. E., Moyer, J. R., Doram, R. C., and Boswell, A. L. (2001). Yellow sweetclover, green manure, and its residues effectively suppress weeds during fallow. Weed Science, 49(4), 406–413. https://doi.org/10.1614/0043-1745(2001)049[0406:YSGMAI]2.0.CO;2

Bourdôt, G. W., Hurrell, G. A., Skipp, R. A., Monk, J., and Saville, D. J. (2011). Mowing during rainfall enhances the control of Cirsium arvense. Biocontrol science and technology, 21(10), 1213-1223. https://doi.org/10.1080/09583157.2011.608119

Bourdôt, G. W., Leathwick, D. M., Hurrell, G. A., and Saville, D. J. (2015). Competitive exclusion of Cirsium arvense in pasture: a simulated neighbour grazing-height experiment. New Zealand Journal of Agricultural Research, 58(1), 1-12. https://doi.org/10.1080/00288233.2014.941507

Brandsæter, L. O., Fogelfors, H., Fykse, H., Graglia, E., Jensen, R. K., Melander, B., Salonen, J., and Vanhala, P. (2010). Seasonal restrictions of bud growth on roots of Cirsium arvense and Sonchus arvensis and rhizomes of Elymus repens. Weed Research, 50(2), 102-109. https://doi.org/10.1111/j.1365-3180.2009.00756.x

Brandsæter, L. O., Bakken, A. K., Mangerud, K., Riley, H., Eltun, R., and Fykse, H. (2011). Effects of tractor weight, wheel placement and depth of ploughing on the infestation with perennial weeds in organic farmed cereals. European Journal of Agronomy, 34(4), 239-246. https://doi.org/10.1016/j.eja.2011.02.001

Brandsæter, L. O., Thomsen, M. G., Wærnhus, K., and Fykse, H. (2012). Effects of repeated clover undersowing in spring cereals and stubble treatments in autumn on Elymus repens, Sonchus arvensis and Cirsium arvense. Crop Protection, 32(1), 104-110. https://doi.org/10.1016/j.cropro.2011.09.022

Brandsæter, L.O., Mangerud, K., Helgheim, M., and Berge, T.W. (2017). Control of perennial weeds in spring cereals through stubble cultivation and mouldboard ploughing during autumn or spring. Crop Protection, 98, 16-23. https://doi.org/10.1016/j.cropro.2017.03.006

Brandsæter, L. O., Mangerud, K., Andersson, L., Børresen, T., Brodal, G., and Melander, B. (2020). Influence of mechanical weeding and fertilisation on perennial weeds, fungal diseases, soil structure and crop yield in organic spring cereals. Acta Agriculturae Scandinavica, Section B–Soil and Plant Science, 70(4), 318-332.

Campos Cuevas, J. (2023). Discovery and development of a novel sustainable contact herbicide based on natural fatty acids [Doctoral dissertation]. Universitat Politècnica de València, Valencia.

Chhokar, R. S., Sharma, R. K., and Sharma, I. (2012). Weed management strategies in wheat-A review. Journal of Cereal Research, 4(2). https://epubs.icar.org.in/index.php/JWR/article/view/35326

Cook, S. K., Davies, L. R., Pickering, F., Tatnell, L. V., Huckle, A., Newman, S., Whiteside, C., White, C., Talbot, D., Holmes, H., Turnbull, P. E., Buckley, D. C., Scrimshaw, J., and Chambers, P. (2018). Weed control options and future opportunities for UK crops (Research Review No. CP 182 / 1807258). In J. H. Clarke, S. Ellis, and S. Clarke (Eds.), ADAS Boxworth, Boxworth, Cambs CB23 4NN.

Coleman, R., and Penner, D. (2006). Desiccant activity of short chain fatty acids. Weed Technology, 20, 410-415. https://doi.org/10.1614/WT-06-195.1

Cordeau, S., Triolet, M., Wayman, S., Steinberg, C., and Guillemin, J.P. (2016). Bioherbicides: Dead in the water? A review of the existing products for integrated weed management. Crop Protection, 87, 44-49. https://doi.org/10.1016/j.cropro.2016.04.016

Ciriminna, R., Fidalgo, A., Ilharco, L. M., and Pagliaro, M. (2019). Herbicides based on pelargonic acid: Herbicides of the bioeconomy. Biofuels, Bioproducts and Biorefining, 13 (6), 1476–1482. https://doi.org/10.1002/bbb.2046

Cripps, M. G., Dowsett, C. A., Jackman, S. D., Van Koten, C., Goeke, D. F., and Houliston, G. J. (2019). Genetic variation in tolerance to defoliation in Cirsium arvense. Weed research, 60(1), 78-84. https://doi.org/10.1111/wre.12391

Cripps, M. G., Gassmann, A., Fowler, S. V., Bourdôt, G. W., McClay, A. S., and Edwards, G. R. (2011). Classical biological control of Cirsium arvense: lessons from the past. Biological Control, 57(3), 165-174. https://doi.org/10.1016/j.biocontrol.2011.03.011

Derscheid, L. A., and Schultz, R. E. (1960). Achene development of Canada thistle and perennial sowthistle. Weeds, 8(1), 55-62. https://doi.org/10.2307/4040507

Detmers, F. (1927). Canada thistle, Cirsium arvense Tourn. Field thistle, creeping thistle. Ohio Agricultural Experiment Station. Bulletin 414. 45 pp.

Devine, M. D., and Born, W. H. V. (1985). Absorption, translocation, and foliar activity of clopyralid and chlorsulfuron in Canada thistle (Cirsium arvense) and perennial sowthistle (Sonchus arvensis). Weed Science, 33(4), 524-530.

Donald, W. W. (1990). Management and control of Canada thistle (Cirsium arvense). Reviews of weed Science, 5, 193-249.

Donald, W. W. (1994). The biology of Canada thistle (Cirsium arvense). Reviews of Weed Science, 6, 77-101.

Donald, W. W., and Khan, M. (1996). Canada thistle (Cirsium arvense) effects on yield components of spring wheat (Triticum aestivum). Weed Science, 44(1), 114-121.

Duke, S. O., and Powles, S. B. (2008). Glyphosate: a once-in-a-century herbicide. Pest Management Science: formerly Pesticide Science, 64(4), 319-325.

Dunbabin, V. (2007). Simulating the role of rooting traits in crop-weed competition. Field Crops Research, 104 (1-3), 44-51. https://doi.org/10.1016/j.fcr.2007.03.014

Eckersten, H., Lundkvist, A., and Torssell, B. (2010). Comparison of monocultures of perennial sow-thistle and spring barley in estimated shoot radiation-use and nitrogen-uptake efficiencies. Acta Agriculturae Scandinavica Section B–Soil and Plant Science, 60(2), 126-135. https://doi.org/10.1080/09064710902721347

Evans, J. E. (1984). Canada thistle (Cirsium arvense): a literature review of management practices. Natural Areas Journal, 11-21.

Evidente, A., and Motta, A. (2001). Phytotoxins from fungi, pathogenic for agrarian, forestal and weedy plants. Bioactive compounds from natural sources, 473-525.

Evidente, A., Andolfi, A., and Cimmino, A. (2011). Fungal phytotoxins for control of Cirsium arvense and Sonchus arvensis. Pest Technology, 5, 1-17.

German Federal Office of Consumer Protection and Food Safety. (2024). Approved pesticides and their uses on crops and pests. Bundesamt für Verbraucherschutz und Lebensmittelsicherheit. https://www.bvl.bund.de/SharedDocs/Downloads/04_Pflanzenschutzmittel/ppp_crops_pests.html (Accessed on August 25, 2024).

Fallahi, E. (1997). Blossom thinning effects of pelargonic acid, endothalic acid, and hydrogen cyanamide in apple and peach. HortScience, 32(3), 524-525.

Favrelière, E., Ronceux, A., Pernel, J., and Meynard, J. M. (2020). Nonchemical control of a perennial weed, Cirsium arvense, in arable cropping systems. A review. Agronomy for Sustainable Development, 40(4), 31. https://doi.org/10.1007/s13593-020-00635-2

Fogelfors, H., and Lundkvist, A. (2008). Selective pressure on Cirsium arvense (L.) Scop. and Sonchus arvensis L. growth characteristics on different types of farmland in Sweden. Acta Agriculturae Scandinavica Section B–Soil and Plant Science, 59(1), 42-49. https://doi.org/10.1080/09064710701827790

Frank, J. R., and Tworkoski, T. J. (1994). Response of Canada Thistle (Cirsium arvense) and Leafy Spurge (Euphorbia esula) Clones to Chlorsulfuron, Clopyralid, and Glyphosate. Weed Technology, 8(3), 565-571. https://doi.org/10.1017/S0890037X00039695

Friesen, G., and Shebeski, L. H. (1960). Economic losses caused by weed competition in Manitoba grain fields. I. Weed species, their relative abundance and their effect on crop yields. Canadian Journal of Plant Science, 40(3), 457-467.

Fykse, H. (1977). Untersuchungen über Sonchus arvensis L., Cirsium arvense (L.) Scop. und Tussilago farfara L. Entwicklungen sowie Translokation von radioaktiv markierten Kohlenhydraten und MCPA. Scientific Reports of the Agricultural University of Norway 56, 1–22.

Gabruck, D. T., Bork, E. W., Hall, L. M., King, J. R., and Hare, D. D. (2013). Interspecific relationships between white clover, Kentucky bluegrass, and Canada thistle during establishment. Agronomy Journal, 105(6), 1467-1474. https://doi.org/10.2134/agronj2013.0172

Graglia, E., Melander, B., and Jensen, R. K. (2006). Mechanical and cultural strategies to control Cirsium arvense in organic arable cropping systems. Weed Research, 46(4), 304-312.

Golubinova, I., and Ilieva, A. (2015) Allelopathic effects of water extracts of Sorghum halepense (L.) Pers., Convolvulus arvensis L. and Cirsium arvense Scop. on early seedling growth of some leguminous crops. Pestic. Phytomed. (Belgrade) 29(1):35–43. https://doi.org/10.2298/PIF1401035G

Gruber, S., and Claupein, W. (2008). Effects of Conservation Tillage on Canada thistle (Cirsium arvense) in Organic Farming. In Book of Abstracts. https://orgprints.org/id/eprint/11730/

Gruber, S., and Claupein, W. (2009). Effect of tillage intensity on weed infestation in organic farming. Soil and Tillage Research, 105(1), 104-111. https://doi.org/10.1016/j.still.2009.06.001

Guncan, A. (1973). Carbohydrate reserves of Sonchus arvensis rhizomes during a vegetative season and comparison with Convolvulus arvensis L. Mededelingen Fakulteit Landbouwwetenschappen Gent, 38(3), 1011-1017. [Paper presented at the 25th International Symposium on Phytopharmacy and Phytiatry, Part I]. https://www.cabidigitallibrary.org/doi/full/10.5555/19742305399 (Accessed on March 29, 2024).

Gustavsson, A.M.D. (1997). Growth and regenerative capacity of plants of Cirsium arvense. Weed Research. 37(4), 229-236. https://doi.org/10.1046/j.1365-3180.1997.d01-37.x

Håkansson, S. (1969). Experiments with Sonchus arvensis L. I. Development and growth, and the response to burial and defoliation in different developmental stages. Lantbrukshögskolans Annaler 35, 989-1030.

Håkansson, S. and Wallgren, B. (1972). Experiments with Sonchus arvensis L. II. Reproduction, plant development and response to mechanical disturbance. Swedish Journal of Agricultural Research, 2, 3-14.

Håkansson, S. (1982). Multiplication, growth and persistence of perennial weeds. In: Holzner, W., Numata, M. (Eds.) Biology and ecology of weeds. Geobotany, vol 2. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-0916-3_11

Håkansson S. (2003). Weeds and weed management on arable land: An ecological approach (Wallingford, Oxon, UK, Cambridge: MA: CABI Publishing). https://doi.org/10.1079/9780851996516.0000

Hamdoun, A.M. (1972). Regenerative capacity of root fragments of Cirsium arvense (L.) Scop. Weed Research, 12(2), 128-136. https://doi.org/10.1111/j.1365-3180.1972.tb01196.x

Hartzler, R. G., and Buhler, D. D. (2007). Ecological management of agricultural weeds. In ecologically based integrated pest management (pp. 37-55). Wallingford UK: CABI. https://doi.org/10.1079/9781845930646.0037

Hasan, M., Ahmad-Hamdani, M. S., Rosli, A. M., and Hamdan, H. (2021). Bioherbicides: An eco-friendly tool for sustainable weed management. Plants, 10(6), 1212. https://doi.org/10.3390/plants10061212

Hatcher, P.E. (2017). Perennial Weeds. In Weed Research (Eds. P.E. Hatcher and R.J. Froud-Williams). 389-412. https://doi.org/10.1002/9781119380702.ch13

Hayden, A. (1934). Distribution and reproduction of Canada thistle in Iowa. American Journal of Botany, 355-373.

Heap, I. M. (2014). Global perspective of herbicide-resistant weeds. Pest Management Science, 70, 1306-1315. https://doi.org/10.1002/ps.3696

Hodgson, J. M. (1977). Canada thistle and its control (No. 523). Department of Agriculture, Agricultural Research Service.

Hodgson, J. M. (1968). The nature, ecology, and control of Canada thistle. United States Department of Agriculture Technical Bulletin 1386, 32 pp.

Hoagland, R. E. (1996). Chemical interactions with bioherbicides to improve efficacy. Weed Technology, 10(3), 651-674.

Hoagland, R. E., Boyette, C. D., Weaver, M. A., and Abbas, H. K. (2007). Bioherbicides: research and risks. Toxin Reviews, 26(4), 313-342. https://doi.org/10.1080/15569540701603991

Johnson Jr, W., Heldreth, B., Bergfeld, W. F., Belsito, D. V., Klaassen, C. D., Hill, R., Liebler, D., Marks Jr, J. G., Shank, R. C., Slaga, T. J., Snyder, P. W., and Andersen, F. A. (2011). Final report of the Cosmetic Ingredient Review Expert Panel on the safety assessment of pelargonic acid (nonanoic acid) and nonanoate esters. International Journal of Toxicology, 30(6_suppl), 228S-269S. https://doi.org/10.1177/1091581811428980

Juneau, K. J. (2013). Integrated management of the invasive species Cirsium arvense, Canada thistle, and Phragmites australis, common reed: Using ecologically based control measures at each stage of the invasion process [Doctoral dissertation, Michigan Technological University].

Kanatas, P., Antonopoulos, N., Gazoulis, I., and Travlos, I. S. (2021). Screening glyphosate-alternative weed control options in important perennial crops. Weed Science, 69(6), 704-718. https://doi.org/10.1017/wsc.2021.55

Kanatas, P., Zavra, S. M., Tataridas, A., Gazoulis, I., Antonopoulos, N., Synowiec, A., and Travlos, I. (2022). Pelargonic acid and caraway essential oil efficacy on barnyardgrass (Echinochloa crus-galli (L.) P. Beauv.) and johnsongrass (Sorghum halepense (L.) Pers.). Agronomy, 12(8), 1755. https://doi.org/10.3390/agronomy12081755

Kardasz, P., Miziniak, W., Bombrys, M., and Kowalczyk, A. (2019). Desiccant activity of nonanoic acid on potato foliage in Poland. Journal of Plant Protection Research, 12-18.

Knudson, J. A. (2009). Canada thistle (Cirsium arvense [L.] Scop.) response to mowing, herbicide, competitive grasses, and soil amendments on wetland, upland, and mesic sites. [Doctoral dissertation, Colorado State University].

Kornecki, T. S., and Balkcom, K. S. (2020). Chapter 9: Planting in cover crop residue. In J. Bergtold and M. Sailus (Eds.), Conservation tillage systems in the Southeast: Production, profitability and stewardship (pp. 119-132). Sustainable Agriculture Research and Education (SARE). Washington, DC, USA.

Kornecki, T. S., and Kichler, C. M. (2022). Effectiveness of cover crop termination methods on no-till cantaloupe. Agriculture, 12(1), 66. https://doi.org/10.3390/agriculture12010066

Korsmo, E. (1930). Unkräuter im Ackerbau der Neuzeit. Berlin. 580 pp.

Korsmo, E. (1954). Anatomy of weeds. Oslo. 413 pp. Mikkelsen, V.M. and F. Laursen. (1966). Markukrudtet i Danmark omkring 1960. Bot. Tidsskrift 62: 1–26.

Kremer, R. J. (2005). The role of bioherbicides in weed management. Biopesticides International, 1(3), 4.

Krueger-Mangold, J., Sheley, R. L., and Roos, B. D. (2002). Maintaining plant community diversity in a waterfowl production area by controlling Canada thistle (Cirsium arvense) using glyphosate. Weed Technology, 16(2), 457-463. http://www.jstor.org/stable/3989575

KTBL. (2020). Soil cultivation and sowing: Definitions of soil cultivation and sowing systems. https://www.ktbl.de/fileadmin/user_upload/Artikel/Pflanzenbau/Bodenbearbeitung/Bodenbearbeitungssysteme_EN.pdf (Accessed on June 7, 2024)

Lacroix, O., Aubertot, J. N., Bohanec, M., Cordeau, S., Corrales, D. C., and Robin, M. H. (2021). IPSIM-Cirsium, a Qualitative Expert-Based Model to Predict Infestations of Cirsium arvense. Frontiers in Agronomy, 3, 655383. https://doi.org/10.3389/fagro.2021.655383

Leathwick, D. M. and Bourdôt, G.W. (2012). A conceptual model for the population dynamics of Cirsium arvense in a New Zealand pasture. New Zealand Journal of Agricultural Research, 55(4), 371-384. https://doi.org/10.1080/00288233.2012.728532

Legleiter, T., Johnson, B., Jordan, T., and Gibson, K. (2012). Successful cover crop termination with herbicides (Purdue External Bulletin WS-50-W). Purdue University. Available online: https://www.extension.purdue.edu/extmedia/ws/ws-50-w.pdf (Accessed on November 7, 2022).

Lemna, W. K. and Messersmith, C. G. (1990). The biology of Canadian weeds. 94. Sonchus arvensis L. Can. J. Plant Sci. 70(2), 509-532. https://doi.org/10.4141/cjps90-060

Liebman, M., and Davis, A. S. (2000). Integration of soil, crop, and weed management in low-external-input farming systems. Weed Research, 40(1), 27–47. https://doi.org/10.1046/j.1365-3180.2000.00164.x

Liew, J., Andersson, L., Boström, U., Forkman, J., Hakman, I., and Magnuski, E. (2012). Influence of temperature and photoperiod on sprouting capacity of Cirsium arvense and Sonchus arvensis root buds. Weed research, 52(5), 449-457. https://doi.org/10.1111/j.1365-3180.2012.00936.x

Liew, J. (2013). Dormancy in reproductive vegetative buds in creeping perennials dominating the agricultural weed flora in Scandinavia. [Doctoral dissertation, Acta Universitatis agriculturae Sueciae, no. 2013:5]. Swedish University of Agricultural Sciences, Uppsala.

Liew, J., Andersson, L., Boström, U., Forkman, J., Hakman, I., and Magnuski, E. (2013). Regeneration capacity from buds on roots and rhizomes in five herbaceous perennials as affected by time of fragmentation. Plant ecology, 214, 1199-1209. https://doi.org/10.1007/s11258-013-0244-4

Líška, E., Hunková, E., and Demjanová, E. (2007). Creeping thistle (Cirsium arvense (L.) Scop.) an important competitor of nutrients consumption in grain maize stands (Zea mays L.). Journal of Central European Agriculture, 8(4), 461-468. https://doi.org/10.5513/jcea.v8i4.486

Lizarazo, C. I., Tuulos, A., Jokela, V., and Mäkelä, P. S. (2020). Sustainable mixed cropping systems for the boreal-nemoral region. Frontiers in sustainable food systems, 4, 103. https://doi.org/10.3389/fsufs.2020.00103

Loddo, D., Jagarapu, K. K., Strati, E., Trespidi, G., Nikolić, N., Masin, R., Berti, A., and Otto, S. (2023). Assessing Herbicide Efficacy of Pelargonic Acid on Several Weed Species. Agronomy, 13(6): 1511. https://doi.org/10.3390/agronomy13061511

Lu, Y. C., Watkins, K. B., Teasdale, J. R., and Abdul-Baki, A. A. (2000). Cover crops in sustainable food production. Food Reviews International, 16(2), 121-157. https://doi.org/10.1081/FRI-100100285

Lukashyk P., Berg M., and Köpke, U. (2008). Strategies to control Canada thistle (Cirsium arvense) under organic farming conditions. Renewable Agriculture and Food Systems 23(1):13-18. https://doi.org/10.1017/S1742170507002013

Magnusson, M. U., Wyse, D. L., and Spitzmueller, J. M. (1987). Canada thistle (Cirsium arvense) propagation from stem sections. Weed Science, 35(5), 637-639. https://doi.org/10.1017/S0043174500060719

Mamolos, A. P., and Kalburtji, K. L. (2001). Competition between Canada thistle and winter wheat. Weed Science, 49(6), 755-759. https://doi.org/10.1614/0043-1745(2001)049[0755:CBCTAW]2.0.CO;2

Martelloni, L., Frasconi, C., Sportelli, M., Fontanelli, M., Raffaelli, M., and Peruzzi, A. (2020). Flaming, glyphosate, hot foam and nonanoic acid for weed control: A comparison. Agronomy, 10(1), 129. https://doi.org/10.3390/agronomy10010129

Masilionyte, L., Maiksteniene, S., Kriauciuniene, Z., Jablonskyte-Rasce, D., Zou, L., and Sarauskis, E. (2017). Effect of cover crops in smothering weeds and volunteer plants in alternative farming systems. Crop Protection, 91, 74-81. https://doi.org/10.1016/j.cropro.2016.09.016

McAllister, R. S., and Haderlie, L. C. (1985). Seasonal variations in Canada thistle (Cirsium arvense) root bud growth and root carbohydrate reserves. Weed Science, 33(1), 44-49.

Melander, B., Holst, N., Rasmussen, I. A., and Hansen, P. K. (2012). Direct control of perennial weeds between crops–Implications for organic farming. Crop Protection, 40, 36-42. https://doi.org/10.1016/j.cropro.2012.04.029

Moore, R.J. (1975). The biology of Canadian weeds: 13. Cirsium arvense (L.) Scop. Canadian Journal of Plant Science, 55(4), 1033-1048. https://doi.org/10.4141/cjps75-163

Mokshin, V. S. (1978). Characteristics of growth of thistle root systems with minimum tillage. Sibirskii Vestnik Sel'skokhozyaistvennoi Nauki, No. 6, 13-17. https://www.cabidigitallibrary.org/doi/full/10.5555/19802329.

Müller, E., and Nentwig, W. (2011). Plant pathogens as biocontrol agents of Cirsium arvense–an overestimated approach? NeoBiota, 11, 1-24. https://doi.org/10.3897/neobiota.11.1803

Muñoz, M., Torres-Pagán, N., Peiró, R., Guijarro, R., Sánchez-Moreiras, A.M., and Verdeguer, M. (2020). Phytotoxic effects of three natural compounds: Pelargonic acid, carvacrol, and cinnamic aldehyde, against problematic weeds in Mediterranean crops. Agronomy, 10(6): 791. https://doi.org/10.3390/agronomy10060791

Muñoz, M., Torres-Pagán, N., Jouini, A., Araniti, F., Sánchez-Moreiras, A.M., and Verdeguer, M. (2022). Control of problematic weeds in Mediterranean vineyards with the bioherbicide pelargonic acid. Agronomy, 12(10): 2476. https://doi.org/10.3390/agronomy12102476

Nadeau, L. B., and Vanden Born, W. H. (1989). The root system of Canada thistle. Canadian Journal of Plant Science, 69(4), 1199-1206.

Neve, P., Matzrafi, M., Ulber, L., Baraibar, B., Beffa, R., Belvaux, X., Torra Farré, J., Mennan, H., Ringselle, B., Salonen, J., Soukup, J., Andert, S., Duecker, R., Gonzalez, E., Hamouzová, K., Karpinski, I., Travlos, I. S., Vidotto, F., and Kudsk, P. (2024). Current and future glyphosate use in European agriculture. Weed Research, 64(3), 181-196. https://doi.org/10.1111/wre.12624

Njoes, A. (1982). Effects of soil cultivation and compaction on grain crops and interactions with nitrogen fertilization. Serie B-Norges Landbrukshoegskole. Institutt for Jordkultur (Norway), (3). https://www.cabidigitallibrary.org/doi/full/10.5555/19831977213

Nkurunziza, L., and Streibig, J. C. (2011). Carbohydrate dynamics in roots and rhizomes of Cirsium arvense and Tussilago farfara. Weed Research, 51(5), 461-468. https://doi.org/10.1111/j.1365-3180.2011.00866.x

Nuzzo, V. (1997). Element stewardship abstract for Cirsium arvense. The Nature Conservancy, Virginia, USA. http://www.invasive.org/gist/esadocs/cirsarv.html

Ofosu, R., Agyemang, E. D., Márton, A., Pásztor, G., Taller, J., and Kazinczi, G. (2023). Herbicide resistance: Managing weeds in a changing world. Agronomy, 13(6), 1595. https://doi.org/10.3390/agronomy13061595

O'Sullivan, P. A., Kossatz, V. C., Weiss, G. M., and Dew, D. A. (1982). An approach to estimating yield loss of barley due to Canada thistle. Canadian Journal of Plant Science, 62:125–731. https://doi.org/10.4141/cjps82-105

O'Sullivan, P. A., Weiss, G. M., and Kossatz, V. C. (1985). Indices of competition for estimating rapeseed yield loss due to Canada thistle. Canadian Journal of Plant Science, 65:145–149. https://doi.org/10.4141/cjps85-020

Owen, M. D., Beckie, H. J., Leeson, J. Y., Norsworthy, J. K., and Steckel, L. E. (2014). Integrated pest management and weed management in the United States and Canada. Pest Management Science, 71(3), 357–376. https://doi.org/10.1002/ps.3928

Paganelli, A., Gnazzo, V., Acosta, H., López, S. L., and Carrasco, A. E. (2010). Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. Chemical Research in Toxicology, 23(10), 1586-1595. https://doi.org/10.1021/tx1001749

Pannacci, E., Ottavini, D., Onofri, A., and Tei, F. (2022). Dose–response curves of pelargonic acid against summer and winter weeds in Central Italy. Agronomy, 12(12), 3229. https://doi.org/10.3390/agronomy12123229

Peerzada, A. M., and Chauhan, B. S. (2018). Thermal weed control: History, mechanisms, and impacts. In Non-chemical weed control. Academic Press. pp. 9-31. https://doi.org/10.1016/B978-0-12-809881-3.00002-4

Peschken, D. P., Thomas, A, G. and Wise, R. F. (1983). Loss in yield of rapeseed (Brassica napus, B. campestris) caused by perennial sowthistle (Sonchus arvensis) in Saskatchewan and Manitoba. Weed Science, 3l: 40-744. https://doi.org/10.1017/S0043174500070284

Pergher, G., Gubiani, R., and Mainardis, M. (2019). Field testing of a biomass-fueled flamer for in-row weed control in the vineyard. Agriculture, 9(10), 210. https://doi.org/10.3390/agriculture9100210

Pilipavičius, V., and Romaneckas, K. (2014). Allelopathic activity of creeping thistle water extracts on germination and early growth of winter wheat. Bulgarian Journal of Agricultural Science, 20(3), 607-612.

Pittman, K. B., Cahoon, C. W., Bamber, K. W., Rector, L. S., and Flessner, M. L. (2020). Herbicide selection to terminate grass, legume, and brassica cover crop species. Weed technology, 34(1), 48-54. https://doi.org/10.1017/wet.2019.107

Powles, S. B. (2008). Evolved glyphosate-resistant weeds around the world: Lessons to be learnt. Pest Management Science, 64, 360-365. https://doi.org/10.1002/ps.1525

Putri, D. P., Widyastuti, Y., Dewi, W. S., and Yunus, A. (2018). The effect of shade and vermicompost application on yield and flavonoid levels of Tempuyung (Sonchus arvensis). IOP Conference Series: Earth and Environmental Science, 142, 012055. https://doi.org/10.1088/1755-1315/142/1/012055.

Ramesh, K., Matloob, A., Aslam, F., Florentine, S. K., and Chauhan, B. S. (2017). Weeds in a changing climate: vulnerabilities, consequences, and implications for future weed management. Frontiers in Plant Science, 8, 95. https://doi.org/10.3389/fpls.2017.00095

Reed, C. F. (1970). Selected weeds of the United States (Agriculture handbook No. 366). U.S. Government Printing Office.

Reimer, M., Ringselle, B., Bergkvist, G., Westaway, S., Wittwer, R., Baresel, J. P., van der Heijden, M. G. A., Mangerud, K., Finckh, M. R., and Brandsæter, L. O. (2019). Interactive effects of subsidiary crops and weed pressure in the transition period to non-inversion tillage: A case study of six sites across Northern and Central Europe. Agronomy, 9(9), 495. https://doi.org/10.3390/agronomy9090495

Ringselle, B., De Cauwer, B., Salonen, J., and Soukup, J. (2020). A review of non-chemical management of couch grass (Elymus repens). Agronomy, 10(8), 1178. https://doi.org/10.3390/agronomy10081178

Rosario-Lebron, A., Leslie, A. W., Yurchak, V. L., Chen, G., and Hooks, C. R. (2019). Can winter cover crop termination practices impact weed suppression, soil moisture, and yield in no-till soybean [Glycine max (L.) Merr.]?. Crop Protection, 116, 132-141. https://doi.org/10.1016/j.cropro.2018.10.020

Salonen, J., and Ketoja, E. (2019). Undersown cover crops have limited weed suppression potential when reducing tillage intensity in organically grown cereals. Organic agriculture, 10(1), 107-121. https://doi.org/10.1007/s13165-019-00262-6

Saxena, S., and Pandey, A. K. (2001). Microbial metabolites as eco-friendly agrochemicals for the next millennium. Applied microbiology and biotechnology, 55, 395-403. https://doi.org/10.1007/s002530000517

Sciegienka, J. K. (2009). Vegetative reproduction and the integrated management of Canada thistle (Master's thesis). Montana State University-Bozeman, College of Agriculture. https://scholarworks.montana.edu/items/4c24d1fd-9b25-4376-aad0-6b4b9fbb6460

Seely, C. I. (1952). Controlling Perennial Weeds with Tillage. Idaho Agricultural Experiment Station, Bulletin No. 288. Special Collections Idaho S 53 (Between E3 - E415). Retrieved from [https://www.lib.uidaho.edu/digital/uiext/items/uiext33160.html]

Shashkov, V. P., Kolmakov, P. P., Volkov, E. D., and Trifonova, L. F. (1977). The influence of rhizomatous weeds in spring wheat crops on the utilization of nitrogen, phosphorus and potassium. Agrokhimiya, 14(3), 57-59.

Short, M. M., Vann, M. C., and Suchoff, D. H. (2020). Organic sucker control: screening different active ingredients for commercial application. Tobacco Science, 57(1), 17-20. https://doi.org/10.3381/TOBSCI-D-20-00008

Sjursen, H., Brandsæter, L. O., and Netland, J. (2012). Effects of repeated clover undersowing, green manure ley and weed harrowing on weeds and yields in organic cereals. Acta Agriculturae Scandinavica, Section B–Soil and Plant Science, 62(2), 138-150. https://doi.org/10.1080/09064710.2011.584550

Surat, W., Kruatrachue, M., Pokethitiyook, P., Tanhan, P., and Samranwanich, T. (2008). Potential of Sonchus arvensis for the phytoremediation of lead-contaminated soil. International Journal of Phytoremediation, 10(4), 325-342. https://doi.org/10.1080/15226510802096184

Taab, A., Andersson, L., and Boström, U. (2018). Modelling the sprouting capacity from underground buds of the perennial weed Sonchus arvensis. Weed Research, 58(5), 348-356. https://doi.org/10.1111/wre.12313

Tataridas, A., Kanatas, P., Chatzigeorgiou, A., Zannopoulos, S., and Travlos, I. (2022). Sustainable crop and weed management in the era of the EU Green Deal: A survival guide. Agronomy, 12(3), 589. https://doi.org/10.3390/agronomy12030589

Tavaziva, V. J. (2012). Effects of competition on compensation point and phenological development in Sonchus arvensis L. [Master thesis]. [Uppsala, Sweden]: Swedish University of Agricultural Sciences. https://stud.epsilon.slu.se/4572/

Tavaziva, V. J., Lundkvist, A., and Verwijst, T. (2019). Effects of selective cutting and timing of herbicide application on growth and development of Cirsium arvense in spring barley. Weed research, 59(5), 349-356. https://doi.org/10.1111/wre.12371

Teasdale, J. R., Brandsæter, L. O., Calegari, A., and Skora Neto, F. (2007). Cover crops and weed management. In M. K. Upadhyaya and R. E. Blackshaw (Eds.), Non-chemical weed management: Principles, concepts, and technology (pp. 49-64). CABI. https://doi.org/10.1079/9781845932909.0049

Tiley, G. E. (2010). Biological flora of the British Isles: Cirsium arvense (L.) scop. Journal of Ecology, 98(4), 938-983.

Thomsen, M. G., Brandsæter, L. O., and Fykse, H. (2011). Sensitivity of Cirsium arvense to simulated tillage and competition. Acta Agriculturae Scandinavica, Section B-Soil and Plant Science, 61(8), 693-700. https://doi.org/10.1080/09064710.2010.543142

Torres-Pagán, N., Muñoz, M., Barbero, S., Mamone, R., Peiró, R., Carrubba, A., Sánchez-Moreiras, A. M., Gómez de Barreda, D., and Verdeguer, M. (2024). Herbicidal Potential of the Natural Compounds Carvacrol, Thymol, Eugenol, p-Cymene, Citral and Pelargonic Acid in Field Conditions: Indications for Better Performance. Agronomy, 14(3), 537. https://doi.org/10.3390/agronomy14030537

Tørresen, K. S., Fykse, H., and Rafoss, T. (2010). Autumn growth of Elytrigia repens, Cirsium arvense and Sonchus arvensis at high latitudes in an outdoor pot experiment. Weed Research, 50(4), 353-363. https://doi.org/10.1111/j.1365-3180.2010.00791.x

Tørresen, K. S., Karlsson, L. M., and Gonzalez-Andujar, J. L. (2017). Seed biology and population dynamics. In P. E. Hatcher and R. J. Froud-Williams (Eds.), Weed research: Expanding horizons (pp. 85–113). Wiley. https://doi.org/10.1002/9781119380702.ch4

Tørresen, K. S., and Gerowitt, B. (2022). Late-autumn ramet sprouting of three arable creeping perennial weed species. Agronomy 12 (9), 2175. https://doi.org/10.3390/agronomy12092175

Travlos, I., Rapti, E., Gazoulis, I., Kanatas, P., Tataridas, A., Kakabouki, I., and Papastylianou, P. (2020). The herbicidal potential of different pelargonic acid products and essential oils against several important weed species. Agronomy, 10(11), 1687. https://doi.org/10.3390/agronomy10111687

Tworkoski, T. (1992). Developmental and environmental effects on assimilate partitioning in Canada thistle (Cirsium arvense). Weed Science, 40(1), 79-85.

Týr, Š. (2008). Actual weed infestation of potato crops in Slovakia. Acta Herbol, 17(2), 125-130.

Van Bruggen, A. H., He, M. M., Shin, K., Mai, V., Jeong, K. C., Finckh, M. R., and Morris, J. G., Jr. (2018). Environmental and health effects of the herbicide glyphosate. Science of the Total Environment, 616–617, 255-268. https://doi.org/10.1016/j.scitotenv.2017.10.309

Vanhala, P., Lötjönen, T., and Hurme, T. (2006). Managing Sonchus arvensis using mechanical and cultural methods. Agricultural and Food Science, 15(4), 444-458. https://doi.org/10.2137/145960606780061498

Vasinauskienė, R., Brazienė, Z., and Avižienytė, D. (2019). The effects of water steam on weeds and fungal diseases in the stands of onion. Zemdirbyste-Agriculture, 106(1). DOI: 10.13080/z-a.2019.106.007

Verwijst, T., Tavaziva, V. J., and Lundkvist, A. (2018). Assessment of the compensation point of Cirsium arvense and effects of competition, root weight and burial depth on below-ground dry weight–leaf stage trajectories. Weed Research, 58(4), 292-303. https://doi.org/10.1111/wre.12312

Verwijst, T., Tavaziva, V.J., and Lundkvist, A. (2017). Effects of selective cutting and herbicide use in spring barley on seed production of Cirsium arvense. Acta Agriculturae Scandinavica, Section B–Soil and Plant Science, 67(6): 562-570. https://doi.org/10.1080/09064710.2017.1318164

Vidme, T. (1961). Control of Sonchus arvensis (L.) with chemicals. Weed Research, 1(4), 275-288.

Vukicevich, E., Lowery, T., Bowen, P., Úrbez-Torres, J. R., and Hart, M. (2016). Cover crops to increase soil microbial diversity and mitigate decline in perennial agriculture. A review. Agronomy for Sustainable Development, 36, 1-14. https://doi.org/10.1007/s13593-016-0385-7

Wedryk, S., and Cardina, J. (2012). Evaluation of tef as a smother crop during transition to organic management. Weed Technology, 26(1), 102-109. https://doi.org/10.1614/WT-D-11-00042.1

Welton, F. A., Morris, V. H., and Hartzler, A. J. (1929). Organic Food Reserves in Relation to the Eradication of Canada Thistles. Bulletin 441, September. Ohio.). Retrieved from [https://kb.osu.edu/bitstream/handle/1811/60879/1/OARDC_bulletin_n441.pdf]

Wesseler, J. (2022). The EU's farm-to-fork strategy: An assessment from the perspective of agricultural economics. Applied Economic Perspectives and Policy, 44(4), 1826-1843. https://doi.org/10.1002/aepp.13239

Whalen, D. M., Bish, M. D., Young, B. G., Conley, S. P., Reynolds, D. B., Norsworthy, J. K., and Bradley, K. W. (2020). Herbicide programs for the termination of grass and broadleaf cover crop species. Weed technology, 34(1), 1-10. https://doi.org/10.1017/wet.2019.73

Weigel, M. M. (2024). Leveraging the specifics of perennial weeds to improve their management [Doctoral dissertation]. University of Rostock, Faculty of Agricultural and Environmental Sciences. Available in print.

Wick, A., Berti, M., Lawley, Y., and Liebig, M. (2017). Integration of annual and perennial cover crops for improving soil health. In M. M. Al-Kaisi and B. Lowery (Eds.), Soil health and intensification of agroecosystems (pp. 127–150). Academic Press. https://doi.org/10.1016/B978-0-12-805317-1.00006-3

Wilson, R. G., and Michiels, A. (2003). Fall herbicide treatments affect carbohydrate content in roots of Canada thistle (Cirsium arvense) and dandelion (Taraxacum officinale). Weed science, 51(3), 299-304. https://doi.org/10.1614/0043-1745(2003)051[0299:FHTACC]2.0.CO;2

White, E., Kumar, G. D., da Silva, A. L. B. R., Kerr, W. L., Cimowsky, S., Widmer, J. A., and Dunn, L. L. (2021). Postharvest reduction of Salmonella enterica on tomatoes using a pelargonic acid emulsion. Foods, 10(178). https://doi.org/10.3390/foods10010178

Zollinger, R. K. (1989). Perennial sowthistle (Sonchus arvensis L.) distribution, biology, and control in Michigan (Doctoral dissertation). Michigan State University, Department of Crop and Soil Sciences.

Zollinger , R. K., and Kells, J. J. (1991). Effect of soil pH, soil water, light intensity, and temperature on perennial sowthistle (Sonchus arvensis L.). Weed Science, 39(3), 376-384.

Zollinger, R. K., and Kells, J. J. (1993). Perennial sowthistle (Sonchus arvensis) interference in soybean (Glycine max) and dry edible bean (Phaseolus vulgaris). Weed Technology, 7(1), 52-57.

Chapter 2: The herbicidal potential of Pelargonic Acid to control *Cirsium arvense* (L.) Scop. in relation to the timing of application and the application volume

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2.1 Abstract

Pelargonic Acid (PA) is a naturally occurring fatty acid that is used as a bio-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-herbicide, *Cirsium arvense*, pelargonic acid, perennial weed

2.2 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 and Masiunas, 2009). In recent years, there has been a growing interest in implementing more environmental-friendly practices towards weed management. Bio-herbicides are alternatives for weed control (Abouziena and Hagaag, 2016). Since 2015, following the authorization of the active ingredient pelargonic acid, one bio-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 and 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.

2.3 Materials 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, 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 kilometers 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.

Table 1. Treatments in Experiments 1 and 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 200l Water
Side-shoot-formation = 21 ≤ BBCH < 30	PA4 = 10.9 kg/ha Pelargonic Acid in 400l 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	

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 belowground 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 (Javaid and 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).

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).

2.4 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 (Table 2).

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 and 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).

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

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 aboveground disturbance of *C. arvense*. According to Verwijst et al. (2018), applying aboveground disturbance at the minimum regenerative capacity of *C. arvense* reduces the carbohydrate reserve of the roots. It prevents root extension and leads to an aboveground biomass reduction. The eight-to-ten-leaf stage is the growth stage in which this plant is most vulnerable to aboveground 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 (Figure 1 and 2).

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 (Figure 1). *C. arvense* 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 until 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 (Figure 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.

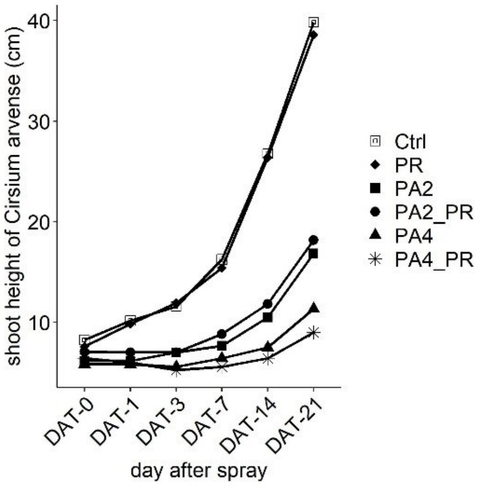

Figure 1. Linear Mixed Model: Effects of Pelargonic acid (PA) with different application volume after adding Paraffin oil as adjuvant on shoot height of *Cirsium arvense*. Treatments: see Table 1. DAT-0 = the day before application of the treatments.

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 and 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).

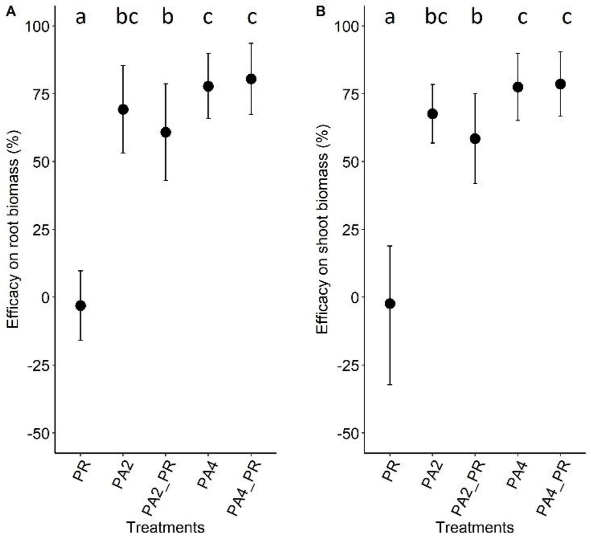

Figure 2. Efficacy of PA with different application volume after adding Paraffin oil as adjuvant. A: Efficacy on root biomass, B: Efficacy on shoot biomass. Treatments: see Table 1. Linear Mixed Model analysis. Different letters denote significant differences (p<0.05) according to the pairwise comparison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

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 belowground 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-herbicides like PA could in addition assist in agro-ecological management by terminating the shoots of perennial weeds without ploughing.

2.5 Acknowledgements

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2.6 References

Abouziena, H. F., and Hagaag, W. M. (2016). Weed control in clean agriculture: A review. Planta Daninha, 34(2), 377–392.

Bakker, D. (1960). A comparative life-history study of Cirsium arvense (L.) Scop. and Tussilago farfara L., the most troublesome weeds in the newly reclaimed polders of the former Zuiderzee. The Biology of Weeds: A Symposium of the British Ecological Society, Blackwell Scientific Publications, Oxford, pp. 205-222.

Bicksler, A. J., Masiunas, J. B., and Davis, A. (2012). Canada thistle (Cirsium arvense) suppression by sudangrass interference and defoliation. Weed science, 60(2), 260-266. https://doi.org/10.1614/WS-D-11-00145.1

Bolat, A., Bayat, A., Tetik, O., Karaagac, H. A., Cerit, I., and Sevilmis, U. (2018). Performance of herbicide spraying methods at different application volumes in maize. Fresenius Environmental Bulletin, 27(5), 2963–2967.

Burroughs, B., Tarone, R., Kesner, J. S., and Garry, V. F. (1999). Herbicides and adjuvants: An evolving view. Toxicology and Industrial Health, 15(1-2), 160–168. https://doi.org/10.1177/074823379901500113

Coleman, R., and Penner, D. (2006). Desiccant activity of short chain fatty acids. Weed Technology, 20, 410-415. https://doi.org/10.1614/WT-06-195.1

Cordeau, S., Triolet, M., Wayman, S., Steinberg, C., and Guillemin, J.P. (2016). Bioherbicides: Dead in the water? A review of the existing products for integrated weed management. Crop Protection, 87, 44-49. https://doi.org/10.1016/j.cropro.2016.04.016

Gustavsson, A.M.D. (1997). Growth and regenerative capacity of plants of Cirsium arvense. Weed Research. 37(4), 229-236. https://doi.org/10.1046/j.1365-3180.1997.d01-37.x

Hodgson, J. M. (1968). The nature, ecology, and control of Canada thistle. United States Department of Agriculture Technical Bulletin 1386, 32 pp.

Javaid, M. M., and Tanveer, A. (2013). Optimization of application efficacy for POST herbicides with adjuvants on three-cornered jack (Emex australis Steinheil) in wheat. Weed Technology, 27(3), 437–444. https://doi.org/10.1614/WT-D-11-00130.1

Kegley, S., Conlisk, E., and Moses, M. (2010). Marin Municipal Water District Vegetation Management Plan Herbicide Risk Assessment. Pesticide Research Institute. https://www.marinwater.org/DocumentCenter/View/259/Herbicide-Risk-Assessment-Chapter-1-Summary-January-10-2010 (Accessed on September 15, 2020).

Kieloch, R., and Domaradzki, K. (2011). The role of the growth stage of weeds in their response to reduced herbicide doses. Acta Agrobotanica, 64(4). https://doi.org/10.5586/aa.2011.068

Knoche, M. (1994). Effect of droplet size and carrier volume on performance of foliage-applied herbicides. Crop Protection, 13(3), 163–178. https://doi.org/10.1016/0261-2194(94)90075-2

McWhorter, C. G., Barrentine, W. L., and Hanks, J. E. (1992). Postemergence grass control with herbicides applied at ULV in paraffinic oil. Weed Technology, 6(2), 262–268. https://www.jstor.org/stable/3987284

Muñoz, M., Torres-Pagán, N., Peiró, R., Guijarro, R., Sánchez-Moreiras, A. M., and Verdeguer, M. (2020). Phytotoxic effects of three natural compounds: Pelargonic acid, carvacrol, and cinnamic aldehyde, against problematic weeds in Mediterranean crops. Agronomy, 10(6): 791. https://doi.org/10.3390/agronomy10060791

R Core Team. (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved March 23, 2021, from https://www.R-project.org/

Sagar, G. R., and Rawson, H. M. (1964). The biology of Cirsium arvense (L.) Scop. In Proceedings of the 7th British Weed Control Conference, Brighton, UK (pp. 553–562).

Tavaziva, V. J., Lundkvist, A., and Verwijst, T. (2019). Effects of selective cutting and timing of herbicide application on growth and development of Cirsium arvense in spring barley. Weed research, 59(5), 349-356. https://doi.org/10.1111/wre.12371

Travlos, I., Rapti, E., Gazoulis, I., Kanatas, P., Tataridas, A., Kakabouki, I., and Papastylianou, P. (2020). The herbicidal potential of different pelargonic acid products and essential oils against several important weed species. Agronomy, 10(11), 1687. https://doi.org/10.3390/agronomy10111687.

Verwijst, T., Tavaziva, V. J., and Lundkvist, A. (2018). Assessment of the compensation point of Cirsium arvense and effects of competition, root weight and burial depth on below-ground dry weight–leaf stage trajectories. Weed Research, 58(4), 292-303. https://doi.org/10.1111/wre.12312

Wilson, R. G., Martin, A. R., and Kachman, S. D. (2006). Seasonal changes in carbohydrates in the root of Canada thistle (Cirsium arvense) and the disruption of these changes by herbicides. Weed Technology, 20, 242–248. https://doi.org/10.1614/WT-05-052R1.1

Chapter 3: The effect of two-year application of pelargonic acid on the growth of *Cirsium arvense* (L.) Scop. and *Sonchus arvensis* L.

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3.1 Abstract

Synthetic herbicides are used for perennial weed management, but owing to environmental and health concerns they face increasing regulatory restrictions. Consequently, there is growing interest in ecologically friendly alternatives including bio-herbicides based on natural compounds such as the active ingredient pelargonic acid (PA). PA acts as a broad-spectrum non-selective contact herbicide. However, when used as a contact herbicide, regrowth of the aboveground parts of plants still presents a challenge. The aim of this study was to investigate the control effect of a two-year application of PA on perennial weeds. The study was conducted between spring 2020 and autumn 2021 as a semi-field experiment. The factors were two levels of weed species (*Cirsium arvense* and *Sonchus arvensis*), three levels of herbicide treatment (untreated control, PA, and glyphosate), and three levels of initial ramet size (5, 10, and 15 cm). The results showed that a two-year application of PA increased its efficacy on *C. arvense* and *S. arvensis* when combined with the smaller initial ramet size (5 cm), but did not prevent regrowth in either species. PA efficacy was greater on *C. arvense* than on *S. arvensis*. The plant coverage decreased by 24% when the initial ramet size was 5 cm for *C. arvense*, while for *S. arvensis* with the same initial ramet size it was reduced by just 4%. For PA-treated *C. arvense* with an initial ramet size of 5 cm, aboveground biomass and belowground biomass were reduced by 43% and 22% respectively. In *S. arvensis*, the reductions in aboveground and belowground biomass for an initial ramet size of 5 cm were 13% and 12% respectively. In general, PA efficacy was not as high as glyphosate efficacy for both species. In conclusion, the results revealed that after PA application the regrowth of shoots from the creeping roots in *C. arvense* and *S. arvensis* decreased when the initial ramet size was 5 cm. This reduction suggests that PA efficacy on these plants increases when it is applied repeatedly on the same patches with smaller initial root fragments.

Keywords: perennial weeds, bio-herbicide, ramet, creeping thistle, sow-thistle

3.2 Introduction

As perennial weeds, *Cirsium arvense* (L.) Scop. and *Sonchus arvensis* L. represent significant threats to agricultural productivity due to their ability to persist and spread over time and compete with crops for resources (Ramesh et al., 2017). *C. arvense* (creeping thistle) and *S. arvensis* (sow thistle) are found in various crops and are considered highly problematic in temperate regions (Liew et al., 2013; Tørresen and Gerowitt, 2022; Andert et al., 2023). Both weeds form patches and mainly have rapid vegetative reproduction through their subterranean reproductive organs (horizontal creeping roots) and abundant seed production (Tørresen and Gerowitt, 2022). Their allelopathic effects on some crops, especially the prevention of seed germination or seedling growth have been reported in previous studies (Szabó and Halbritter, 2015; Bashir et al., 2018; Egushova and Anokhina, 2022).

There are various control methods for these weeds, including chemical, mechanical, and cultural methods (Melander et al., 2012). The common chemical control method for perennial weeds is the use of systemic herbicides, e.g., the non-selective active ingredient glyphosate in the intercropping period

(Beckie et al., 2020). Furthermore, certain systemic herbicides are registered for application during the cropping period, e.g., the active ingredient metsulfuron-methyl can be used in cereals, effectively controlling a broad spectrum of weeds including *C. arvense* (Bhullar et al., 2013; Zargar et al., 2019). For decades, synthetic herbicides have been crucial for perennial weed control owing to their practical and financial advantages (Loddo et al., 2023). However, for herbicides such as glyphosate, stricter regulations concerning their registration and usage have been implemented in numerous countries due to concerns about herbicide resistance and their adverse effects on the environment and human health (Antier et al., 2020; Beckie et al., 2020). The 'Farm to Fork' strategy is one of the key components of the European Green Deal that reflects the growing interest in sustainable and ecologically friendly weed control solutions, including synthetic herbicide substitutions (European Commission, 2020; Radicetti and Mancinelli, 2021). Alternative approaches include mechanical methods, cultural tools, and alternative herbicides, which are non-chemical, natural, or less toxic (Synowiec et al., 2017; Ibáñez and Blázquez, 2018; Beckie et al., 2020).

Bio-herbicides are products of natural origin for weed control that are either microorganisms or products derived from living organisms, including the natural metabolites produced by these organisms (Cordeau et al., 2016). The herbicidal effects of natural substances, such as plant essential oils and organic acids, have been the subject of many studies in recent years. (Barton et al., 2014; Synowiec et al., 2017; Casella et al., 2023; Kouki et al., 2023). Among tested natural active ingredients, pelargonic acid (PA) is the sole ingredient available on the market (Loddo et al., 2023). PA is a naturally occurring fatty acid found in foods such as vegetables and fruits and has been approved as a safe food agent in numerous countries (Ciriminna et al., 2019). PA degrades quickly in the environment and does not cause long-term runoff contamination in rainy seasons (European Food Safety Authority, 2021). Bio-herbicides that contain the active ingredient PA are known as burn down herbicides and are increasingly used for weed control, e.g. on gardens, lawns, and walkways (Ciriminna et al., 2019).

In light of PA's potential as a bio-herbicide and its ability to contribute to the reduction of synthetic herbicides, researchers have been exploring its efficacy, and have shown that PA is the most successful bio-herbicide available (Webber et al., 2014a; Carroll et al., 2022; Muñoz et al., 2022; Pannacci et al., 2022). However, its effectiveness varies between weed species (Webber et al., 2014a, 2014b). Monocotyledon weeds such as *Alopecurus myosuroides* Huds. and *Lolium rigidum* Gaud. are less sensitive to PA, and may display reduced and transient symptoms at higher doses (Travlos et al., 2020; Loddo et al., 2023). Dicotyledon weeds also exhibit considerable differences in sensitivity to PA (Webber et al., 2014a, 2014b; Loddo et al., 2023). Furthermore, there is evidence of regrowth after the application of PA for most weeds (Ciriminna et al., 2019; Muñoz et al., 2020; Travlos et al., 2020; Muñoz et al., 2022; Loddo et al., 2023). Due to the occurrence of regrowth, previous studies have suggested sequential applications of PA with short intervals within a growing season (Barker and Prostak, 2009; Webber et al., 2014a, 2014b). Earlier investigations mainly concentrated on annual weeds, particularly examining the impact of PA with a focus on the aboveground parts of plants (Webber et al., 2014a, 2014b; Ciriminna et al., 2019; Muñoz et al., 2020; Travlos et al., 2020; Muñoz et al., 2022; Pannacci et al., 2022; Loddo et al., 2023). The presence of creeping roots in *C. arvense* and *S. arvensis* negatively affects the success of control methods (Liew et al., 2013). Adventitious buds are formed on the horizontal creeping roots, from which new shoots can emerge (Brandsæter et al., 2010; Liew et al., 2013). The activities of adventitious buds and the emergence of shoots differ from species to species (Brandsæter et al., 2010). Furthermore, the ability of creeping roots to sprout varies significantly during the season (Brandsæter et al., 2010; Liew et al., 2013). For *S. arvensis*, the sprouting capacity appears to decrease in late summer to early autumn (Håkansson, 1969; Håkansson and Wallgren, 1972; Brandsæter et al., 2010), while sprouting for *C. arvense* does not decrease as long as environmental conditions allow it (Brandsæter et al., 2010). The root fragment of the creeping perennial is called a ramet, which is genetically identical to the mother plant (Tørresen and Gerowitt, 2022), and it is often induced by mechanical soil disturbance (Håkansson, 2003). Large ramets can rapidly produce new *C. arvense* plants, while smaller ramets often do not survive to produce vegetative offspring due to their low carbohydrate reserves (Hamdoun, 1972). Similar to *C. arvense*, the emergence and number of sprouts in *S. arvensis* depend on the dry matter content of the roots (Lemna and Messersmith, 1990;

Vanhala et al., 2006). When the creeping roots are fragmented by tillage, the resulting smaller ramets are less viable and have less dry matter, making them more likely to die (Vanhala et al., 2006). Furthermore, there is a phenological stage at which belowground biomass reaches its minimum dry weight before it begins to increase again (Tavaziva, 2012). In both species, this stage occurs when they have between four and seven leaves (Håkansson, 2003). Depleting the belowground carbohydrate reserves through fragmentation of the regenerative structures and applying treatments to the lowest belowground biomass have been suggested by previous research as offering better control (Håkansson, 1969; Gustavsson, 1997; Brandsæter et al., 2010). To achieve successful control, it is essential to apply control methods to these perennial weeds when they are most sensitive to disturbance (Verwijst et al., 2018).

An earlier study investigated the effects of plant growth stage, application volume and the addition of adjuvant on PA efficacy to control *C. arvense*. The results demonstrated that PA efficacy is greater when applied on a 4-8 leaf-stage plant using an increased application volume, and also by adding an adjuvant (Ganji et al., 2022). The present study investigated the feasibility of repeated PA application over two consecutive years on the same spot (patch of *C. arvense* and *S. arvensis*) considering their initial ramet size. Its aim was to determine the PA herbicidal impact on the entire life cycle of the perennial weed species and their regrowth patterns that might not be identified in shorter-term studies. Moreover, the efficacy of PA as a potential herbicidal treatment was compared with that of the commonly used active ingredient glyphosate (GLY). This design facilitated a comprehensive comparison of the effects of PA treatment and two reference conditions: the untreated control (UC), representing the baseline or natural state, and GLY treatment, which serves as a standard for effective perennial weed control (Hudek et al., 2021; Kanatas et al., 2021).

The present study focused on perennial weeds with creeping roots and examined the influence of initial ramet size on PA efficacy after two applications within two consecutive years on the same spot (patch of *C. arvense* and *S. arvensis*), with the aim of improving knowledge about the control of perennial weeds with creeping roots using PA. Considering the regenerative capacity of the creeping roots, the objectives of this study were to determine (i) whether the initial ramet size influences PA efficacy and regrowth of *C. arvense* and *S. arvensis* after PA application and (ii) whether there are any differences between these perennial weeds in terms of PA efficacy and regrowth patterns after repeated application.

It was hypothesized that repeated application of PA over two consecutive years reduces growth parameters, aboveground and belowground biomass, and flower numbers in both species. It was expected that *S. arvensis* would be more susceptible to PA than *C. arvense* due to seasonal variations that impact the sprouting abilities of *S. arvensis* by demonstrating reduced capacity in late summer to early autumn (Håkansson, 1969; Håkansson and Wallgren, 1972; Brandsæter et al., 2010). For this reason, it was assumed that regrowth after herbicide application would differ between these species. Finally, it was also assumed that smaller *C. arvense* and *S. arvensis* ramets have a lower regenerative capacity, which reduces regrowth and increases PA efficacy.

3.3 Materials and Methods

A two-year pot experiment (from spring 2020 to autumn 2021) under semi-field conditions was conducted in the experimental field of Rostock University in northeast Germany (location Rostock: 54° 4' 6.726" N, 12° 4' 54.0876" E).

Figure 3 provides a concise overview of the experimental workflow used in this semi-field experiment. The process included (1) the preparation of the semi-field experiment by planting ramets in pots, (2) the application of the herbicide treatments in each experimental year, and (3) visual assessments to measure the control efficacy of the herbicides compared with the untreated control.

The experiment was a factorial, completely randomized block design with four replications. The factors were plant species with two levels, treatments with three levels, and ramets with three levels of initial size: 5, 10, and 15 cm (Table 3). For this research, Beloukha® (680 g/L pelargonic acid) and

Roundup Powerflex® (480 g/L glyphosate and 393.6 g/L acid equivalent) were used as the PA and GLY treatments, respectively.

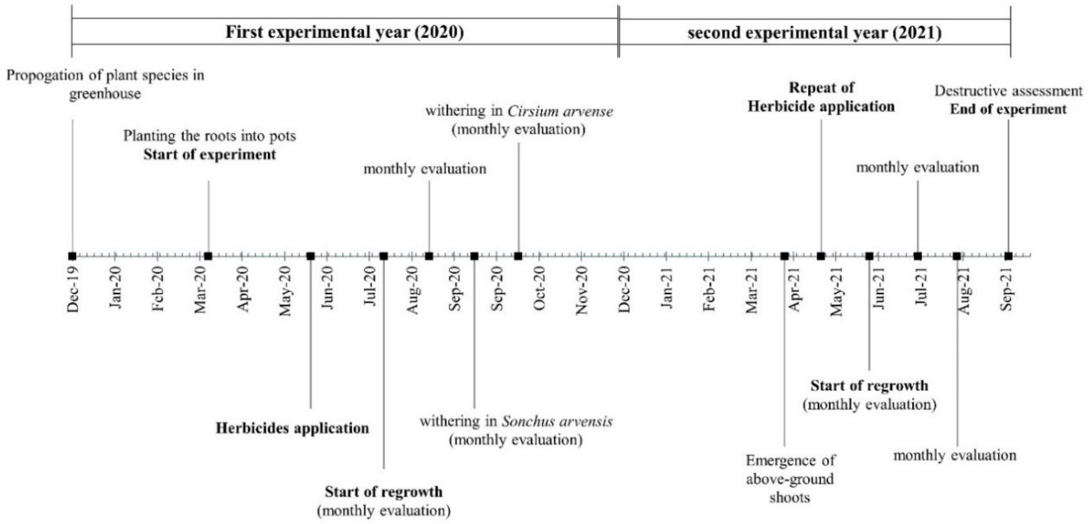


Figure 3. Experimental set up, assessments, and activities over the two years of the experiment.

Table 3. Overview of factorial experiment design with plant species, herbicide treatments, and initial ramet size as factors

Factors	**Levels of factor**	**Abb.**[1]	**Description of herbicides**	
			Used amount	**Active ingredient content (g/L)**
Treatments (Herbicides)	Untreated control	UC	-	-
	Pelargonic acid	PA	16 L/ha	680 g/L pelargonic acid
	Glyphosate	GLY	3 L/ha	480 g/L glyphosate
Plant Species	*Cirsium arvense*			
	Sonchus arvensis			
Ramet initial size	5 cm			
	10 cm			
	15 cm			

[1] Abbreviation for treatment names

Ramet planting in pots

In December 2019, creeping roots of *C. arvense* were collected from the University of Rostock's experimental field and those of *S. arvensis* from a field near Güstrow (53°46'14.6"N, 12°09'59.3"E). The collected roots were stored by propagation in pots in the greenhouse. In February 2020, the pots were buried in the soil and filled with a mixture of ½ field soil, ¼ potting soil, and ¼ compost. The pot size was 200 L with a diameter of 80 cm (pot surface area 0.503 m^2). The distance between pots was one meter. The creeping roots obtained from propagated greenhouse pots (cleaned and washed to eliminate the remaining soil) were used for the semi-field experiment. Roots that were more than 3 mm in diameter were fragmented into ramets 5, 10, and 15 cm in length, and weighed. The term ramet is used in the text instead of root fragment because the root fragments used in this experiment were derived from one progenitor of each plant species, and shared the same genotypes as the parent plants (Tørresen and Gerowitt, 2022). Finally, one single ramet was planted directly at a 5 cm soil depth in each pot. The pots were irrigated immediately after the ramets were planted and again when there was a need for irrigation on warm days during the growing seasons in both experimental years. After the establishment of the pots in the soil and until the end of the experiment in the second year, other weeds were removed from the pots by hand. Before herbicide application in both experimental years, fertilization was undertaken to achieve nutrient conditions comparable with spring cereal fields. Hakaphos Blau® as an NPK fertilizer (15% N, 10% P, and 15% K) was applied to the soil at the amount of 16.65 g per pot as a balanced nutrient solution. These rates correspond to 50 kg of nitrogen per hectare, which is less fertilization than regular spring cereal fields as there was no crop in the pots to compete for the nutrients.

Application of herbicide treatments

At the end of May 2020, herbicide treatments were applied to the *S. arvensis* pots. Owing to the late emergence of *C. arvense* sprouts, the herbicide application for this species was performed two weeks later. The plant growth stage at the time of herbicide applications for both plant species was the four-to-eight-leaf-stage considering the compensation point according to Håkansson (2003) and BBCH 14-18 according to Meier (2018), and each pot had one or two plants (shoots from the same ramet).

At the beginning of May 2021, the herbicide treatments were applied again. Each pot was treated with the same herbicide treatment as the previous year. The plant growth stage at herbicide application time was four-to-eight-leaf, but each pot had many shoots emerged from the same ramet which was recorded. In both years, a plot-spraying device with a pressure of 2.1 bar and a speed of 4 kilometers per hour was utilized for the herbicide applications. The application volume for the treatments was 200 L/ha. The operated flat jet nozzle was size 02.

Assessments

The herbicide treatments were assessed by visually estimating the percentage of necrotization. The assessments were conducted at 1, 7, 14, and 21 days after treatment (DAT) of the herbicides. A value of 0% necrotization was equivalent to completely vital vegetation, while a value of 100% represented completely dead vegetation. The level of necrotization was interpreted as herbicide efficacy. The pots were evaluated for regrowth from the ramets starting 28 days after herbicide treatment (28th_DAT). To be able to monitor the regrowth pattern and effect of the treatments over the long-term experimental period and identify the possible variability in regrowth pattern, data on plant height, shoot density per m^2, and BBCH stage (Meier, 2018) were collected monthly until the end of the growing season. The monthly evaluations were performed from July to October 2020 and from April to September 2021. To obtain information on how the weed species population was established and regrew after herbicide application during the growing season, in August of each year before the withering of the plants, the shoot density per m^2, the percentage coverage of the soil surface by the plant, and the number of flowers were assessed. All emerged shoots were counted and then shoot density per m^2 was calculated according to a pot surface area of 0.503 m^2. Counting of the flowers indicates the reproductive capacity of the weed species, and helps to understand the potential for seed production after application of the treatments. By undertaking this assessment in August, the weed's reproductive success was estimated, before it completed its life cycle. Aboveground and belowground biomass was measured per pot using the destructive method at the end of the experiment in 2021, which determined the effectiveness of the herbicide treatment at controlling weed growth. For this, the collected plant materials were placed in an oven at 60 °C for at least 24 hours, and the dried biomass was then measured. As mentioned in the experimental setup, ramets were weighed before being planted in the pots and this was used as a covariate in the statistical analysis. Figure 3 gives an overview of the above-mentioned experimental setup and assessments.

Weather conditions

Figure 4 shows the weather conditions for the entire experimental period. The average air temperature for the growing season (March to September) was 13.2 °C in 2020 and 13.1 °C in 2021. In the 2020 growing season, the minimum average soil temperatures were 5.9 °C and 5.6 °C respectively at 5 cm and 20 cm belowground level in March. In August, the maximum average temperatures at 5 cm and 20 cm belowground level were 19.6 °C and 18.8 °C respectively. In 2021, the minimum soil temperature at these belowground levels averaged 2.3 °C and 2.1 °C, respectively, and the maximum was 20.7 °C in 5 cm and 19.8 °C in 20 cm soil depth in July. The total amount of precipitation in 2020 was 428 mm. Of this amount, 321 mm occurred in the growing season. The precipitation amount from January until the end of September 2021 was 500 mm, of which 376 mm occurred in the growing season (Figure 4).

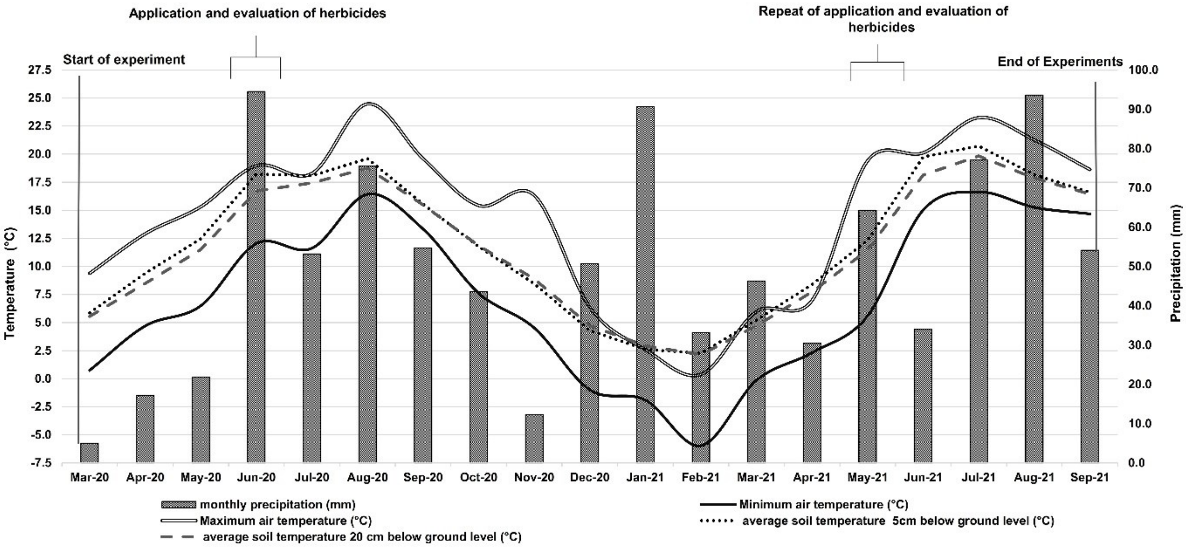


Figure 4. Air temperature (°C), soil temperatures at 5 cm and 20 cm belowground level, and precipitation (mm) during the experimental period in 2020 and 2021. Information for both years was obtained from the research weather station of the University of Rostock's Department of Hydrology and Applied Meteorology.

Statistical analysis

Data on herbicide efficacy (necrotization percentage) were analyzed using linear mixed model analysis (LMM), with weed species, herbicide treatments, initial ramet size, DATs and their interaction as fixed effects, and replicates as random effects. In this analysis, the following model (Equation 1) was fitted for two consecutive years, 2020 and 2021. For each year, the same model structure with shared fixed effects was used, but allowed for separate random intercepts (b_m) to capture year-specific variability:

$$\text{Herbicide efficacy}(\%)_{\text{ijklm}} = \mu + S_i \times H_j \times RS_k \times D_l + b_m + \epsilon_{ijkl} \ldots\ldots\ldots\ldots\ldots\ldots \text{(1)}$$

where μ is the overall mean; S_i, H_j, RS_k and D_l represent the fixed effects for "weed species", "herbicide treatment", "initial ramet size", and "DATs" respectively; b_m represents the random intercepts for the grouping variable "block", and ϵ_{ijkl} is the error term that accounts for unexplained variability in the model.

The long-term effectiveness of PA was revealed through a separate analysis at 21^{st}_DAT for each experimental year, providing information to understand the sustained impact of weed control over the course of the experiment. The same model structure (Equation 2) with shared fixed effects was utilized to analyze the herbicide efficacy in the final assessment at 21^{st}_DAT as follows:

$$\text{Herbicide efficacy at } 21^{st}\text{\_DAT}(\%)_{\text{ijkl}} = \mu + S_i \times H_j \times RS_k + b_l + \epsilon_{ijk} \ldots\ldots\ldots\ldots\ldots \text{(2)}$$

where μ is the overall mean; S_i, H_j and RS_k are the fixed effects for "weed species", "herbicide treatment", "initial ramet size", and "DATs" respectively; b_m represents the random intercepts for the grouping variable "block", and ϵ_{ijkl} is the error term.

As the experimental years were not independent of each other, in order to examine the effect of factors on the measured variables after regrowth, which were shoot density per m^2, the percentage coverage of the soil surface by the plant, and the number of flowers, the data from the measurements at the end of the experiment were utilized for statistical analysis using LMM (Equation 3-7). Three factors (weed species, herbicide treatment, and initial ramet size) and their interaction were fixed effects in this analysis. The random effects were replicates (blocks) and the initial weight of ramets (as covariates). It was assumed that the association between the initial weight of ramets and measured variables depended on the magnitude of weight, thus the heterogeneity of weight was modeled as a random effect in the data analysis of this research. The model equations are as follows:

$$\text{Aboveground biomass}_{ijk} = \mu + S_i \times H_j \times RS_{ij} + b_k + \epsilon_{ijk} \quad (3)$$

$$\text{Belowground biomass}_{ijk} = \mu + S_i \times H_j \times RS_{ij} + b_k + \epsilon_{ijk} \quad (4)$$

$$\text{Shoot density}_{ijk} = \mu + S_i \times H_j \times RS_{ij} + b_k + \epsilon_{ijk} \quad (5)$$

$$\text{Soil coverage (\%)}_{ijk} = \mu + S_i \times H_j \times RS_{ij} + b_k + \epsilon_{ijk} \quad (6)$$

$$\text{Flower head}_{ijk} = \mu + S_i \times H_j \times RS_{ij} + b_k + \epsilon_{ijk} \quad (7)$$

where μ is the overall mean; S_i is the effect of species; H_j is the effect of herbicide treatment; RS_{ij} is the effect of ramet size; b_k represents the random intercept for block and initial weight of ramets; ϵ_{ijk} is the random error term.

For the analysis of the monthly evaluation of plant height, in addition to the fixed effects of weed species, herbicide treatments, initial ramet size, and their interactions, the fixed effect of the experimental year and its interaction with the fixed effects of these three factors were included in the model (Equation 8). To account for variability within different levels of block and month, random intercepts were included. This allowed the model to capture random variations in plant height within these nested grouping variables. In addition, the model assumed a first-order autoregressive correlation structure (AR1) within each combination of block and month. This choice of correlation structure accounted for potential temporal autocorrelation in plant height measurements within the same block and month combinations. The model equation is as follows:

$$\text{Height}_{ijkl} = \mu + S_i \times H_j \times RS_k \times Y_l + b_m + \epsilon_{ijkl} \quad (8)$$

where μ is the overall mean; S_i is the effect of species; H_j is the effect of herbicide treatment; RS_k is the effect of ramet size; Y_l is the effect of year; b_m represents the random intercept for block and month in which the subscript "m" represents different levels of the month nested within block, and ϵ_{ijkl} is the random error term.

All the models were fitted using the restricted maximum likelihood (REML) method. To evaluate the goodness of fit of the linear mixed models (LMMs), the variance decomposition method was employed to calculate conditional R-squared values for all models. The variance decomposition method was chosen because it has the ability to dissect the total variance in the response variable into components related to fixed effects, random effects, and residual error. The same method was used to calculate marginal R-squared values for all models except the model for plant height. The log-likelihood method was utilized due to the unique characteristics of this particular model, which required a different approach for evaluating goodness of fit. The log-likelihood method was more appropriate in this specific case, as it was able to capture the subtle details of the data better, resulting in a more precise evaluation of the model's performance.

For all the data, pairwise comparisons were conducted using Tukey's HSD tests on the results of LMMs to identify significant differences between treatments by including all the interactions of fixed effects. R version 4.3.0 (R Core Team, 2023) was used to conduct all statistical analyses, and the packages "nlme" (Pinheiro and Bates, 2023), "lme4" (Bates et. al, 2015), "lmerTest" (Kuznetsova et al., 2017), and "emmeans" (Lenth et al., 2018) were used for the LMMs and pairwise comparisons.

3.4 Results

Herbicide efficacy

The results of herbicide efficacy at all DATs showed that the main effect of herbicide treatment on herbicide efficacy was statistically significant, which means that the relationship between herbicide treatment and herbicide efficacy varied at different DATs. The results did not express a significant difference between initial ramet sizes or between the weed species *C. arvense* and *S. arvensis* (Supplementary Table 1). However, ramet sizes and species will be presented separately in the text due to biological concerns.

Herbicide efficacy across multiple assessment days in both years showed that the PA treatment had highest efficacy on both weed species at 1st_DAT in 2020, which decreased over the experimental period. PA treatment efficacy for both species was significantly higher than the GLY treatment and UC for all ramet sizes at 1st and 7th_DAT in 2020. The PA treatment showed a statistically significantly higher efficacy on the 5 cm ramet size in *C. arvense* compared with all UC treatments until 21st_DAT in 2020 (Supplementary Table 1). The PA treatment efficacy between various ramet sizes was not significant. For both weed species in 2020, the differences between 1st_DAT and 14th/21st_DAT were significant for PA treatment efficacy on all ramet sizes. PA treatment efficacy at 7th_DAT was significantly different from 21st_DAT for *C. arvense*, ramet sizes 10 cm and 15 cm, and for all ramet sizes of *S. arvensis* (Supplementary Table 1 and Figure 5).

After repeated application in 2021, the effect of the PA treatment showed a similar trend and efficacy on both weed species. The GLY treatment in this experiment showed 90 -100% efficacy for all ramet sizes in both species starting at 7th_DAT in 2020 and on a day between 7th and 14th_DAT in 2021. The plants with the smallest ramet size treated with GLY in 2020 did not regrow in 2021. The *C. arvense* plants with initial ramet sizes of 10 cm and 15 cm treated with GLY did not regrow in the second year of the experiment either. Therefore, GLY treatment efficacies for all ramet sizes of *C. arvense* and the 5 cm ramet size in *S. arvensis* are not displayed on the graph in the second year (Figure 5).

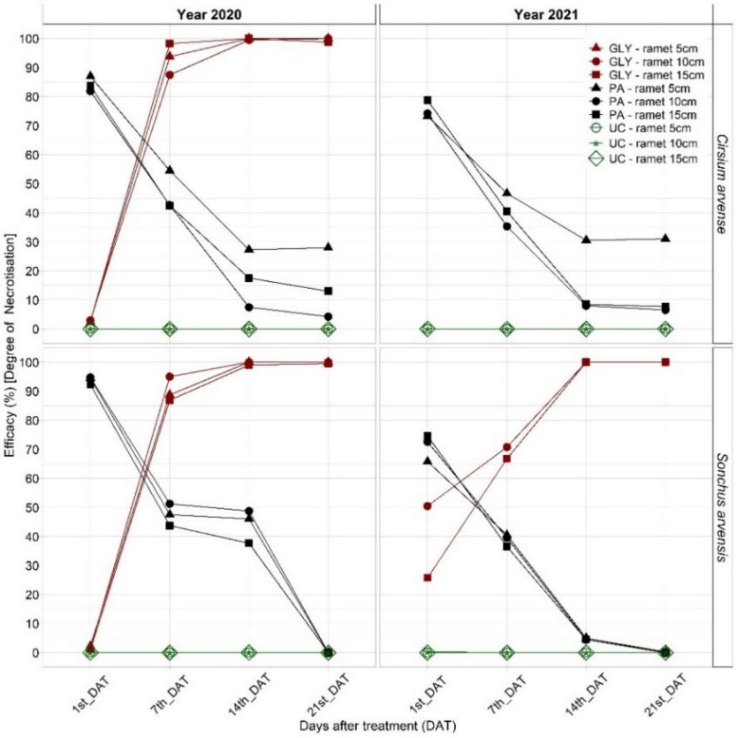


Figure 5. PA treatment efficacy (%) [degree of necrotization] compared with the untreated control and GLY treatment at 1st, 7th, 14th, and 21st_DAT after application on *C. arvense* and *S. arvensis* in the first year and repeated application in the second year of experiment. PA: Pelargonic acid; GLY: glyphosate; UC: untreated control.

For the evaluation of herbicide efficacy at 21st_DAT in both 2020 and 2021, individual effects of weed species and ramet size were found to be non-significant (Supplementary Figure 1). At 21st_DAT in both years, the PA treatment exhibited an effect that was not statistically significant, whereas the GLY treatment demonstrated a statistically significant impact. In 2020, the interaction effects between *S. arvensis* and the PA treatment as well as between the 5 cm ramet size and the PA treatment did not exhibit a statistical significance. In 2021, the interaction effects between the GLY treatment and the initial ramet size of 10 cm showed a significant positive influence. There was a statistically significant positive association between the 5 cm ramet size and herbicide efficacy when the PA treatment was applied (Supplementary Figure 1 and Figure 5).

Regrowth after herbicide application

i. Nondestructive regrowth evaluation

For shoot density per m^2, the main effects of weed species and the interaction between weed species with the GLY treatment were significant, while other fixed effects and their interactions did not have a significant effect (Figure 6). According to these results, the difference between species was statistically significant. With the untreated control and considering the same initial ramet size, the shoot density of *S. arvensis* was on average 249.8 shoots per m^2 greater than *C. arvense*. With the PA treatment, after regrowth the shoot density of *C. arvense* for all initial ramet sizes was significantly lower than for *S. arvensis*. There was no statistically significant difference between the PA and UC treatments in all initial ramet sizes for both weed species. However, when not accounting for initial ramet size, application of PA compared with untreated control led to a maximum 9% reduction in shoot density of *C. arvense* per m^2. Shoot density in the GLY treatments was significantly lower than in all the other treatments. (Figure 6).

The results showed significant differences in soil coverage between the two plant species and between the three levels of herbicide (Figure 7). The average soil coverage of *S. arvensis* was 27.8% higher than *C. arvense* when *S. arvensis* with the same initial ramet size was exposed to the UC treatment. The average soil coverage by plants treated with GLY was 65.4% lower than that of UC plants in the same weed species and with the same initial ramet size. This difference was also statistically significant. Although PA-treated *C. arvense* plants covered an average lower percentage of the soil surface on average than PA-treated *S. arvensis* plants, the difference between the two species was not significant for any ramet size. In *C. arvense*, the initial ramet size of 5 cm treated with PA covered around 50% of the soil surface, while for the same ramet size under UC around 70% of the soil surface was covered. *S. arvensis* with an initial ramet size of 10 cm also showed the lowest soil coverage compared with UC with the same initial ramet size. However, the differences in both cases were not statistically significant (Figure 7).

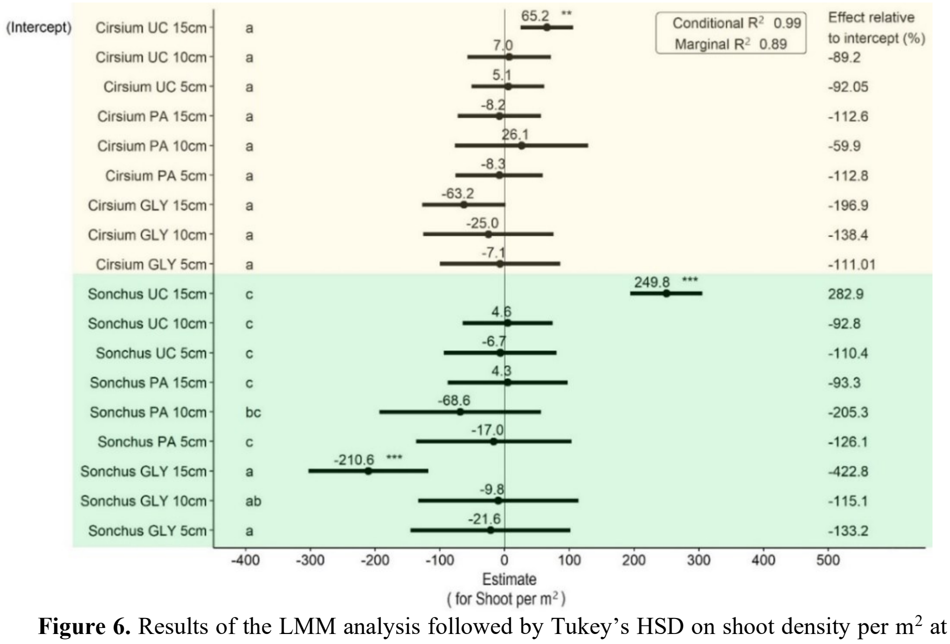


Figure 6. Results of the LMM analysis followed by Tukey's HSD on shoot density per m^2 at the end of the experiment. Significance codes obtained from the LMM analysis for main effects shown in this graph are as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Groups with different letters are significantly different at $p < 0.05$ level based on Tukey's HSD test. PA: Pelargonic acid; GLY: glyphosate; UC: untreated control; Cirsium: *Cirsium arvense*; Sonchus: *Sonchus arvensis*. 5 cm, 10 cm and 15 cm represent the initial size of ramets.

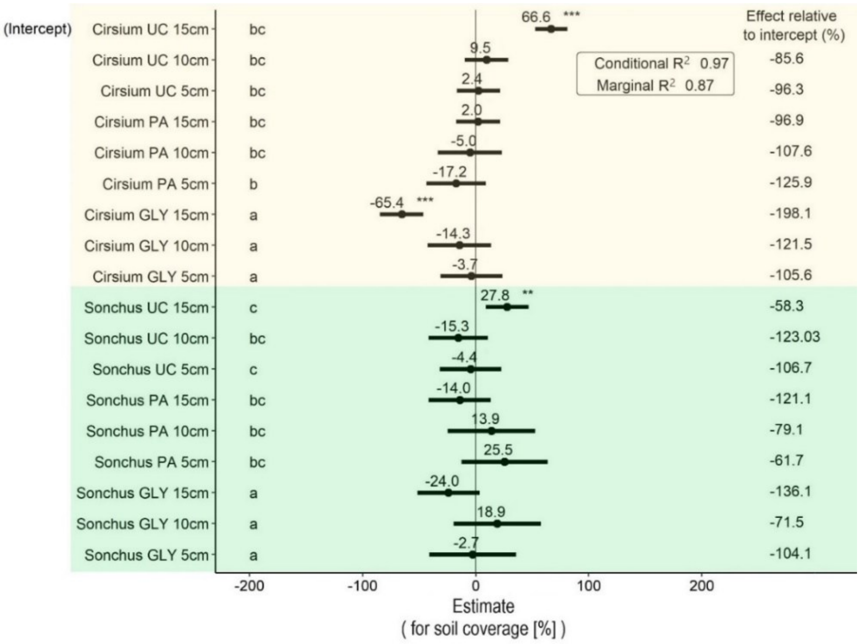


Figure 7. Results of the LMM analysis followed by Tukey's HSD on soil surface coverage (%) at the end of the experiment. Significance codes obtained from the LMM analysis for main effects shown in this graph are as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Groups with different letters are significantly different at $p < 0.05$ level based on Tukey's HSD test. PA: Pelargonic acid; GLY: glyphosate; UC: untreated control; Cirsium: *Cirsium arvense*; Sonchus: *Sonchus arvensis*. 5 cm, 10 cm and 15 cm represent the initial size of ramets.

The main effect of herbicide treatments on the number of flowers was significant (Figure 8). When comparing untreated plants with the same initial ramet size, *S. arvensis* produced approximately 10 flowers more than *C. arvense* on average. PA-treated plants of *S. arvensis* in 5 cm and 15 cm ramet sizes produced a smaller number of flowers compared with UC of the same ramet sizes, while a smaller number of flowers were produced by *C. arvense* with initial ramet sizes of 5 cm and 10 cm, but these differences were not statistically significant (Figure 8).

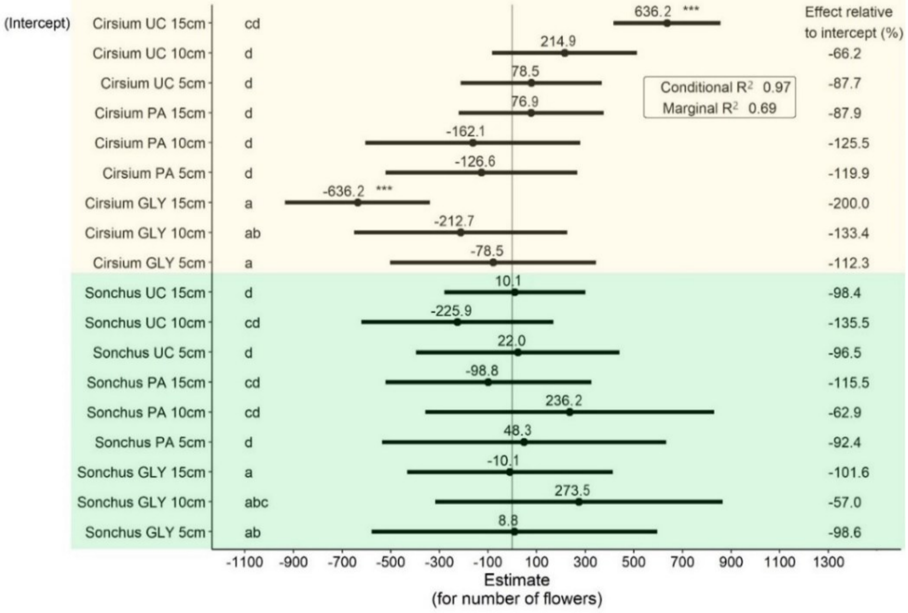


Figure 8. Results of the LMM analysis followed by Tukey's HSD on the number of produced flowers at the end of the experiment. Significance codes obtained from the LMM analysis for main effects shown in this graph are as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Groups with different letters are significantly different at $p < 0.05$ level based on Tukey's HSD test. PA: Pelargonic acid; GLY: glyphosate; UC: untreated control; Cirsium: *Cirsium arvense*; Sonchus: *Sonchus arvensis*. 5 cm, 10 cm and 15 cm represent the initial size of ramets.

ii. *Destructive evaluation of biomass*

The results for aboveground and belowground biomass demonstrated no evidence of differences between the two weed species after regrowth (Figure 9, Supplementary Tables 2 and 3). The fixed effect of herbicide treatments was significant. No significant difference was found in aboveground or belowground biomass between the PA treatments and UC treatments. The effect of initial ramet size on both biomass categories was not significant either. However, the results showed a significant interaction effect between PA treatment and ramet size of 5 cm in the case of aboveground biomass. The results also suggested that there might be an interaction effect on aboveground biomass between species, PA treatment, and ramet size of 5 cm, but this was not strong enough to be considered statistically significant. In the case of belowground biomass, there was a statistically significant interaction effect between species and ramet size of 10 cm (Supplementary Tables 2 and 3). The results showed no significant differences in aboveground and belowground biomass between PA-treated plants and UC treatments for any ramet size (Figure 9). Nevertheless, when comparing the UC treatments with PA treatments in *S. arvensis*, the lowest aboveground biomass of 236.7 g and belowground biomass of 85.4 g belonged to PA-treated plants with an initial ramet size of 5cm, which corresponds to reduction of 13% and 12% in aboveground and belowground biomass respectively. The lowest aboveground biomass for *C. arvense* was 181.9 g obtained from PA-treated plants with an initial ramet size of 5 cm, but the lowest belowground dry biomass was 68.2 g obtained from an initial ramet size of 15 cm treated with PA. When comparing untreated control and PA-treated plants in *C. arvense*, the application of PA decreased the belowground biomass of *C. arvense* by 22% with a 5 cm initial ramet size and by 16% when the ramet sizes were larger. The aboveground biomass of *C. arvense* with a 5 cm initial ramet size was reduced by 43%, while for the larger ramet sizes, it was just 2% (Figure 9).

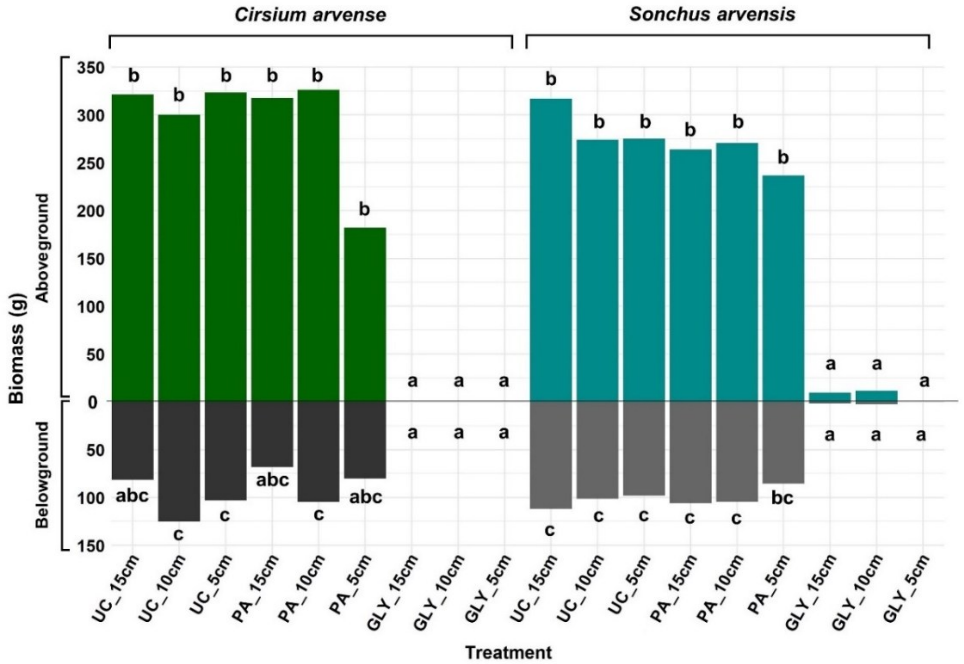


Figure 9. Aboveground and belowground biomass of *C. arvense* and *S. arvensis* after regrowth. Different letters show significant differences between treatments at $p < 0.05$ using Tukey's HSD test separately for aboveground biomass and belowground biomass. PA: Pelargonic acid; GLY: glyphosate; UC: untreated control; 5 cm, 10 cm and 15 cm represent the initial size of ramets.

Plant height during the experimental period

The main effects of experimental year, weed species, and herbicide treatments on plant height were statistically significant (Figure 10, Supplementary Table 4). Other significant interaction effects on plant height were found between species and the year 2021, and between herbicide treatments and the year 2021. Specifically, *S. arvensis* had almost the same height in 2021 compared with 2020, while *C. arvense* was taller in 2021 than in 2020. Generally, *C. arvense* tended to be significantly taller than *S.*

arvensis. In the case of herbicide treatments, the PA treatment had a more negative effect on plant height in 2020 than in 2021. The application of the PA and GLY treatments resulted in significantly shorter plants compared with the UC, while initial ramet size did not have a significant effect on height (Supplementary Table 4). In 2021, PA-treated plants of *C. arvense* with an initial ramet size of 10 cm were significantly shorter than *C. arvense* plants with the same initial ramet size in the UC treatment. For *S. arvensis*, there were no significant differences between the PA and UC treatments (Figure 10, Supplementary Table 4).

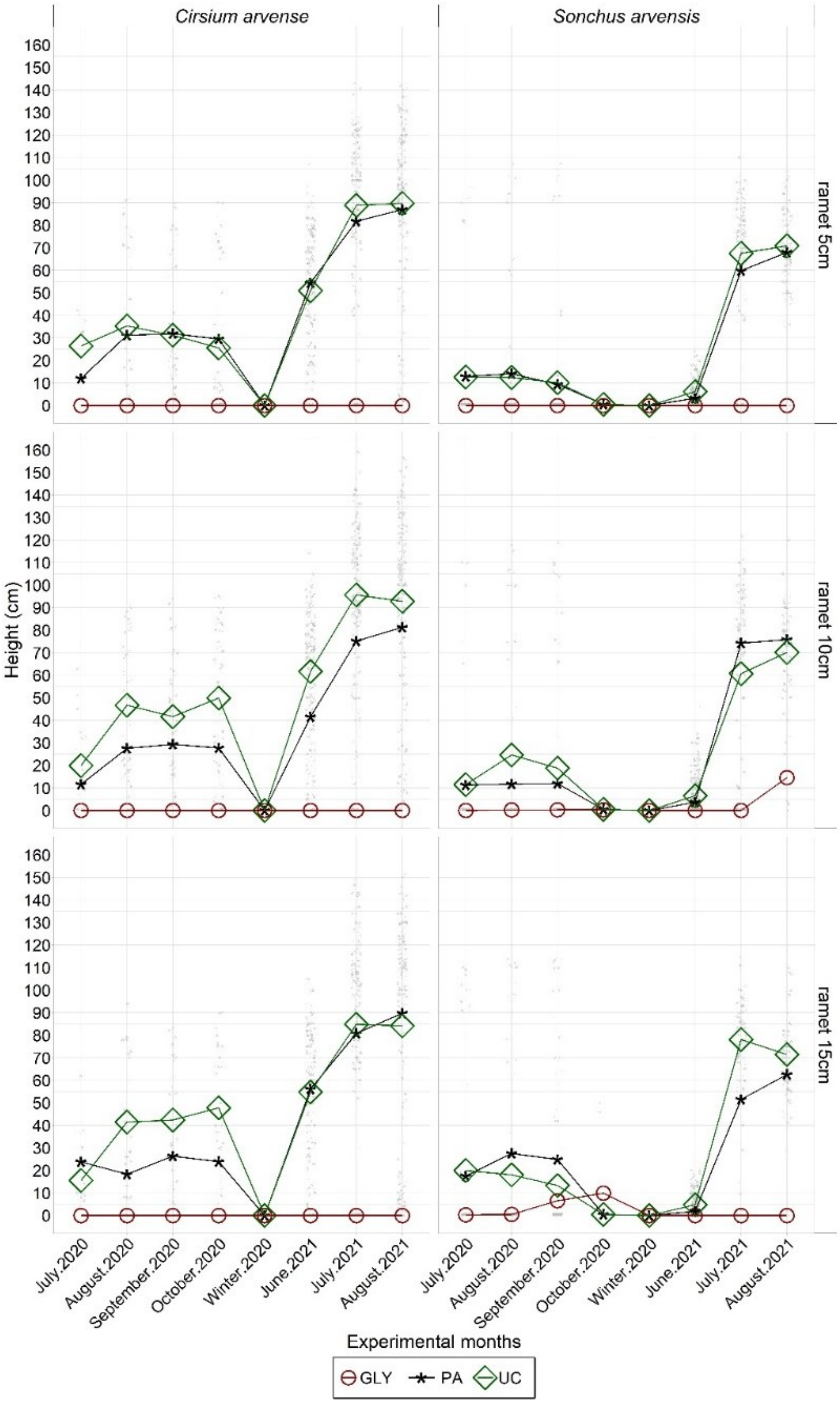


Figure 10. Monthly measured plant heights (cm) of regrown *C. arvense* and *S. arvensis* after herbicide applications during the experimental period. PA: Pelargonic acid; GLY: glyphosate; UC: untreated control.

3.5 Discussion

The aim of this study was to investigate the effects of twice application of PA on *C. arvense* and *S. arvensis* in two consecutive growing seasons. Their regrowth after PA applications was evaluated by combining two approaches: targeting the aboveground parts of weed species with twice application of PA, and addressing the belowground root systems using three different initial ramet sizes. By adopting different initial ramet sizes, the objective was to obtain insights into the effects of fragmenting the creeping root system of perennials, as it would be affected by mechanical disturbance (e.g., tillage practices) in real on-farm situations. However, the use of initial ramet sizes and their effect on PA efficacy did not represent mechanical control, rather, offered a valuable perspective and understanding of the fragmentation effects of creeping roots.

This study provided evidence that PA application reduces the aboveground and belowground biomass, as well as flower production in *C. arvense* and *S. arvensis*. This was remarkable when the initial ramet size was smaller, suggesting that PA exhibits an enhanced efficacy on these plants when applied on the same patches repeatedly in combination with smaller initial root fragments. However, the quantity and persistence of these effects were lower than those produced by the GLY treatment. After application of GLY in the first experimental year, *C. arvense* did not regrow regardless of ramet size, and *S. arvensis* demonstrated regrowth only for ramet sizes of 10 cm and 15 cm. In November 2023, the European Commission announced the extension of the glyphosate license for another decade after months of debate. However, this renewed license would be accompanied by new limitations and rules. The statement also highlighted that governments retained the authority to restrict the use of glyphosate in their own countries if they deemed the risks too high (Casassus, 2023), particularly concerning the preservation of biodiversity (Sullivan and Sullivan, 2003; Andert et al. 2022; El Jaouhari et al., 2023). Given the strong criticism regarding the use of glyphosate, as well as the new limitations and rules imposed by the commission, it is still crucial to find sustainable alternatives for it (Antier et al., 2020; Beckie et al., 2020; Casassus, 2023). Glyphosate is a non-selective systemic herbicide. Its effect is not visible in the early days after application because the plant takes some days to absorb and distribute glyphosate inside its tissues, which varies depending on the plant type (annual, perennial), growth stage, and environmental conditions (Sprankle et al., 1975; Satchivi et al., 2000; Fadin et al., 2018). PA causes necrotic lesions on plant aerial parts by attacking and destroying cell membranes of the plant epidermis, and causing rapid tissue dehydration (Ciriminna et al., 2019; Campos et al., 2022b). The PA mode of action implies that as a burndown herbicide, it could be a fast but temporary solution for controlling weeds, especially when there are work bottlenecks such as weather conditions or time constraints (Webber et al., 2014c; Pannacci et al., 2022; Campos et al., 2022a). Although PA may offer various advantages, it is occasionally misinterpreted as being similar to glyphosate and other pre-emergence herbicides, creating misleading expectations about its effectiveness and thus improper use (Campos et al., 2022b). A single application of PA does not provide lasting weed control (Patton et al., 2019; Loddo et al., 2023). Nevertheless, the present results show that it has the potential to be used in combination with other approaches, and thus offers an alternative to glyphosate against perennial weeds. Its combination with the smallest ramet size reduced growth parameters compared with the untreated control, even though there was no second fragmentation or soil mechanical treatments during the two-year experimental period. This finding is similar to the findings previously reported by Kanatas et al. (2020) that the use of a stale seedbed method integrated with the application of PA decreases perennial weeds. Other studies using PA as a weed control tool suggest the use of PA as a valuable tool for weed management approaches that use multiple tactics (Kanatas et al., 2022; Pannacci et al., 2022; Loddo et al., 2023). Due to its rapid burn-down effect, PA has a wide range of practical applications in weed management (Crmaric et al., 2018; Krauss et al., 2020), such as precision spot weeding (Webber and Webber, 2011; European Food Safety Authority, 2021), crop desiccation, and sucker control in plants (Coleman and Penner, 2008; Short et al., 2020). Given its effectiveness on many annual herbaceous weeds, it could potentially be used to manage weed growth in stubble and for pre-sowing herbicide applications (Andert and Gerowitt, 2020). The use of soil cultivation tools on a farm infested with perennial weeds such as *S. arvensis* leads to root fragmentation and buries the fragmented roots deep in the soil (Brandsæter et al., 2017), or brings them to the soil surface and enhances the decay of root fragments (Vanhala et al., 2006). It is likely that small perennial weed plants remain in the field after harvest, and if stubble cultivation does not control these small plants, they will accumulate nutrient reserves in their creeping roots for the next growing season (Håkansson, 2003; Vanhala et al., 2006). During this period, PA application might help achieve successful perennial weed control because PA can be applied on the 4-8-leaf-stage plants (Ganji et al., 2022) that are not only at their sensitive aboveground stage (Håkansson, 2003; Tavaziva, 2012), but also face a lack of nutrient reserves due to fragmentation by soil cultivation tools (Brandsæter et al., 2010). In this study, enhancement in PA efficacy was observed when applied on plants at the 4-8-leaf-stage that grew from initial smaller ramet sizes, proving the sensitivity of plants because of their smaller amount of nutrient reserves according to the abovementioned research findings.

It was anticipated that *S. arvensis* would probably be more susceptible to PA than *C. arvense* due to seasonal variations affecting its sprouting ability. As anticipated, the regrowth of weed species after herbicide did differ, but contrary to expectations, *C. arvense* seemed to be more susceptible to PA than *S. arvensis* (Figures 9 and 10). The assessments of plant growth parameters after regrowth suggested that shoot density per m^2 and soil coverage varied based on the weed species (Figures 6 and 7). When comparing weed species considering the same initial ramet size, *S. arvensis* has a higher shoot density per m^2. When comparing the effects of PA treatment on shoot density between the two species, *C. arvense* has a lower shoot density than *S. arvensis*. According to the investigation carried out by Liew et al. (2013), the higher shoot density in *S. arvensis* compared with *C. arvense* is due to the presence of a higher bud density on adventitious roots of *S. arvensis*. In *S. arvensis*, there was an observable effect of 5 cm ramet size on PA efficacy. There was a lower shoot density per m^2 in PA-treated *S. arvensis* with 5 cm initial ramet compared with the untreated control. Previous studies have confirmed that a longer root fragment of *S. arvensis* produces more shoots than a shorter one (Anbari et al. 2011). In the untreated conditions of the current study, *S. arvensis* generally exhibited greater soil coverage than *C. arvense*. PA application resulted in a lower percentage of coverage for both species, although it was lower for *C. arvense* plants, particularly with smaller initial ramets, than for *S. arvensis* plants. These results are in agreement with the findings of Ward and Mervosh (2012) whose application of a PA treatment for two consecutive years on the same plot effectively reduced the coverage of *Microstegium vimineum*.

In general, PA-treated plants produced a smaller number of flowers than untreated control plants (Figure 8). Among PA-treated plants in both species, the smaller ramet size exhibited a smaller number of flowers. In a semi-field experiment using boxes, Anbari et al. (2011) tested the sprouting and shoot development of *S. arvensis* in relation to initial ramet size. They reported a positive correlation between the flower number and ramet length in *S. arvensis* and proved that the fragmentation of creeping roots delayed growth and reduced flower production (Anbari et al., 2011). These results are in agreement with the results of the present study.

The 5 cm initial ramet size enhanced PA efficacy, and a reduction in the aboveground and belowground biomass of both species under the mentioned treatments was observed, although it was not statistically significant (Figure 9). In previous studies conducted by Gustavsson (1997) on *C. arvense* and by Anbari et al. (2011) on *S. arvensis*, a smaller aboveground biomass was produced by a shorter ramet size than by a longer ramet size. The results of the present study in relation to aboveground biomass ae in agreement with these findings. For *C. arvense*, the lowest belowground biomass was found for the 15 cm initial ramet size when comparing PA treatments with each other. Additionally, among untreated *C. arvense* plants, the biomass for the initial ramet size of 15 cm was the lowest. One reason for this could be environmental conditions. *C. arvense* biomass increases when more water is available (Sciegienka et al., 2011). When the temperature is lower and the photoperiod is shorter, then the root biomass of *C. arvense* is higher than shoot biomass. With an increase in temperature, the shoot growth increases and results in taller and more robust plants, which rapidly form flower heads (Hunter and Smith, 1972). In the present study, the aboveground biomass produced by plants with the initial ramet size of 15 cm was high due to favorable temperatures and high precipitation in both experimental years (particularly the high precipitation one month before biomass measurements). Moreover, PA-treated *C. arvense* with an initial ramet size of 15 cm produced a larger number of flowers than untreated *C. arvense* with the same initial ramet size. Furthermore, the produced aboveground biomass was almost similar between these two treatments, while the belowground biomass in PA-treated *C. arvense* was smaller than that of the untreated plants. It can thus be inferred that PA-treated *C. arvense* with the initial ramet size of 15 cm attempted to ensure its survival by producing more flowers, which leads to more aboveground biomass production and more belowground depletion. Additionally, it should be considered that there can be an effect of root longevity, and the creeping roots of *C. arvense* cannot live longer than one to two years (Moore, 1975; Bourdôt et al., 2000; Leathwick and Bourdôt, 2012).

Plant height is a direct indicator of herbicide impact, providing detailed information on how the herbicide influences the physical structure of plants. Therefore, the dynamic changes in monthly plant height after herbicide application provided insights into the regrowth patterns of both weed species in both experimental years (Figure 10). The average height of *S. arvensis* was similar in both years, while the height of *C. arvense* was greater in 2021. PA application reduced plant height compared with untreated plants in both years. However, this plant height reduction in 2020 was greater than in 2021. The results showed that the effects of species and herbicides varied depending on the year. This could be due to the variations in weather conditions between 2020 and 2021, and unexpected environmental effects, as discussed by Hunter and Smith (1972) and Sciegienka et al. (2011). The effect of PA on the height of weed species in the present study is in line with earlier studies on *Lolium rigidum* Gaud. and *Avena sterilis* L., which reported a lower height in PA-treated plants compared with untreated plants (Travlos et al., 2020). Overall, the ramet size of 5 cm produced shorter plants in all treatments. This supports the findings of Sciegienka et al. (2011) on *C. arvense* that a smaller root fragment size produces shorter plants.

The herbicide efficacy analysis determined the high efficacy of PA compared with the untreated control and revealed the negative relationship of PA efficacy with days after applications in both years (Figure 5). Due to its rapid effect, PA efficacy was higher compared with other treatments at the beginning and until 7th day after application, but then declined over time due to the occurrence of regrowth. The findings of previous research on both annual and perennial species have demonstrated that PA reaches its maximum efficacy within several hours of application and remains effective for up to one week, although the plant regrowth subsequently reduces its efficacy (Muñoz et al., 2020; Travlos et al., 2020; Muñoz et al., 2022; Pannacci et al., 2022; Ganji et al., 2022 and 2023; Loddo et al., 2023).

3.6 Conclusions

It is concluded that a two-year application of PA on the same specific spot in combination with a smaller ramet size facilitates the development of integrated weed management (IWM) strategies. To reduce the infestation level of perennial weeds, PA application could be combined with mechanical fragmentation of creeping roots in the intercropping period. From today's perspective, however, PA is registered on the European market for use as a plant desiccant in potatoes and to kill suckers in perennial crops, such as hops and grapevine. It is currently not registered for other applications in arable crops. Since current policies towards restricting the use of synthetic herbicides in arable farming enforce the use of alternatives such as bio-herbicides, efforts at economic and political levels are required. To ensure the proper use of this active substance, it is crucial to educate farmers about integrated weed management, conduct field applications at recommended times, and adhere to label instructions. This would help a suitable niche market for this active ingredient to be established.

On the market, bio-herbicides based on PA are costly, and their application rate per hectare is higher compared with synthetic herbicides, and may not be cost-effective for large-scale applications in arable farming. Therefore, expanding the application time from multiple repeated applications in one year to a two-year repeated applications on the same spot might assist with financially balancing PA application costs and achieving acceptable weed control. Further research studies should be undertaken to perform financial comparisons of PA applications and synthetic herbicides. Additional studies regarding enhancements in the technical aspects of PA application, such as the incorporation of adjuvants or the adjustment of water volume to achieve more comprehensive plant coverage, are essential for more successful weed control.

3.7 Acknowledgements

We sincerely thank our colleagues in the Crop Health group at the University of Rostock for their technical assistance. We also would like to acknowledge the support of the Department of Hydrology and Applied Meteorology at the University of Rostock for providing weather data.

3.8 Supplementary tables and figures

Supplementary Table 1. Results of the LMM analysis followed by Tukey's HSD on herbicide efficacy at all days after applications (DATs) in both years

	Year 2020			**Year 2021**		
Fixed effects	**Estimate**	**P(t)**	**Tukey's HSD**	**Estimate**	**P(t)**	**Tukey's HSD[1]**
Cirsium UC 15cm – 1st DAT (Intercept)	4.9e-13	1	a	-4.7e-13	1	a
Sonchus UC 15cm – 1st DAT	5.5e-13	1	a	7.6e-13	1	a
Cirsium PA 15cm – 1st DAT	83.7	2e-16 ***	f	78.75	3.36e-12 ***	fg
Cirsium GLY 15cm – 1st DAT	2.5	0.716	a	100	2e-16 ***	g
Cirsium UC 10cm – 1st DAT	-4.6e-13	1	a	6.8e-13	1	a
Cirsium UC 5cm – 1st DAT	-3.1e-13	1	a	6.2e-13	1	a
Cirsium UC 15cm – 14th DAT	-4.1e-13	1	a	5.03e-13	1	a
Cirsium UC 15cm – 21st DAT	-5.1e-13	1	a	5.4e-13	1	a
Cirsium UC 15cm – 7th DAT	-4.4e-13	1	a	4.3e-13	1	a
Sonchus PA 15cm – 1st DAT	8.5	0.381	f	-4.1	0.784658	efg
Sonchus GLY 15cm – 1st DAT	-1.5	0.877	a	-74.2	1.69e-06 ***	abcd
Sonchus UC 10cm – 1st DAT	-5.6e-13	1	a	0.2	0.986786	a
Sonchus UC 5cm – 1st DAT	-6.9e-13	1	a	-9.6e-13	1	a
Cirsium PA 10cm – 1st DAT	-1.8	0.847	ef	-4.5	0.765635	efg
Cirsium GLY 10cm – 1st DAT	0.5	0.959	a	-3e-13	1	g
Cirsium PA 5cm – 1st DAT	3.2	0.738	f	-5.5	0.715624	efg
Cirsium GLY 5cm – 1st DAT	-3.9e-14	1	a	-2.4e-13	1	g
Sonchus UC 15cm – 14th DAT	-6.8e-13	1	a	-8.3e-13	1	a
Sonchus UC 15cm – 21st DAT	-5.1e-13	1	a	-9.9e-13	1	a
Sonchus UC 15cm – 7th DAT	-6.3e-13	1	a	-7.2e-13	1	a
Cirsium PA 15cm – 14th DAT	-66.2	8.39e-11 ***	abc	-70.2	5.58e-06 ***	abc
Cirsium GLY 15cm – 14th DAT	97.5	2e-16 ***	f	-3.5e-14	1	g
Cirsium PA 15cm – 21st DAT	-70.7	5.58e-12 ***	ab	-71	4.48e-06 ***	abc
Cirsium GLY 15cm – 21st DAT	96.2	2e-16 ***	f	-8.3e-14	1	g
Cirsium PA 15cm – 7th DAT	-41.2	3.11e-5 ***	cd	-38.2	0.011897 *	abcdef
Cirsium GLY 15cm – 7th DAT	95.7	2e-16 ***	f	9.2e-14	1	g
Cirsium UC 10cm – 14th DAT	4.2e-13	1	a	-7.5e-13	1	a
Cirsium UC 5cm – 14th DAT	2.2e-13	1	a	-7e-13	1	a
Cirsium UC 10cm – 21st DAT	5.3e-13	1	a	-7.5e-13	1	a
Cirsium UC 5cm – 21st DAT	3.9e-13	1	a	-7.4e-13	1	a
Cirsium UC 10cm – 7th DAT	4.02e-13	1	a	-6.3e-13	1	a
Cirsium UC 5cm – 7th DAT	2.6e-13	1	a	-6e-13	1	a
Sonchus PA 10cm – 1st DAT	4.3	0.75	f	2.2	0.917917	efg
Sonchus GLY 10cm –1st DAT	8.7e-13	1	a	24.5	0.251822	cdef
Sonchus PA 5cm –1st DAT	-1	0.942	f	-3.3	0.87438	defg
Sonchus GLY 5cm –1st DAT	1.2	0.927	a	74.2	0.000603 ***	g
Sonchus PA 15cm – 14th DAT	11.6	0.396	bcd	0.1	0.995328	ab
Sonchus GLY 15cm – 14th DAT	0.5	0.971	f	74.2	0.000603 ***	g
Sonchus PA 15cm – 21st DAT	-21.4	0.118	a	-3.3	0.87438	a
Sonchus GLY 15cm – 21st DAT	2.2	0.87	f	74.2	0.000603 ***	g
Sonchus PA 15cm – 7th DAT	-7.2	0.597	cd	0.1	0.995328	abcdef
Sonchus GLY 15cm – 7th DAT	-9.8	0.472	f	41	0.055825	defg
Sonchus UC 10cm – 14th DAT	6.7e-13	1	a	-0.2	0.990656	a
Sonchus UC 5cm – 14th DAT	8.5e-13	1	a	1.09e-12	1	a
Sonchus UC 10cm – 21st DAT	4.5e-13	1	a	-0.2	0.990656	a
Sonchus UC 5cm – 21st DAT	5.5e-13	1	a	1.3e-12	1	a
Sonchus UC 10cm – 7th DAT	6.9e-13	1	a	-0.2	0.990656	a
Sonchus UC 5cm – 7th DAT	8.02e-13	1	a	9.4e-13	1	a
Cirsium PA 10cm – 14th DAT	-8.1	0.554	a	4	0.851368	abc
Cirsium GLY 10cm – 14th DAT	-1	0.942	f	3.3e-13	1	g
Cirsium PA 5cm – 14th DAT	6.5	0.636	abcd	27.5	0.198534	abcde
Cirsium GLY 5cm – 14th DAT	2.1e-13	1	f	2.7e-13	1	g
Cirsium PA 10cm – 21st DAT	-6.8	0.616	a	3.2	0.878996	abc
Cirsium GLY 10cm – 21st DAT	0.7	0.956	f	3.2e-13	1	g
Cirsium PA 5cm – 21st DAT	11.7	0.392	abcd	28.7	0.178968	abcde
Cirsium GLY 5cm – 21st DAT	1.2	0.927	f	3.1e-13	1	g
Cirsium PA 10cm – 7th DAT	2	0.884	cd	-0.6	0.974308	abcdef
Cirsium GLY 10cm – 7th DAT	-11.2	0.413	f	1.2e-13	1	g
Cirsium PA 5cm – 7th DAT	8.7	0.524	de	11.6	0.584166	bcdef
Cirsium GLY 5cm – 7th DAT	-4.	0.743	f	9.6e-14	1	g
Sonchus PA 10cm – 14th DAT	16.7	0.39	d	-1.7	0.955094	ab
Sonchus GLY 10cm – 14th DAT	1.5	0.938	f	-24.5	0.417409	g
Sonchus PA 5cm – 14th DAT	-0.4	0.983	cd	-18.1	0.548421	ab
Sonchus GLY 5cm – 14th DAT	-0.2	0.99	f	-74.2	0.014595 *	g
Sonchus PA 10cm – 21st DAT	4.3	0.822	a	-1.2	0.968293	a
Sonchus GLY 10cm – 21st DAT	-0.7	0.969	f	-24.5	0.417409	g
Sonchus PA 5cm – 21st DAT	-14	0.471	a	-19.8	0.510528	a
Sonchus GLY 5cm – 21st DAT	-2	0.918	f	-74.2	0.014595 *	g
Sonchus PA 10cm – 7th DAT	3	0.877	d	5.9	0.842792	abcdef
Sonchus GLY 10cm – 7th DAT	18.8	0.331	f	-20.5	0.497337	defg
Sonchus PA 5cm – 7th DAT	-7.2	0.709	d	1.1	0.968623	abcdef
Sonchus GLY 5cm – 7th DAT	5.1	0.792	f	-41	0.175362	g
Fitting quality of LMM models **Conditional R^2**	0.9507225			0.8899877		
Marginal R^2	0.9485036			0.8890006		

Significance codes for P(t): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

[1] Groups with different letters are significantly different at $p < 0.05$ level based on Tukey's HSD test.

Supplementary Table 2. Results of the LMM analysis followed by Tukey's HSD on the aboveground biomass at the end of experiment

Fixed effects			Estimate	P(t)	Effect Relative to Intercept (%)	Tukey's HSD[1]
Cirsium	**UC**	**15cm (Intercept)**	321.1	2.04e-14 ***		b
Sonchus	**UC**	**15cm**	-5.1	0.9066	-101.6	b
Cirsium	**PA**	**15cm**	-3.1	0.9417	-101.0	b
Cirsium	**GLY**	**15cm**	-321.1	1.28e-09 ***	-200.0	a
Cirsium	**UC**	**10cm**	-20.9	0.6314	-106.5	b
Cirsium	**UC**	**5cm**	-3.4	0.9372	-101.1	b
Sonchus	**PA**	**15cm**	-48.8	0.4298	-115.2	b
Sonchus	**GLY**	**15cm**	14.5	0.8137	-95.5	a
Sonchus	**UC**	**10cm**	-19.6	0.7483	-106.1	b
Sonchus	**UC**	**5cm**	-37.5	0.5433	-111.7	b
Cirsium	**PA**	**10cm**	29.7	0.6329	-90.7	b
Cirsium	**GLY**	**10cm**	20.9	0.7360	-93.5	a
Cirsium	**PA**	**5cm**	-132.3	0.0349 *	-141.2	b
Cirsium	**GLY**	**5cm**	3.4	0.9556	-98.9	a
Sonchus	**PA**	**10cm**	17.6	0.8401	-94.5	b
Sonchus	**GLY**	**10cm**	21.4	0.8056	-93.3	a
Sonchus	**PA**	**5cm**	146.1	0.0972	-54.5	b
Sonchus	**GLY**	**5cm**	28.1	0.7458	-91.2	a
Fitting quality of LMM models		**Conditional R^2**	0.90			
		Marginal R^2	0.77			

Significance codes for P(t): * p < 0.05, ** p < 0.01, *** p < 0.001

[1] Groups with different letters are significantly different at p < 0.05 level based on Tukey's HSD test.

Supplementary Table 3. Results of the LMM analysis followed by Tukey's HSD on the belowground biomass at the end of experiment

Fixed effects			Estimate	P(t)	Effect Relative to Intercept (%)	Tukey's HSD[1]
Cirsium	**UC**	**15cm (Intercept)**	76.1	2.99e-05***		abc
Sonchus	**UC**	**15cm**	33.1	0.12504	-56.56	c
Cirsium	**PA**	**15cm**	-7.9	0.71912	-110.29	abc
Cirsium	**GLY**	**15cm**	-76.1	0.00099***	-197.91	a
Cirsium	**UC**	**10cm**	49.3	0.02768*	-35.13	c
Cirsium	**UC**	**5cm**	21.5	0.31668	-71.75	c
Sonchus	**PA**	**15cm**	4.7	0.8786	-93.75	c
Sonchus	**GLY**	**15cm**	-31.1	0.31883	-143.00	a
Sonchus	**UC**	**10cm**	-61.8	0.03838*	-181.11	c
Sonchus	**UC**	**5cm**	-32.4	0.29392	-142.55	c
Cirsium	**PA**	**10cm**	-8.3	0.79843	-111.01	c
Cirsium	**GLY**	**10cm**	-47.5	0.14351	-164.66	a
Cirsium	**PA**	**5cm**	-17.5	0.54626	-123.01	abc
Cirsium	**GLY**	**5cm**	-21.5	0.4914	-130.32	a
Sonchus	**PA**	**10cm**	21.6	0.62106	-71.73	c
Sonchus	**GLY**	**10cm**	52.1	0.23309	-29.51	a
Sonchus	**PA**	**5cm**	7.8	0.85489	-89.72	bc
Sonchus	**GLY**	**5cm**	35.1	0.41657	-51.76	a
Fitting quality of LMM models		**Conditional R^2**	0.97			
		Marginal R^2	0.68			

Significance codes for P(t): * p < 0.05, ** p < 0.01, *** p < 0.001

[1] Groups with different letters are significantly different at p < 0.05 level based on Tukey's HSD test.

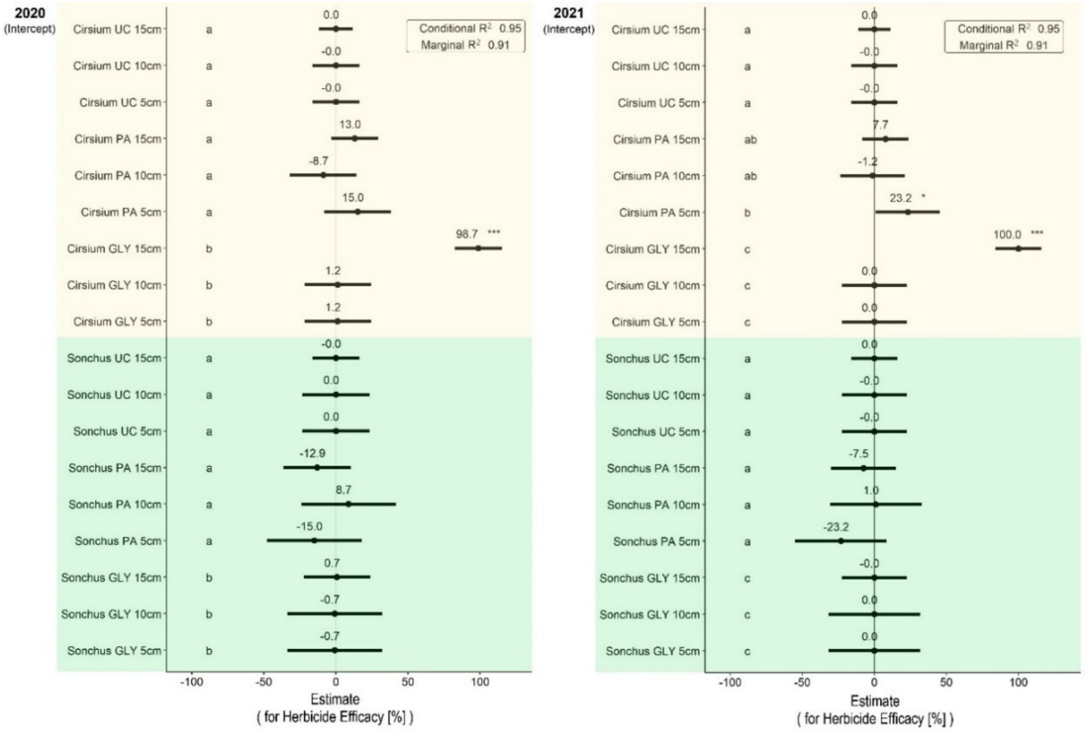
Supplementary Table 4. Results of the LMM analysis followed by Tukey's HSD on monthly measured plant heights over 2020 and 2021

Fixed effects	Estimate	P(t)	Effect Relative to Intercept (%)	Tukey's HSD[1]
Cirsium UC 15cm in year 2020 (Intercept)	34.4	0 ***		ghij
Sonchus UC 15cm in year 2020	-18.1	0.001 **	-152.6	defg
Cirsium UC 10cm in year 2020	-3.7	0.4932	-111.0	fghi
Cirsium UC 5cm in year 2020	-5.7	0.2653	-116.6	fghi
Cirsium GLY 15cm in year 2020	-39.4	0 ***	-214.3	abcd
Cirsium PA 15cm in year 2020	-14.8	0.0115 *	-142.9	efg
Cirsium UC 15cm in year 2021	49.9	0 ***	44.8	kl
Sonchus UC 10cm in year 2020	-3.05	0.6793	-108.9	cdef
Sonchus UC 5cm in year 2020	-9.7	0.1649	-128.4	bcde
Sonchus GLY 15cm in year 2020	-7.8	0.3428	-122.9	a
Sonchus PA 15cm in year 2020	3.6	0.6366	-89.5	cde
Cirsium GLY 10cm in year 2020	-0.9	0.9166	-102.9	abc
Cirsium GLY 5cm in year 2020	6.1	0.4497	-82.1	abcde
Cirsium PA 10cm in year 2020	0.3	0.9683	-99.1	defg
Cirsium PA 5cm in year 2020	6.3	0.4233	-81.5	efg
Sonchus UC 15cm in year 2021	-12.5	0.0701	-136.5	j
Cirsium UC 10cm in year 2021	7.05	0.3024	-79.5	l
Cirsium UC 5cm in year 2021	1.1	0.8573	-96.6	kl
Cirsium GLY 15cm in year 2021	-28.5	0.0013 **	-182.9	cdefg
Cirsium PA 15cm in year 2021	13.7	0.0566	-60.1	kl
Sonchus GLY 10cm in year 2020	4.6	0.7042	-86.5	a
Sonchus GLY 5cm in year 2020	17.7	0.1212	-48.4	ab
Sonchus PA 10cm in year 2020	1.5	0.8876	-95.6	bcde
Sonchus PA 5cm in year 2020	4.06	0.7011	-88.2	bcde
Sonchus UC 10cm in year 2021	-4.6	0.6198	-113.4	ij
Sonchus UC 5cm in year 2021	10.6	0.2394	-69.1	ij
Sonchus GLY 15cm in year 2021	28.3	0.0196 *	-17.8	bcdefg
Sonchus PA 15cm in year 2021	-11.3	0.2438	-132.8	hij
Cirsium GLY 10cm in year 2021	-7.4	0.5696	-121.6	cdefg
Cirsium GLY 5cm in year 2021	2.6	0.8153	-92.2	cdefg
Cirsium PA 10cm in year 2021	-15.9	0.1144	-146.3	k
Cirsium PA 5cm in year 2021	-9.3	0.3426	-127.2	kl
Sonchus GLY 10cm in year 2021	23.6	0.1767	-31.4	defgh
Sonchus GLY 5cm in year 2021	-18.1	0.27	-152.8	cdefg
Sonchus PA 10cm in year 2021	25.4	0.0583	-26.1	j

Sonchus PA 5cm in year 2021		3.5	0.7926	-89.7	hij
Fitting quality of LMM models	**Conditional R^2**	0.5389057			
	Marginal R^2	0.8706745			

Significance codes for P(t): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

[1]Groups with different letters are significantly different at $p < 0.05$ level based on Tukey's HSD test.



Supplementary Figure 1. Results of the LMM analysis followed by Tukey's HSD on herbicide efficacy at 21st_DAT in both years. Significance codes obtained from the LMM analysis for main effects shown in this graph are as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Within each year, groups with different letters are significantly different at $p < 0.05$ level based on Tukey's HSD. PA: Pelargonic acid; GLY: glyphosate; UC: untreated control; Cirsium: *Cirsium arvense*; Sonchus: *Sonchus arvensis*. 5 cm, 10 cm and 15 cm represent the initial size of ramets.

3.9 References

Anbari, S., Lundkvist, A., and Verwijst, T. (2011). Sprouting and shoot development of Sonchus arvensis in relation to initial root size. Weed Research. 51(2), 142-150. https://doi.org/10.1111/j.1365-3180.2010.00837.x

Andert, S. and Gerowitt, B. (2020). Controlling arable weeds with natural substances as bio-based herbicides. Julius-kühn-Archiv 464, 407-414. https://doi.org/10.5073/jka.2020.464.061

Andert, S., de Mol, F., Koning, L., and Gerowitt, B. (2022). Weed response in winter wheat fields on a gradient of glyphosate use in the recent past. Agriculture, Ecosystems and Environment, 333, 107977. https://doi.org/10.1016/j.agee.2022.107977

Andert, S., Guguin, J., Hamacher, M., Valantin-Morison, M., and Gerowitt, B. (2023). How farmers perceive perennial weeds in Northern France and Eastern Germany. Frontiers in Agronomy, 5, 1247277. https://doi.org/10.3389/fagro.2023.1247277

Antier, C., Kudsk, P., Reboud, X., Ulber, L., Baret, P. V., and Messéan, A. (2020). Glyphosate use in the European agricultural sector and a framework for its further monitoring. Sustainability, 12(14), 5682. https://doi.org/10.3390/su12145682

Barker, A.V. and Prostak, R.G. (2009). Alternative management of roadside vegetation. HortTechnology. 19(2), 346-352. https://doi.org/10.21273/HORTTECH.19.2.346

Barton, A. F., Clarke, B. R., Dell, B., and Knight, A. R. (2014). Post-emergent herbicidal activity of cineole derivatives. Journal of pest science, 87, 531-541. https://doi.org/10.1007/s10340-014-0566-6

Bashir, T., Anum, W., Ali, I., Ghaffar, A., Ali, L., Raza, M. U., Javed, Z.,Zafar, A., and Shabir, A. (2018). Allelopathic effects of perennial sow thistle (Sonchus arvensis L.) on germination and seedling growth of maize (Zea mays L.). Allelopathy Journal, 43(1), 105-116.

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1), 1–48. https://doi.org/10.48550/arXiv.1406.5823

Beckie, H.J., Flower, K.C., and Ashworth, M.B. (2020). Farming without glyphosate?. Plants. 9(1): 96. https://doi.org/10.3390/plants9010096

Bhullar, M. S., Kaur, S., Kaur, T., Singh, T., Singh, M., and Jhala, A. J. (2013). Control of broadleaf weeds with post-emergence herbicides in four barley (Hordeum spp.) cultivars. Crop Protection, 43, 216-222. https://doi.org/10.1016/j.cropro.2012.10.005

Bourdôt, G.W., Leathwick, D.M., and Hurrell, G.A. (2000). Longevity of Californian thistle roots. New Zealand Plant Protection, 53, 258–261. https://doi.org/10.30843/nzpp.2000.53.3704

Brandsæter, L.O., Fogelfors, H., Fykse, H., Graglia, E., Jensen, R.K., Melander, B., Salonen, J., and Vanhala, P. (2010). Seasonal restrictions of bud growth on roots of Cirsium arvense and Sonchus arvensis and rhizomes of Elymus repens. Weed Research, 50(2), 102-109. https://doi.org/10.1111/j.1365-3180.2009.00756.x

Brandsæter, L.O., Mangerud, K., Helgheim, M., and Berge, T.W. (2017). Control of perennial weeds in spring cereals through stubble cultivation and mouldboard ploughing during autumn or spring. Crop Protection, 98, 16-23. https://doi.org/10.1016/j.cropro.2017.03.006

Campos, J., Bodelon, L., Verdeguer, M., and Baur, P. (2022a). Mechanistic aspects and effects of selected tank-mix partners on herbicidal activity of a novel fatty acid ester. Plants, 11(3), 279. https://doi.org/10.3390/plants11030279

Campos, J., Mansour, P., Verdeguer, M., and Baur, P. (2022b). Contact herbicidal activity optimization of methyl capped polyethylene glycol ester of pelargonic acid. Journal of Plant Diseases and Protection, 130(1), 93-103. https://doi.org/10.1007/s41348-022-00661-0

Carroll, D. E., Kaminski, J. E., and Borger, J. A. (2022). Efficacy of natural herbicides on dandelion (Taraxacum officinale GH Weber ex Wiggers) and white clover (Trifolium repens L.) populations. International Turfgrass Society Research Journal, 14(1), 759-769. https://doi.org/10.1002/its2.8

Casassus, B. (2023). EU allows use of controversial weedkiller glyphosate for 10 more years. Nature. https://doi.org/10.1038/d41586-023-03589-z

Casella, F., Vurro, M., Valerio, F., Perrino, E. V., Mezzapesa, G. N., and Boari, A. (2023). Phytotoxic effects of essential oils from six lamiaceae species. Agronomy, 13(1), 257. https://doi.org/10.3390/agronomy13010257

Ciriminna, R., Fidalgo, A., Ilharco, L. M., and Pagliaro, M. (2019). Herbicides based on pelargonic acid: Herbicides of the bioeconomy. Biofuels, Bioproducts and Biorefining, 13 (6), 1476–1482. https://doi.org/10.1002/bbb.2046

Coleman, R., and Penner, D. (2006). Desiccant activity of short chain fatty acids. Weed Technology, 20, 410-415. https://doi.org/10.1614/WT-06-195.1

Cordeau, S., Triolet, M., Wayman, S., Steinberg, C., and Guillemin, J.P. (2016). Bioherbicides: Dead in the water? A review of the existing products for integrated weed management. Crop Protection, 87, 44-49. https://doi.org/10.1016/j.cropro.2016.04.016

Crmaric, I., Keller, M., Krauss, J., and Delabays, N. (2018). Efficacy of natural fatty acid based herbicides on mixed weed stands. Julius-kühn-Archiv 458, 328–333. https://doi.org/10.5073/jka.2018.458.048

European Food Safety Authority (EFSA), Alvarez, F., Arena, M., Auteri, D., Borroto, J., Brancato, A., Carrasco Cabrera, L., Castoldi, A.F., Chiusolo, A., Colagiorgi, A., Colas, M., Crivellente, F., De Lentdecker, C., Egsmose, M., Fait, G., Gouliarmou, V., Ferilli, F., Greco, L., Ippolito, A., Istace, F., Jarrah, S., Kardassi, D., Kienzler, A., Leuschner, R., Lava, R., Linguadoca, A., Lythgo, C., Magrans, O., Mangas, I., Miron, I., Molnar, T., Padovani, L., Parra Morte, J.M., Pedersen, R., Reich, H., Santos, M., Sharp, R., Szentes, C., Terron, A., Tiramani, M., Vagenende, B., and Villamar-Bouza, L. (2021). Conclusion on the peer review of the pesticide risk assessment of the active substance pelargonic acid (nonanoic acid). EFSA Journal, 19(8): 6813. https://doi.org/10.2903/j.efsa.2021.6813

Egushova, E. A., and Anokhina, O. V. (2022). Allelopathic effect of weed extracts on vegetable seeds. IOP Conference Series: Earth and Environmental Science, 1010, 012104. https://doi.org/10.1088/1755-1315/1010/1/012104

El Jaouhari, M., Damour, G., Tixier, P., and Coulis, M. (2023). Glyphosate reduces the biodiversity of soil macrofauna and benefits exotic over native species in a tropical agroescosystem. Basic and Applied Ecology, 73, 18-26. https://doi.org/10.1016/j.baae.2023.10.001

European Commission (2020). Farm to fork strategy: for a fair, healthy and environmentally-friendly food system. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions, 381, 1-9. https://food.ec.europa.eu/horizontal-topics/farm-fork-strategy_en#Publications (Accessed on December 03, 2023).

Fadin, D. A., Tornisielo, V. L., Barroso, A. A. M., Ramos, S., Dos Reis, F. C., and Monquero, P. A. (2018). Absorption and translocation of glyphosate in Spermacoce verticillata and alternative herbicide control. Weed Research, 58(5), 389-396. https://doi.org/10.1111/wre.12329

Ganji, E., Andert, S., and Gerowitt, B. (2022). The herbicidal potential of Pelargonic Acid to control Cirsium arvense (L.) Scop. in relation to the timing of application and the application volume. Julius-kühn-Archiv 468, 86-93. https://doi.org/10.5073/20220117-074121

Ganji, E., Grenzdörffer, G., and Andert, S. (2023). Estimating the Reduction in Cover Crop Vitality Followed by Pelargonic Acid Application Using Drone Imagery. Agronomy, 13(2), 354. https://doi.org/10.3390/agronomy13020354

Gustavsson, A. M. D. (1997). Growth and regenerative capacity of plants of Cirsium arvense. Weed Research. 37(4), 229-236. https://doi.org/10.1046/j.1365-3180.1997.d01-37.x

Håkansson, S. (1969). Experiments with Sonchus arvensis L. I. Development and growth, and the response to burial and defoliation in different developmental stages. Lantbrukshögskolans Annaler 35, 989-1030.

Håkansson, S. and Wallgren, B. (1972). Experiments with Sonchus arvensis L. II. Reproduction, plant development and response to mechanical disturbance. Swed. J. Agric. Res. 2, 3-14.

Håkansson S. (2003). Weeds and weed management on arable land: An ecological approach (Wallingford, Oxon, UK, Cambridge: MA: CABI Publishing). https://doi.org/10.1079/9780851996516.0000

Hamdoun, A. M. (1972). Regenerative capacity of root fragments of Cirsium arvense (L.) Scop. Weed Research, 12(2), 128-136. https://doi.org/10.1111/j.1365-3180.1972.tb01196.x

Hudek, L., Enez, A., and Bräu, L. (2021). Comparative analyses of glyphosate alternative weed management strategies on plant coverage, soil and soil biota. Sustainability, 13(20), 11454. https://doi.org/10.3390/su132011454

Hunter, J.H. and Smith, L.W. (1972). Environment and herbicide effects on Canada thistle ecotypes. Weed Science, 20(2), 163-167. https://www.jstor.org/stable/4042184

Ibáñez, M. D. and Blázquez, M. A. (2018). Phytotoxicity of essential oils on selected weeds: Potential hazard on food crops. Plants, 7(4), 79. https://doi.org/10.3390/plants7040079

Kanatas, P., Travlos, I., Papastylianou, P., Gazoulis, I., Kakabouki, I., and Tsekoura, A. (2020). Yield, quality and weed control in soybean crop as affected by several cultural and weed management practices. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 48(1), 329-341. https://doi.org/10.15835/nbha48111823

Kanatas, P., Antonopoulos, N., Gazoulis, I., and Travlos, I. S. (2021). Screening glyphosate-alternative weed control options in important perennial crops. Weed Science, 69(6), 704-718. https://doi.org/10.1017/wsc.2021.55

Kanatas, P., Zavra, S. M., Tataridas, A., Gazoulis, I., Antonopoulos, N., Synowiec, A., and Travlos, I. (2022). Pelargonic acid and caraway essential oil efficacy on barnyardgrass (Echinochloa crus-galli (L.) P. Beauv.) and johnsongrass (Sorghum halepense (L.) Pers.). Agronomy, 12(8), 1755. https://doi.org/10.3390/agronomy12081755

Kouki, H., Amri, I., Souihi, M., Pieracci, Y., Trabelsi, I., Hamrouni, L., Flamini, G., Hirsch, A.M., and Mabrouk, Y. (2023). Chemical composition, antioxidant, herbicidal and antifungal activities of leaf essential oils from three Tunisian Eucalyptus species. Journal of Plant Diseases and Protection, 1-12. https://doi.org/10.1007/s41348-023-00772-2

Krauss, J., Eigenmann, M., and Keller, M. (2020). Pelargonic acid for weed control in onions: factors affecting selectivity. Julius Kühn-Archiv 464, 415–419. https://doi.org/10.5073/jka.2020.464.062

Kuznetsova A., Brockhoff P. B., and Christensen R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. J. Stat. Softw. 82(13), 1–26. doi:10.18637/jss.v082.i13.

Leathwick, D. M. and Bourdôt, G.W. (2012). A conceptual model for the population dynamics of Cirsium arvense in a New Zealand pasture. New Zealand Journal of Agricultural Research, 55(4), 371-384. https://doi.org/10.1080/00288233.2012.728532

Lemna, W. K. and Messersmith, C. G. (1990). The biology of Canadian weeds. 94. Sonchus arvensis L. Canadian Journal of Plant Science, 70(2), 509-532. https://doi.org/10.4141/cjps90-060

Lenth, R., Singmann, H., Love, J., Buerkner, P., and Herve, M. (2018). Package "Emmeans". R Package Version 4.0-3. http://cran.r-project.org/package=emmeans

Liew, J., Andersson, L., Boström, U., Forkman, J., Hakman, I., and Magnuski, E. (2013). Regeneration capacity from buds on roots and rhizomes in five herbaceous perennials as affected by time of fragmentation. Plant ecology, 214, 1199-1209. https://doi.org/10.1007/s11258-013-0244-4

Loddo, D., Jagarapu, K. K., Strati, E., Trespidi, G., Nikolić, N., Masin, R., Berti, A., and Otto, S. (2023). Assessing Herbicide Efficacy of Pelargonic Acid on Several Weed Species. Agronomy, 13(6): 1511. https://doi.org/10.3390/agronomy13061511

Meier, U. (2018). Growth stages of mono- and dicotyledonous plants: BBCH Monograph. Quedlinburg: Open Agrar Repositorium. doi: 10.5073/20180906-074619.

Melander, B., Holst, N., Rasmussen, I. A., and Hansen, P. K. (2012). Direct control of perennial weeds between crops–Implications for organic farming. Crop Protection, 40, 36-42. https://doi.org/10.1016/j.cropro.2012.04.029

Moore, R.J. (1975). The biology of Canadian weeds.: 13. Cirsium arvense (L.) Scop. Canadian Journal of Plant Science, 55(4), 1033-1048. https://doi.org/10.4141/cjps75-163

Muñoz, M., Torres-Pagán, N., Peiró, R., Guijarro, R., Sánchez-Moreiras, A. M., and Verdeguer, M. (2020). Phytotoxic effects of three natural compounds: Pelargonic acid, carvacrol, and cinnamic aldehyde, against problematic weeds in Mediterranean crops. Agronomy, 10(6): 791. https://doi.org/10.3390/agronomy10060791

Muñoz, M., Torres-Pagán, N., Jouini, A., Araniti, F., Sánchez-Moreiras, A. M., and Verdeguer, M. (2022). Control of problematic weeds in Mediterranean vineyards with the bioherbicide pelargonic acid. Agronomy, 12(10): 2476. https://doi.org/10.3390/agronomy12102476

Patton, A. J., Braun, R. C., and Weisenberger, D. V. (2019). Single applications of natural postemergence weed control options do not provide effective ground ivy control. Crop, Forage and Turfgrass Management, 5(1), 1-7. https://doi.org/10.2134/cftm2018.12.0101

Pannacci, E., Ottavini, D., Onofri, A., and Tei, F. (2022). Dose–response curves of pelargonic acid against summer and winter weeds in Central Italy. Agronomy, 12(12), 3229. https://doi.org/10.3390/agronomy12123229

Pinheiro, J., Bates, D., and R Core Team (2023). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-163. https://CRAN.R-project.org/package=nlme.

R Core Team (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org

Radicetti, E., and Mancinelli, R. (2021). Sustainable weed control in the agro-ecosystems. Sustainability, 13(15), 8639. https://doi.org/10.3390/su13158639

Ramesh, K., Matloob, A., Aslam, F., Florentine, S. K., and Chauhan, B. S. (2017). Weeds in a changing climate: vulnerabilities, consequences, and implications for future weed management. Frontiers in Plant Science, 8, 95. https://doi.org/10.3389/fpls.2017.00095

Satchivi, N. M., Wax, L. M., Stoller, E. W., and Briskin, D. P. (2000). Absorption and translocation of glyphosate isopropylamine and trimethylsulfonium salts in Abutilon theophrasti and Setaria faberi. Weed Science, 48(6), 675-679. https://doi.org/10.1614/0043-1745(2000)048[0675:AATOGI]2.0.CO;2

Sciegienka, J., Keren, E., and Menalled, F. (2011). Impact of root fragment dimension, weight, burial depth, and water regime on Cirsium arvense emergence and growth. Canadian Journal of Plant Science, 91(6), 1027-1036. https://doi.org/10.4141/cjps2011-059

Short, M. M., Vann, M. C., and Suchoff, D. H. (2020). Organic sucker control: screening different active ingredients for commercial application. Tobacco Science, 57(1), 17-20. https://doi.org/10.3381/TOBSCI-D-20-00008

Sprankle, P., Meggitt, W. F., and Penner, D. (1975). Absorption, action, and translocation of glyphosate. Weed Science, 23(3), 235-240. https://doi.org/10.1017/S0043174500052930

Sullivan, T. P., and Sullivan, D. S. (2003). Vegetation management and ecosystem disturbance: impact of glyphosate herbicide on plant and animal diversity in terrestrial systems. Environmental Reviews, 11(1), 37-59. https://doi.org/10.1139/a03-005

Synowiec, A., Kalemba, D., Drozdek, E., and Bocianowski, J. (2017). Phytotoxic potential of essential oils from temperate climate plants against the germination of selected weeds and crops. Journal of pest science, 90, 407-419. https://doi.org/10.1007/s10340-016-0759-2

Szabó, K., and Halbritter, P. S. R. (2015). Allelopathic effects of Cirsium arvense (L.) Scop. in Hun-gary. Bulgarian Journal of Agricultural Science, 21(5), 1012-1021.

Tavaziva, V. J. (2012). Effects of competition on compensation point and phenological development in Sonchus arvensis L. [Master thesis]. [Uppsala, Sweden]: Swedish University of Agricultural Sciences. https://stud.epsilon.slu.se/4572/

Tørresen, K. S. and Gerowitt, B. (2022). Late-Autumn Ramet Sprouting of Three Arable Creeping Perennial Weed Species. Agronomy, 12(9): 2175. https://doi.org/10.3390/agronomy12092175

Travlos, I., Rapti, E., Gazoulis, I., Kanatas, P., Tataridas, A., Kakabouki, I., and Papastylianou, P. (2020). The herbicidal potential of different pelargonic acid products and essential oils against several important weed species. Agronomy, 10(11): 1687. https://doi.org/10.3390/agronomy10111687

Vanhala, P., Lötjönen, T., and Hurme, T. (2006). Managing Sonchus arvensis using mechanical and cultural methods. Agricultural and Food Science, 15(4), 444-458. https://doi.org/10.2137/145960606780061498

Verwijst, T., Tavaziva, V. J., and Lundkvist, A. (2018). Assessment of the compensation point of Cirsium arvense and effects of competition, root weight and burial depth on below-ground dry weight–leaf stage trajectories. Weed Research, 58(4), 292-303. https://doi.org/10.1111/wre.12312

Ward, J. S. and Mervosh, T. L. (2012). Nonchemical and herbicide treatments for management of Japanese stiltgrass (Microstegium vimineum). Invasive Plant Science and Management, 5(1), 9-19. https://doi.org/10.1614/IPSM-11-00018.1

Webber, C. L., Taylor, M. J., and Shrefler, J. W. (2014a). Weed control in yellow squash using sequential post directed applications of pelargonic acid. HortTechnology. 24(1), 25-29. https://doi.org/10.21273/HORTTECH.24.1.25

Webber, C. L., Taylor, M. J., and Shrefler, J. W. (2014b). Weed control in sweet bell pepper using sequential post directed applications of pelargonic acid. HortTechnology. 24(6), 663-667. https://doi.org/10.21273/HORTTECH.24.6.663

Webber, C. L., Shrefler, J. W., and Taylor, M. J. (2014c). Adjuvants Affect Duckweed (Lemna minor) Control with Pelargonic Acid. Journal of Agricultural Science, 6(12), 1. https://doi.org/10.5539/jas.v6n12p1

Webber, C. L. and Webber, D. M. (2011). Duckweed control with over-the-top application of pelargonic acid. Journal of Environment, Monitoring, and Restoration, 7, 78-86.

Zargar, M., Bayat, M., Lyashko, M., and Chauhan, B. (2019). Postemergence herbicide applications impact Canada thistle control and spring wheat yields. Agronomy Journal, 111(6), 2874-2880. https://doi.org/10.2134/agronj2019.02.0125

Chapter 4: Estimating the Reduction in Cover Crop Vitality Followed by Pelargonic Acid Application Using Drone Imagery

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4.1 Abstract

Cultivation of cover crops is a valuable practice in sustainable agriculture. In cover crop management, the method of desiccation is an important consideration, and one widely used method for this is the application of glyphosate. With use of glyphosate likely to be banned soon in Europe, the purpose of this study was to evaluate the herbicidal effect of pelargonic acid (PA) as a bio-based substitute for glyphosate. This study presents the results of a two-year field experiment (2019 and 2021) conducted in northeast Germany. The experimental setup included an untreated control, three different dosages (16, 8, and 5 L/ha) of PA, and the active ingredients glyphosate and pyraflufen. A completely randomised block design was established. The effect of the herbicide treatments was assessed by a visual estimate of the percentage of crop vitality and a comparison assessment provided by an Ebee+ drone. Four vegetation indices (VIs) calculated from the drone images were used to verify the credibility of colour (RGB)-based and near-infrared (NIR)-based vegetation indices. The results of both types of assessment indicated that pelargonic acid was reasonably effective in controlling cover crops within a week of application. In both experimental years, the PA (16 L/ha) and PA_2T (double application of 8 L/ha) treatments demonstrated their highest herbicidal effect for up to seven days after application. PA (16 L/ha) vitality loss decreased over time, while PA_2T (double application of 8 L/ha) continued to exhibit an almost constant effect for longer due to the second application one week later. The PA dosage of 5 L/ha, pyraflufen, and a mixture of the two exhibited a smaller vitality loss than the other treatments. However, except for glyphosate, the herbicidal effect of all the other treatments decreased over time. At the end of the experiment, the glyphosate treatment (3 L/ha) demonstrated the lowest estimated vitality. The results of the drone assessments indicated that vegetation indices (VIs) can provide detailed information regarding crop vitality following herbicide application and that RGB-based indices, such as EXG, have the potential to be applied efficiently and cost-effectively utilising drone imagery. The results of this study demonstrate that pelargonic acid has considerable potential for use as an additional tool in integrated crop management.

Keywords: bioherbicide; desiccation; RGB indices; NIR indices; vegetation indices

4.2 Introduction

Cultivation of cover crops is a valuable and sustainable agricultural practice that offers agroecosystems numerous benefits. However, several factors need to be considered in the management of cover crops, including the method used to desiccate them (Legleiter et al., 2012; Ulcuango et al., 2021). While being desirable in agroecosystems, many cover crops have the potential to become troublesome weeds, and thus may reduce the yields of subsequent crops if consideration is not given to their proper desiccation (Legleiter et al., 2012; Ulcuango et al., 2021). Moreover, in climate-smart conservation tillage systems, cover crop desiccation is a tool used to prepare a weed-free seedbed (Rosario-Lebron et al., 2019, Tataridas et al., 2022).

Cover crops are either damaged or killed by frost in winter (frost-sensitive cover crops) or actively desiccated by mowing, tillage or application of chemical herbicides. The application of a non-selective herbicide (e.g., the active ingredient glyphosate) is a common method for desiccating cover crops because, compared with other desiccation methods, it can offer suitable levels of control at almost any time or plant growth stage (Tataridas et al., 2022; Balkcom et al., 2020; Pittman et al., 2020). However, a delay in desiccating the cover crop carries risks associated with the next crop emergence, such as water moisture depletion (Schmitt et al., 2021; Eash et al., 2021; Rosario-Lebron et al., 2019) and nitrogen immobilization (Eash et al., 2021). Therefore, in the event of delayed desiccation (e.g., closer to the sowing time of the next crop), herbicide application is the method most commonly used due to its rapid effects (Balkcom et al., 2020; Kornecki and Balkcom, 2020). The results of previous studies reveal that using the non-selective active ingredient glyphosate for cover crop desiccation provides the best control of grass cover crop species (Cornelius and Bradley, 2017; Palhano et al., 2018; Whalen et al 2020), but studies have also shown that glyphosate does not provide an adequate level of effectiveness for the desiccation of broadleaf cover crop species (Cornelius and Bradley, 2017; Palhano et al., 2018; Whalen et al., 2020).

Furthermore, in recent years, the use of synthetic herbicides, especially glyphosate, has raised several environmental and health concerns (Van Bruggen et al., 2018; Paganelli et al., 2010), such that European governments, especially the German government, have announced that use of this active ingredient should be very limited or even prohibited by the end of 2023 (Antier et al., 2015). A severe glyphosate restriction or ban means that alternatives need to be identified (Beckie et al., 2020). One of the substitutes for glyphosate is the application of bioherbicides (Beckie et al., 2020) since they offer advantages such as rapid degradation (Cordeau et al., 2016). Pelargonic acid (PA) is a bio-based non-selective contact herbicide that is rapidly degraded in the soil (DT50 < 2 days) (Savage and Zorner, 1996; Krauss et al., 2020) and exhibits damage on the green tissue after 15-60 minutes at temperatures above 15 °C on a sunny day (Savage and Zorner, 1996; Ciriminna et al., 2019). Its mode of action is to move through the cuticle and cell membrane, reduce intra-cellular pH and ultimately cause rapid membrane dysfunction, leading to the loss of membrane integrity and cell death (Ciriminna et al., 2019; Travlos et al., 2020). Owing to this mode of action, PA is recognised as an extremely rapid burndown herbicide, with plants starting to collapse between one and three hours after application (Savage and Zorner, 1996). Most bioherbicides are unable to provide adequate control because of a lack of aggressiveness; therefore, efficacy can be improved by mixing a bioherbicide and a chemical herbicide (Hoagland, 1996). There is potential for enhancing PA efficacy with the addition of another active ingredient (Coleman and Penner, 2008). Therefore, this study investigated a mixture of pyraflufen and reduced PA dosage. Pyraflufen is a post-emergence contact herbicide used to control broadleaf weed species (Moretti et al., 2014).

One approach for evaluating the effect of bio-based and chemical herbicides for cover crop desiccation is to undertake a visual evaluation (Duddu et al., 2019). Another assessment method is to use an unmanned aerial vehicle (UAV), which is a relatively new technology and a useful tool for evaluating the effectiveness of various types of crop management (Rodene et al., 2022; Marino and Alvino, 2019) e.g., herbicide effectiveness (Duddu et al., 2019, Streibig et al., 2014). The advantages of this method are its accuracy, flexibility and cost-effectiveness (Duddu et al., 2019; Rodene et al., 2022; Marino and Alvino, 2019). Aerial images obtained by UAV can detect differences in levels of plant health that may not be possible by visual observation (Duddu et al., 2019; Rodene et al., 2022). Spectral data collected by UAVs are normally evaluated in the form of vegetation indices (VIs) (Rodene et al., 2022). Evaluation of vegetation can be conducted precisely using various VIs, but this greatly depends on the experimental questions (Mink et al., 2020) because each index has its own unique characteristics and usage purposes (Rodene et al., 2022). Near infrared (NIR) and red-edge-based indices are used to measure plant health and biotic and abiotic stresses because they are better at showing plant reaction to stress within these bands (Meena et al., 2020). RGB-based indices, which are calculated using visible reflectance bands (Guo et al., 2021), are the simplest and most commonly used UAV method for monitoring vegetation because they do not require a special, expensive multispectral camera. Commonly derived parameters are plant cover and stress (Guo et al., 2021; Rasmussen et al., 2016). The important aspect regarding an assessment of plant stress or herbicide effectiveness is that the drones are equipped with multispectral sensors, which are capable of measuring different characteristics, including detailed plant vigour (Mink et al., 2020). The introduction of a stress factor, such as herbicide application, influences sensor measurements because sensor systems analyse the plant canopy's green colour rather than herbicide injuries on the plants (Streibig et al., 2014). Therefore, VIs calculated from these data can provide reliable information on monitored plant vitality (Mink et al., 2020). In the present study, cover crop vitality was estimated visually by the greenness of the plants, as in the drone assessments.

There are different variables in the environment that need to be considered when using different VIs, such as limiting factors (Xue et al., 2017). The NIR and red-based indices can be affected by low vegetation coverage due to the presence of soil in the background (Thenkabail et al., 2000). It should be noted that beside the effects of soil, some indices are sensitive to atmospheric effects (Xue et al., 2017).

Given the unique properties of various VIs, the normalised difference vegetation index (NDVI) and leaf chlorophyll index (LCI) were chosen as NIR/red-edge-based indices, and the visible atmospherically resistant index (VARI) and excess green index (EXG) were selected as RGB-based indices to evaluate herbicide treatments in this study.

Thus far, little is known about cover crop desiccation by bioherbicides. By focusing on cover crop management in the European context, this study aimed to close this knowledge gap by evaluating the herbicidal potential of pelargonic acid for cover crop desiccation compared with other chemical herbicides, using 'Rostock' (northeast Germany) as a case study site. Owing to its rapid degradation effect, it is anticipated that PA will be used as a bio-based alternative for cover crop desiccation purposes. This study site was chosen because of its moderate maritime climate in which cover crops are not usually damaged by frost in winter.

A further objective of this research was to verify the credibility of RGB and NIR-based vegetation indices derived from drone images to measure the effect of herbicides in cover crop desiccation at different assessment times after application. It was hypothesised that VIs derived from drone imagery can provide detailed information about crop vitality at different assessment times after application of herbicides and can be used as an alternative to the visual rating method.

4.3 Materials and Methods

Study site

The experiment was conducted under field conditions in the summer of 2019 and repeated in the summer of 2021 in northeast Germany (location 'Rostock': 54º3'39.76" N, 12º4'58.14" E) (Figure 11). In both years, the soil type in the fields was loamy sand. Local weather conditions in 'Rostock' are favourable for arable crop production due to its moderate maritime climate. Winter cover crops are commonly used in crop rotations that include summer crops (e.g., maize, legumes, summer cereals or root and tubers). The average air temperature for the growing season was 13.3 °C (minimum 2.4 °C and maximum 24.3 °C) in 2019 and 12.3 °C (minimum 0 °C and maximum 26.5 °C) in 2021. The total amount of precipitation in the growing season until application of the treatments was 186 mm in 2019 and 241 mm in 2021. After the experiment started, the field received a further 24 mm and 33.8 mm more rain respectively in 2019 and 2021 (Figure 12).

For the experiment in 2019, clover grass (DSV COUNTRY field grass 2055) was sown in April at a rate of 20 kg/ha in 10 rows per plot with a 15 cm row space, using a System Hege 34. "DSV COUNTRY field grass 2055" contains 20% perennial ryegrass (*Lolium perenne*), 30% red clover (*Trifolium pratense*), 20% timothy grass (*Phleum pratense*) and 30% meadow fescue (*Festuca pratensis*). For the experiment in 2021, the same clover grass was sown using the same method in autumn 2020 and was cut once in June 2021 (before the start of experiment) to maintain similar plant sizes and conditions as in 2019.

Figure 11. Study area of Rostock, northeast Germany.

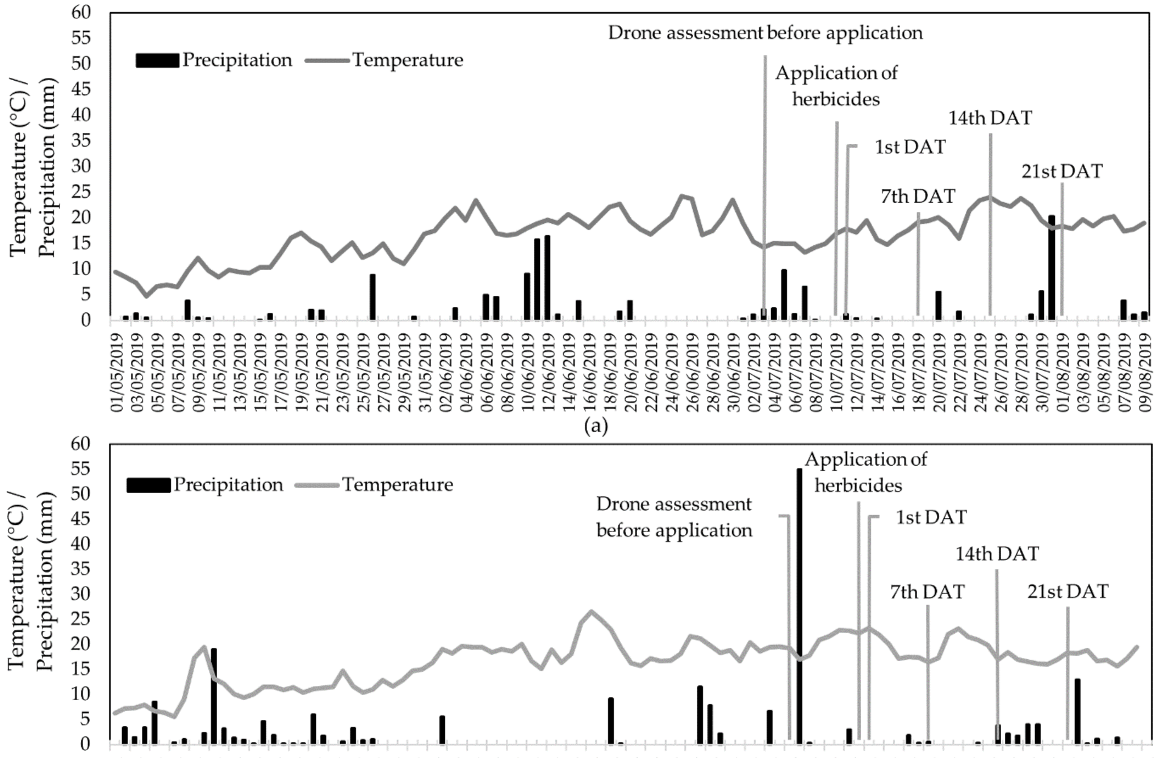


Figure 12. Air temperature (°C) and precipitation during the growing season (mm) in (a) 2019 and (b) 2021. Information for both years was obtained from the research weather station of the Department of Hydrology and Applied Meteorology of the University of Rostock.

Experimental setup

The experimental design for both experimental years consisted of a randomised complete block design with four replications. The size of the experimental area was around 460 m^2 and included 28 plots of 6 m^2 in four columns (blocks) and seven rows. The plant growth stage at the time of herbicide application in both years ranged from BBCH 45 to 49. The herbicide treatments applied in this experiment are given in Table 4. The treatments included three different dosages (16, 8 and 5 l/ha) of pelargonic acid, with the 8 l/ha dosage applied twice (the second time within a week of the first application). The commercial product Beloukha® was used for treatments containing pelargonic acid.

For the treatments including the active ingredient pyraflufen, Quickdown® was used. Roundup Ultra® and Roundup Powerflex® were used as glyphosate in 2019 and 2021 respectively. Treatments were applied by means of a plot-spraying device with a pressure of 2.1 bar and speed of 4 kilometers per hour. The application volume for the treatments including Quickdown® was 300 l/ha and for the rest was 200 l/ha. Flat jet nozzles size 02 and 03 were used for 200 l/ha and 300 l/ha application volumes respectively.

Table 4. Herbicide treatments used in the experiments in 2019 and 2021

Treatment	Amount used (L /ha)	Active ingredient content (g/L)
UC	-	Untreated control
PA	16 l/ha	680 g/l pelargonic acid
PA_R	5 l/ha	680 g/l pelargonic acid
PA_2T[1]	8 l/ha[1]	680 g/l pelargonic acid
PYR	0.8 l/ha	24.2 g/l pyraflufen
PYR + PA_R	0.8 l/ha PYR + 5 l/ha PA	24.2 g/l pyraflufen + 680 g/l pelargonic acid
GLY	3 l/ha	480 /l glyphosate

[1] Second application within a week of the first application

Assessments

The effect of the herbicide treatments was assessed by estimating the percentage of crop vitality. This assessment was conducted before application and at 1, 7, 14 and 21 days after application (DAT) of the herbicide treatments by visual observation. The "Göttinger Schätzrahmen (Bartels et al., 1983)" was placed three times randomly on each plot as a non-destructive sampling method and the percentage of the vitality of all plant species inside the frame was evaluated. A value of 0% vitality represented completely dead vegetation and a value of 100% was equivalent to completely vital vegetation. In addition to the visual observation, an assessment of the herbicide treatments was conducted using drone surveys on all the above-mentioned DATs except 21st_DAT in 2021.

An Ebee+ drone from the company Sensefly was used for the recordings. The drone has an RTK system, which in principle allows highly accurate ground control-free georeferencing. The fixed-wing aircraft weighs about 1,100 g and can carry only one payload at a time, i.e. either a colour digital camera (S.O.D.A.) with 20 MPix or a Sequoia multi-spectral camera manufactured by the company Parrot. Thus, two flights were made on each flight date: The first with the multispectral camera, followed immediately by a second with the RGB camera. Planned with a longitudinal and transversal overlap of 80% each, one flight flew over the whole of the University of Rostock's test field, which was approximately 8 ha. The speed of the fixed wing drone Ebee+ is set to 14 m/s by default, relative to the surrounding air. Depending on the wind speed and wind direction, the ground speed may vary from 11-18 m/s. Thus, the forward lap differs by between 60 and 80%. Due to the 80% side lap, there will always be enough overlap to ensure high-quality 3D-data and near-nadir viewing perspectives throughout the image blocks. The flight altitude for both flights was about 70 m, resulting in a ground resolution of 2 cm for the RGB images and 7 cm for the multispectral images. During postprocessing, the image data were photogrammetrically processed. The RGB data were processed using Metashape 1.5.2 software from Agisoft, while the multispectral data were processed using Pix4DMapper 4.5.2 software, in order to obtain absolute reflectance values. Several permanent control points were included in the bundle block adjusment to ensure the accuracy of the georeferencing. The positional and vertical accuracy at the control points was between 1 and 2 cm. Sunlight and cloud conditions during the drone flights, collected by the authors, are given in Table 5. The image surveys were acquired within two hours around solar noon (11:00-14:30 CEST). The sun elevation above the horizon was around 50-60 °.

Table 5. Sunlight and cloud conditions during the drone flights, collected by the authors

Date	2019	2021
Before application	Completely cloudy	Completely cloudy
1st_DAT	Partly cloudy	Partly cloudy
7th_DAT	Completely cloudy	Partly cloudy
14th_DAT	Completely cloudy	Partly cloudy
21st_DAT	Sunny	-

Vegetation indices

The vegetation indices used to evaluate crop vitality after application of the treatments in these experiments were as follows:

NDVI (normalised difference vegetation index) (Xue et al., 2017), calculated as:

$$NDVI = \frac{NIR - R}{NIR + R} \tag{1}$$

where NIR is the near-infrared band reflectance and R is the red band reflectance;

VARI (visible atmospherically resistant index) (Gitelson et al., 2002), calculated as:

$$VARI = \frac{G - R}{G + R - B} \tag{2}$$

where R, G, and B are the normalised red, green and blue bands of the image respectively;

EXG (excess green index) (Woebbecke et al., 2017), obtained from the following formula:

$$ExG = 2 * G \quad - R \quad - B \tag{3}$$

In this formula, to make the index more reliable and normalise the differences in image acquisition or in illumination and exposure conditions, G , R and B were used as the transformed values of the R (red), G (green) and B (blue) bands. These values were calculated as follows:

$$G \quad = \frac{G}{G_{Max}}, \qquad R \quad = \frac{R}{R_{Max}}, \qquad B \quad = \frac{B}{B_{Max}} \tag{4}$$

where R, G, and B are the average light intensity of red, green and blue, and R_{Max}, G_{Max}, and B_{Max} represent the maximum values of R, G and B respectively (Yang, 2018; Gil and Pacheco, 2020);

LCI (leaf chlorophyll index) (Narmilan et al., 2022), calculated using the following formula:

$$LCI = \frac{NIR - RedEdge}{NIR + R} \tag{5}$$

where RedEdge is the red-edge reflectance, and NIR and R represent the near-infrared band reflectance and red band reflectance respectively.

The values obtained from the above formulae were considered absolute values for VIs. The relative values of each VI were then calculated using two different approaches: the relative VI values for each plot on each assessment day were calculated relative to the absolute VI values calculated before the drone flights, and then the relative VI values were calculated relative to the absolute VI values obtained from the untreated control plots on the same assessment day.

Statistical analysis

The statistical analyses were performed using the Statistic program R version 4.2.2 (R Core Team, 2022). The distribution of visually estimated vitality data analysed by the Shapiro-Wilk normality test (Shapiro et al., 1968) showed that the data deviated significantly from a normal distribution ($p<0.01$). Therefore, the Kruskal-Wallis test (R package 'agricolae' (de Mendiburu and Yaseen, 2020)) was used to compare the herbicide treatments on each assessment day. The data distribution for all calculated VIs proved to be the same ($p<0.01$). Therefore, to check differences between treatments using VIs, a Kruskal-Wallis analysis was conducted on absolute VI values. These results can be found at Appendix A.

Linear and polynomial regressions were fitted to the data to investigate the relationships between all the VIs and between the VIs and estimated vitality on each DAT. The vitality loss of the treatments was considered to be 0% for all plots on the day before application. Due to singularities in the dataset, the coefficient of determination (R^2) could not be determined to explain the relationship between VIs and estimated vitality before the application of the treatments in either year.

4.4 Results

Visually estimated reduction in cover crop vitality

At 1st_DAT in 2019, all the herbicide treatments except GLY caused vitality losses. The lowest visually estimated vitality was 68%, which was from PA (full dosage). The effect of this herbicide treatment increased over time and reached an estimated vitality of 38% at 7th_DAT, before starting to decrease until the end of the experiment (Figure 13).

The estimated vitality of the PYR+PA_R treatment showed a decremental trend from 90% to 70% until 14th_DAT. After that time, however, the trend became incremental. PA_2T exhibited the same trend as PYR+PA_R, although the vitality loss caused by PA_2T was 10%, which was significantly higher than PYR+PA_R on 14th_DAT. The estimated vitality was extremely high for the GLY and PYR treatments at 1st_DAT in 2019. The herbicidal effect of the PYR treatment increased slightly up to 14th_DAT, but vitality loss was ≈10% at 21st_DAT (Figure 13).

At the end of the experiment, GLY showed the lowest estimated vitality (≈ 30%), while among other herbicide treatments, the lowest vitality (≈84%) was obtained by PA_2T. Nevertheless, during the first week following application, the vitality loss obtained by PA proved to be 40%, which was significantly higher than that obtained by GLY (Figure 13). PA_R showed the lowest vitality loss among the herbicide treatments of 2% to 5%.

The repetition of the experiment in 2021 revealed similar results. A greater vitality loss was obtained by treatments containing pelargonic acid at 1st_DAT. Except for PA_R, this herbicidal effect was visible until 14th_DAT. GLY was the only treatment that did not exhibit a significant vitality loss compared with the untreated control at 1st_DAT. The herbicidal effect of PA decreased from a loss of vitality of 83% (1st_DAT) to 58% (7th_DAT), and then to 25% at the end of experiment. The lowest vitality for PYR was 7th_DAT at 60%, which was significant. The effect of this treatment slowly decreased after that time. PA_2T displayed over 50% vitality loss during the first week following application and later declined gradually over time. PYR+PA_R showed a similar decline during the experiment. One week after application, GLY started to exhibit a significant loss in crop vitality. The minimum vitality obtained by GLY was 2% at the end of the experiment (Figure 13).

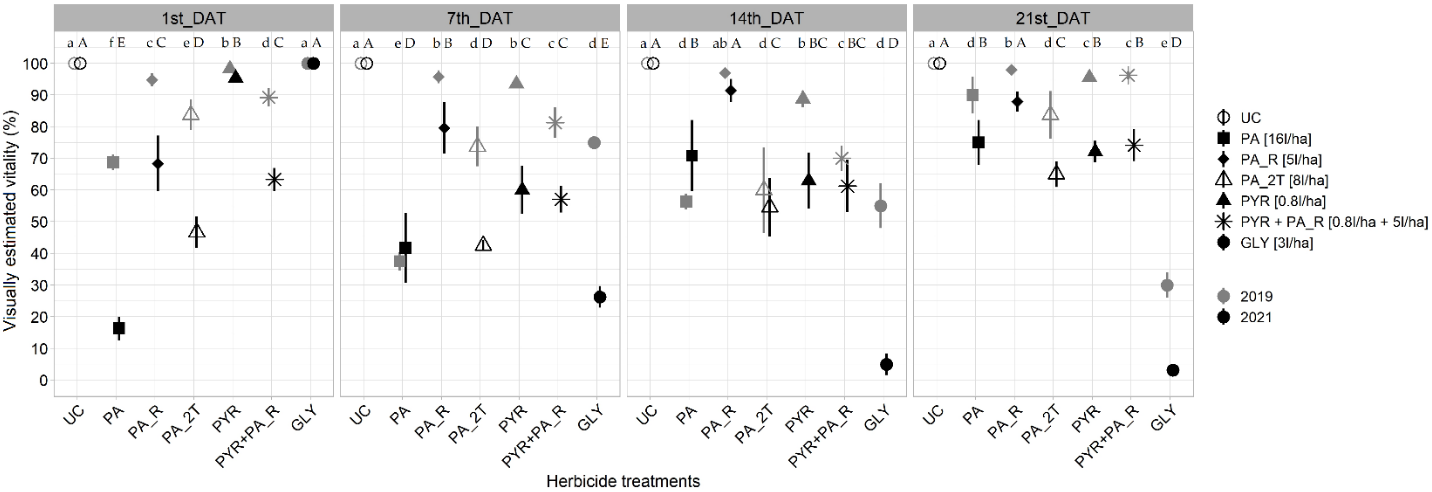


Figure 13. Visually estimated crop vitality after application of herbicides on the cover crop in both years. Small letters show significant differences between treatments within each day in 2019 and capital letters show significant differences between treatments within each day in 2021 at p<0.05 using the Kruskal-Wallis test. Error bars indicate the standard deviation for each treatment within each assessment day.

Vegetative indices calculated from drone images

To be able to compare visual observations with VIs, relative VI values compared with the untreated control plot were used. These are shown in Figures 14 and 15. To make the visualisation comprehensible, the estimated vitality values were converted into decimal numbers and then used in the figures for illustration purposes. Furthermore, as these figures show, the estimated vitality obtained from visual observations and the drone-based approach (VIs) was very similar. The estimated vitality graph (Figures 14a, 15a) shows that the effect of the treatments containing PA wore off over time, depending on the application rate and time of observation. In comparison, the onset of vitality loss caused by GLY came after a delay. Both experimental years showed similar results for PA and GLY. However, in 2021, PA showed a more satisfactory herbicidal effect and the herbicidal effect of PA_2T was more constant during the experiment (Figure 14a). EXG exhibited a trend closer to the estimated vitality in both years (Figures 14, 15) while the trends for NDVI and VARI were closer to the estimated vitality in 2021 (Figure 15).

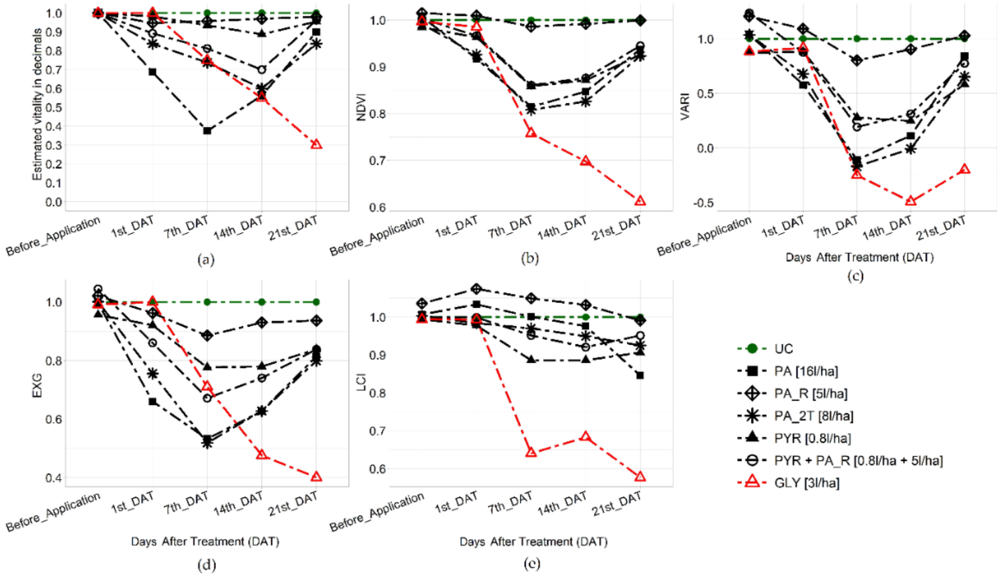


Figure 14. (a) Estimated crop vitality obtained by visual observation in 2019; (b), (c), (d), (e): calculated VIs relative to the untreated control obtained by a drone in 2019.

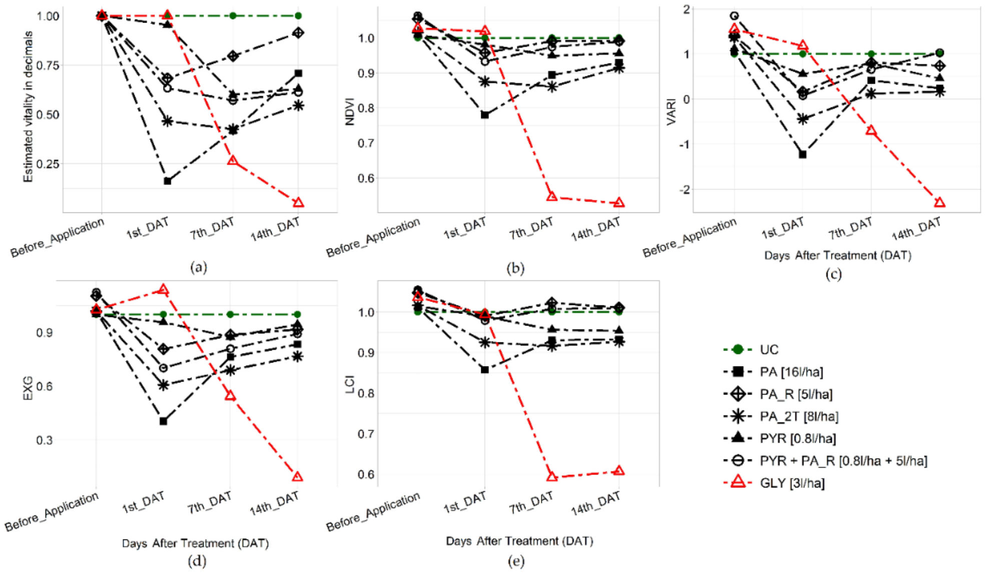


Figure 15. (a) Estimated crop vitality obtained by visual observations in 2021; (b), (c), (d), (e): calculated VIs relative to the untreated control obtained by a drone in 2021.

Figure 16 illustrates changes in plant vitality within the experimental plots before and after herbicide application in 2021, expressed by RGB imagery. Figure 17 shows the NDVI map of the experimental plots before and after herbicide application in 2021. In both figures, the effect of PA applied on four plots at 1st_DAT can be seen. These two figures also show the start of vitality loss in the plot treated with GLY from 7th_DAT up to the end of the experiment.

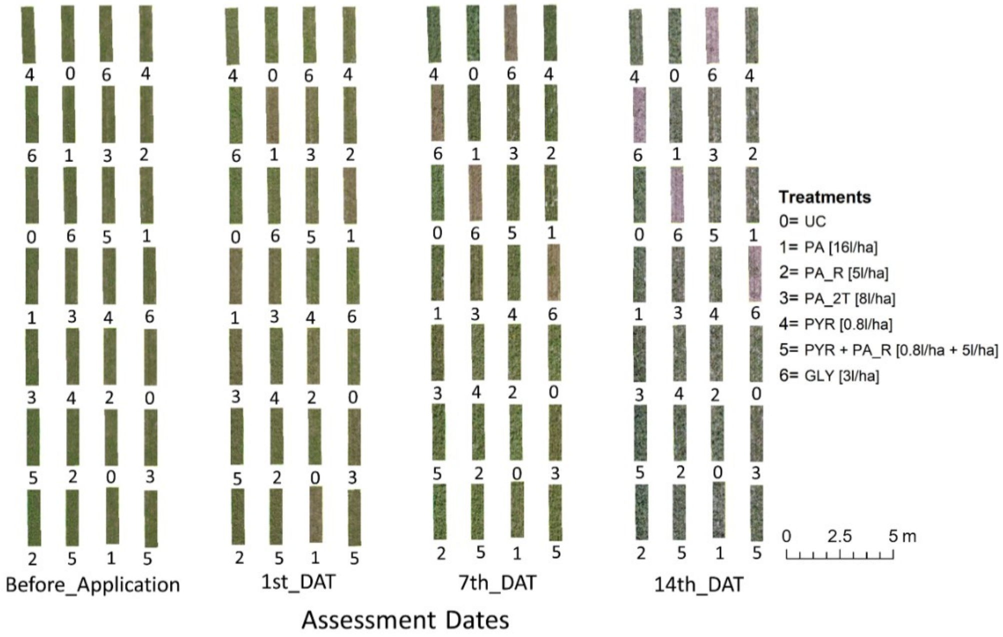


Figure 16. Changes in plant vitality due to different herbicides during the experiment in 2021, expressed by RGB.

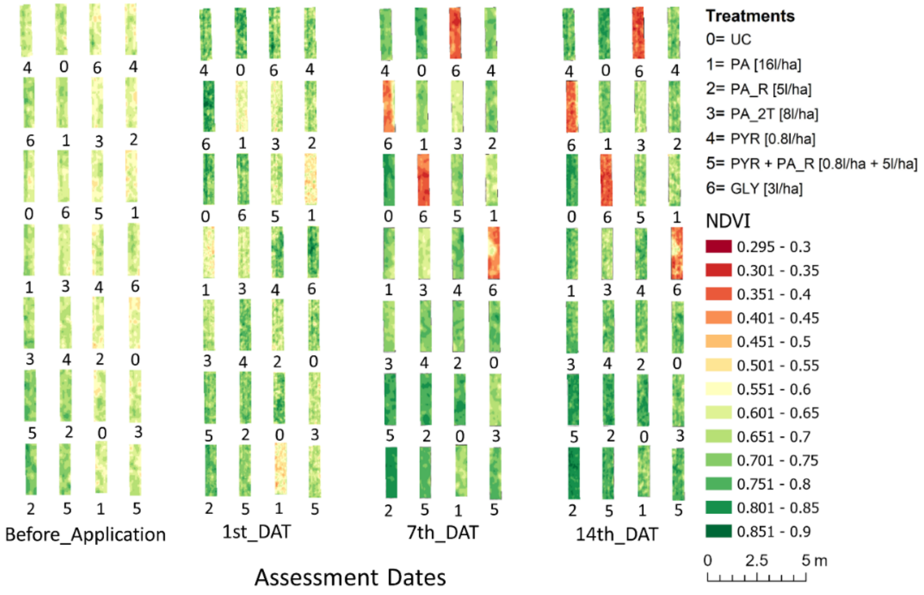


Figure 17. Changes in plant vitality due to different herbicides during the experiment in 2021, expressed by NDVI.

Relationship between vegetative indices and visually estimated plant vitality

The results of the regression analysis showed that in both years there were significant positive relationships ($p<0.05$) between NDVI and estimated visual plant vitality on all assessment days. In 2019, R^2 at 21st_DAT was significantly higher than all other DATs ($R^2= 0.93$, $p<0.05$). Coefficients of determination (R^2) were 0.64 and 0.54 for 7th_DAT and 14th_DAT respectively. The smallest correlation between these two variables occurred on 1st_DAT (Figure 18a). In 2021, the highest R^2 was 0.96, which was determined for 14th_DAT (Figure 19a).

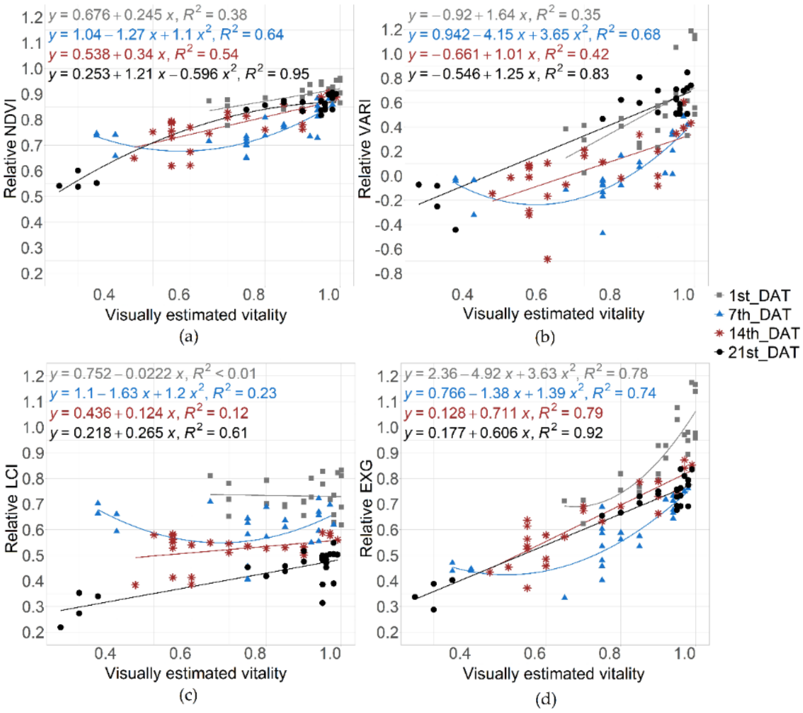


Figure 18. Relationship between VIs (relative values compared with before application) and visually estimated vitality in 2019. Linear and polynomial regression analysis ($p<0.05$).

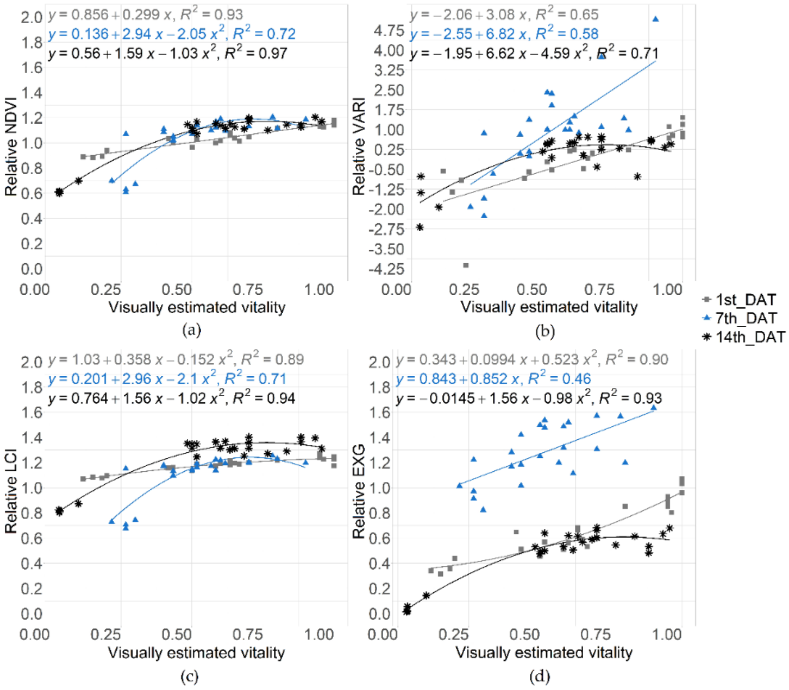


Figure 19. Relationship between VIs (relative values compared with before application) and visually estimated vitality in 2021. Linear and polynomial regression analysis ($p<0.05$).

The relationship between VARI and estimated vitality was also positive in both years. The weakest relationship between these two variables occurred on 1st_DAT in 2019 (R^2 = 0.35, $p<0.05$). This correlation slightly improved to its strongest level at 7th_DAT ($R^2=0.68$, $p<0.05$) (Figure 18b). The results for 2021 showed a different relationship between VARI and estimated vitality. The strongest correlation occurred on 1st_DAT ($R^2=0.84$, $p<0.05$) and declined over time (Figure 19b). For 1st, 7th and

14th_DAT, coefficient determination values for the correlation between LCI and estimated vitality were very low, with an incremental trend and change to a high correlation with R^2 of 0.61 at 21st_DAT (Figure 18c). In 2021, their relationship was significantly positive ($p<0.05$) at 1st_DAT, 7th_DAT and 14th_DAT with R^2 of 0.89, 0.67 and 0.94 respectively (Figure 19c). Regression analysis revealed a significant relationship ($p<0.05$) between EXG and estimated vitality in 2019 for all assessment days (Figure 18d). In 2021, the EXG index exhibited a significantly high correlation at 1st_DAT and 14th_DAT. R^2 for 7th_DAT was 0.46 (Figure 19d).

Relationship between vegetative indices

The results of the regression analysis showed that there were significant ($p<0.05$) and strongly positive relationships between NDVI, VARI and EXG on almost all assessment days in both years (Figures 20 and 21). In 2019, the correlation between NDVI and VARI was higher than that between NDVI and EXG (Figure 20a, c). LCI displayed a strong correlation with NDVI on all DATs in 2019 (Figure 20e). Its relationship with VARI was not as strong as that with NDVI. At 1st and 7th_DAT, LCI exhibited a low correlation, which improved at 14th_DAT and reached its highest level (R^2= 0.65) at 21st_DAT (Figure 20d).

In 2021, the results of the regression analysis showed that LCI had a significant positive relationship ($P<0.05$) with NDVI on all assessment days (Figure 21e). The relationship was similar between LCI and VARI (Figure 21f). The relationship between EXG and LCI was significantly positive, but R^2 for 1st_DAT was 0.43 (Figure 21b). In general, LCI displayed a higher correlation with NDVI and VARI than with EXG.

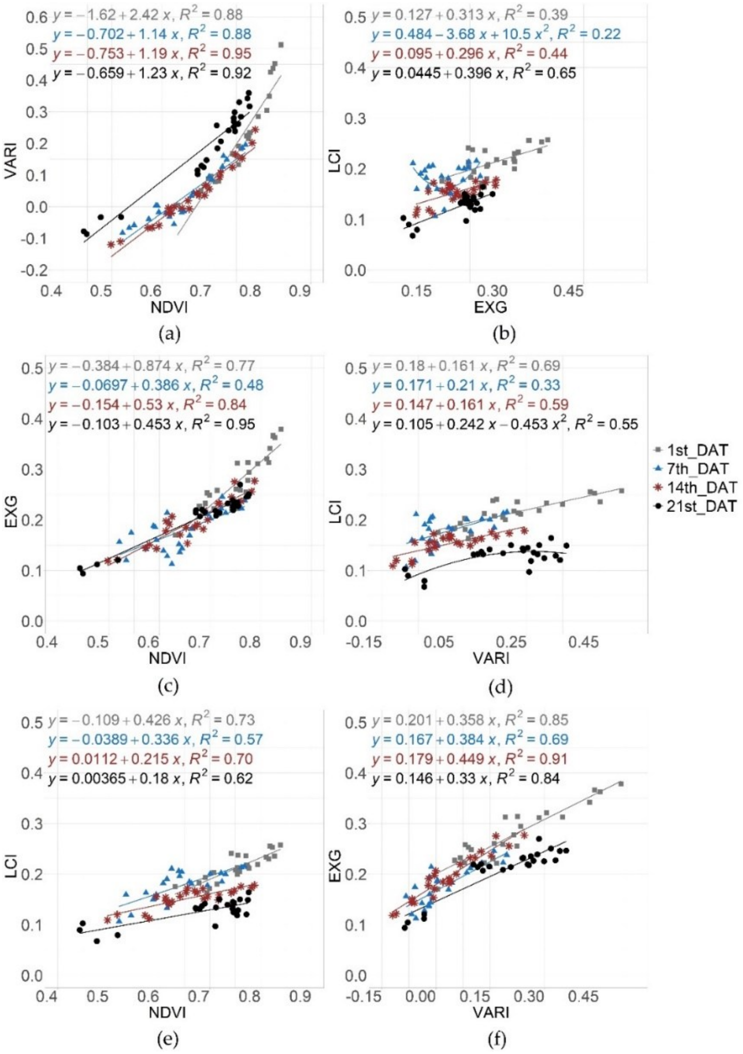
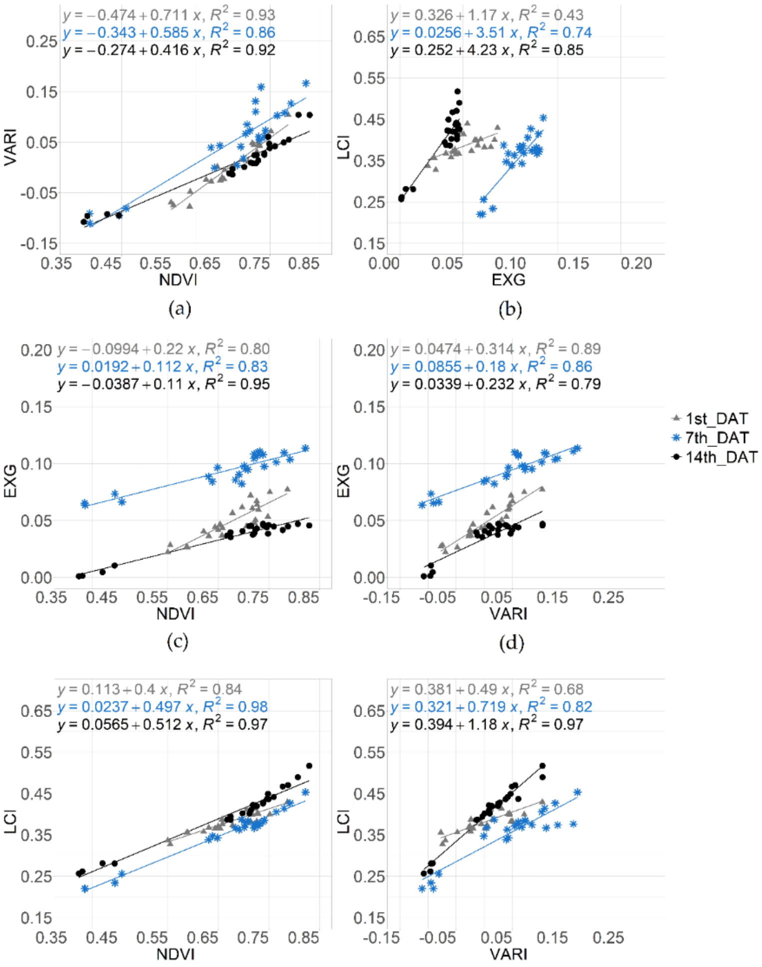


Figure 20. Relationship between VIs (absolute values) in 2019. Linear and polynomial regression analysis ($p<0.05$)



(a) (b)

(c) (d)

(e) (f)

Figure 21. Relationship between VIs (absolute values) in 2021. Linear regression analysis ($p<0.05$).

4.5 Discussion

The aim of this study was to test the effectiveness of pelargonic acid as a desiccation tool on the cover crop in comparison with other treatments. The results indicated that pelargonic acid was reasonably effective at controlling cover crop growth within a week of application. In both experimental years, PA (16 l/ha) and PA_2T (double application of 8 l/ha) showed their highest herbicidal effect up to 7th_DAT. PA vitality loss decreased over time while PA_2T continued to exhibit an almost constant effect for longer because of the second application one week later. Weber et al. (2014) report that repeated application of pelargonic acid could result in a high level of herbicidal control (Webber et al., 2014). Due to the previously mentioned fact that glyphosate-based products will be severely restricted and probably banned in Europe in the near future (Antier et al., 2020), this study compared PA as a bio-based alternative with glyphosate (GLY). In contrast to PA, both in visual observations and using the drone-based approach (VIs), the herbicidal effect of GLY was not visible in the first few days of application because GLY absorption by the plant and subsequent translocation inside the plant take some days to exhibit significant effects, which is dependent on the type of plant (annual, perennial, developmental stage) and environmental factors (Fadin et al., 2018; Satchivi et al., 2000; Sprankle et al., 1975). This means that PA as a non-selective contact herbicide could potentially be a short-term but rapid destruction alternative to glyphosate, especially in volatile weather conditions or when there are work bottlenecks (lack of labour and/or machinery).

The results demonstrated an initial rise and subsequent fall in the efficacy of PYR and its mixture with PA_R in both assessment methods. This decrease in PYR efficacy could be explained by cover crop regrowth on 7th_DAT. However, pyraflufen efficacy has been proven to be relatively dependent on plant growth stage at the time of spraying (Moretti et al., 2014; Murata et al., 2002), which might explain the lower efficacy of PYR in the present study. The results obtained from the PYR+PA_R treatment in this experiment also demonstrated that mixing pyraflufen with other post-emergence herbicides increases herbicidal efficacy (Moretti et al., 2014). However, the efficacy enhancement caused by mixing pyraflufen with pelargonic acid was around 5% greater than the single PYR treatment and 17% higher than the single PA_R treatment.

Since visual assessments of herbicidal effects are subject to human error and require a lot of time and effort (Duddu et al., 2019), the applicability of VIs for assessing herbicide effectiveness on cover crops was investigated. The VIs "NDVI, VARI, LCI and EXG" were individually compared with the visual vitality estimation and with each other.

This research showed that EXG's correlation with estimated vitality proved to be better than the correlation of other VIs with estimated vitality. The reason for this is that EXG reacts more to leaf discoloration (Yun et al., 2016), i.e., plant vitality (Streibig et al., 2014). Yun et al. (2016) report that EXG values derived from UAV low-flight altitude RGB images for experimental plots without herbicide application were higher than those with herbicide application (Yun et al., 2016). Their study proves that EXG values can be used to ascertain the impact of herbicide use on plant growth (Yun et al., 2016). According to Streibig et al. (2014) and Rasmussen et al. (2016), this index can be used to quantify crop injury from herbicide use. It is an efficient VI because it can be obtained using consumer-grade cameras mounted on UAVs (Rasmussen et al., 2016). Based on research by Yang (2018), EXG can distinguish vegetation from the surrounding environment precisely. Torres-Sánchez et al. (2014) have also reported that the EXG index obtained from RGB images can be used to visualise the vegetation fraction and vegetation growth accurately. At the start of the experiment in 2019, there was a large number of flowering weeds in the experimental plots. Furthermore, before application in the same year, the field received less precipitation and was exposed to higher temperatures, which may explain cover crop coverage in the experimental field being less, revealing more of the soil background after application of the herbicide treatments. These results demonstrate that the presence of flowering weeds and soil background in this study did not negatively affect the EXG index.

Other than EXG, according to Henry et al. (2004) and Lewis et al. (2014), one of the common vegetative indices for detecting injury symptoms on plants caused by herbicides is NDVI (Travlos et al., 2021). In this experiment, on 1st_DAT in 2019, the application of PA treatments showed significantly more vitality loss in the visual observation (estimated vitality) than in the NDVI index. In addition, the correlation between estimated vitality and both NDVI and VARI was low on 1st_DAT in 2019. NDVI is calculated by mathematically comparing the amount of red light (which vegetation absorbs) and reflected near infrared light (which vegetation reflects) (Weier and Herring, 2000). This index is sensitive to plant chlorophyll content; therefore, plant vitality and biomass are often detectable using this vegetative index (Götze et al., 2010). The chlorophyll pigments in a healthy plant absorb most of the visible red light, while the cell of the plant reflects most of the near infrared light. This means that when a plant is very photosynthetically active, less NIR will be reflected (Travlos et al., 2021). Two factors might have affected NDVI in this experiment: the presence of weeds (mostly at flowering stage) and the fact that the plants were damaged because of herbicide application. However, in some plots the plant cells were not permanently destroyed, just damaged, which had a small effect on reflected NIR, and therefore NDVI did not show as much as damage as the visual observation suggested. In contrast to NDVI, VARI has a low sensitivity to atmospheric effects (Gitelson et al., 2002), but the reason for the low correlation with estimated vitality on 1st_DAT for this index was due to the fact that a high weed population negatively impacts VARI and produce lower values (Erunova et al., 2021). VARI can also be greatly affected by the background colour and a change in leaf colour. Its value increases with the development of vegetation coverage in the background during the vegetative stages unless inflorescence appears in the field (Sakamoto et al., 2011). Therefore, both NDVI and VARI showed a higher correlation with estimated vitality at 7th_DAT, when weed density was reduced following herbicide applications and the cover crop started to regrow and produce a vegetative coverage. In previous studies, NDVI has been found to be more effective than VARI in evaluations of crop health in fields (Rodene et al., 2022).

With regard to the leaf chlorophyll index (LCI), previous studies have shown that this index has a high correlation with plant chlorophyll content (Datt et al., 2003). Changes in a plant's physical and biochemical structure caused by a stress factor could influence chlorophyll as an important pigment in photosynthesis (Meena et al., 2020), therefore the LCI value will decrease due to the reduction in this pigment content (Datt et al., 2003). Nonetheless, in the present study, LCI showed heterogeneity in both experimental years. This may be due to the scale at which this index was tested for its correlation with estimated vitality and other vegetative indices. The correlation between VIs and plant parameters might reflect variability on different scales, meaning that those that are strongly correlated with a plant parameter on a certain scale may exhibit a very weak correlation on another scale. In the case of LCI, when the amount of foliage in the plant canopy is low, LCI has a low correlation with a plant's chlorophyll on a canopy scale, while it shows a high correlation with chlorophyll content on a leaf scale (Xue et al., 2017; Xiao et al., 2013). In this study, the soil was not completely covered by the cover crop in 2019 due to less precipitation and higher temperatures in the growing season and also to the existence of a high weed infestation. Therefore, during the period of 1st to 14th_DAT, LCI was heavily influenced and exhibited weak correlations with estimated vitality. After cover crop regrowth, LCI showed a higher correlation with estimated vitality on 21st_DAT. Previous studies have demonstrated the negative effects of low vegetation coverage and the presence of soil background on NIR and red-based indices (Thenkabail et al., 2000).

The relationship analysis of VIs in the current study revealed that NDVI and VARI were highly correlated on all assessment days in both years. NDVI and VARI also correlated linearly with EXG on all assessment days in both years. The high correlation between EXG and VARI indices is in line with the findings of Zhang et al. (2019) in an assessment of turf grass performance (Zhang et al., 2019) and another study estimating the aboveground biomass of wheat (Lu et al., 2019).

4.6 Conclusions

The cover crop's high susceptibility to the PA and PA_2T treatments in the early days after application was proven by both the visual and drone-based assessments. The results confirmed that pelargonic acid has the potential to be a more sustainable alternative to synthetic herbicides. The possible ban of glyphosate in Europe requires further research to be undertaken on bio-herbicides. Pelargonic acid has been registered on the European market for use as a plant desiccant in potatoes and to kill suckers in perennial crops such as hop and grapevine. However, it is currently not registered for other management purposes in arable crops. The results of this study demonstrate that pelargonic acid has considerable potential to be an additional tool in integrated crop management. Future use registrations, e.g., for cover crop desiccation or the control of monocotyl and dicotyl weeds in arable crops, would allow the use of pelargonic acid as a substitute for glyphosate. Moreover, details on its technical application (water temperature, adjuvants and the effect of weather conditions during/after application) require further investigations to ensure its suitability for on-farm use.

With regard to the drone assessment, it was concluded that VIs obtained by drone imagery can be used to monitor the effectiveness of herbicide desiccation methods in cover crops. Furthermore, the EXG index demonstrated its ability to visualise the effect of herbicides with a high degree of accuracy on all assessment days. As an RGB-based index obtainable with customer-grade cameras, EXG presents a more cost-effective option than NIR-based indices and offers an alternative to visual observation. LCI is the most sensitive and least robust VI to bi-directional reflectance distribution function (BRDF), shade and other irregularities such as flowering weeds in the crop cover. However, if the vegetation cover is homogeneous, it is a very good VI for determining plant stress and changes in leaf chlorophyl.

4.7 Acknowledgements

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4.8 Appendix A

Supplementary Table 5. Results of a comparison of treatments using absolute values of vegetation indices at different DAT in 2019

VI	Treatment	Mean					Mean rank							
		Before	1st_DAT	7th_DAT	14th_DAT	21st_DAT	1st_DAT		7th_DAT		14th_DAT		21st_DAT	
NDVI	UC	0.847	0.790	0.757	0.776	0.771		a	24.75	a	25	a	24.25	a
	PA	0.851	0.721	0.614	0.656	0.723	9.25	a	11	bc	11.75	b	13	b
	PA_R	0.859	0.796	0.746	0.770	0.771	20	a	24	a	24	a	24.75	a
	PA_2T	0.838	0.727	0.610	0.641	0.712	8.5	a	9.25	bc	10	b	11.25	b
	PYR	0.833	0.758	0.649	0.676	0.714	14	a	12.5	bc	13.75	b	12.75	b
	PYR+PA_R	0.848	0.760	0.649	0.679	0.729	14.5	a	14	b	14.5	b	13	b
	GLY	0.844	0.779	0.575	0.542	0.472	17.25	a	6	c	2.5	c	2.5	c
VARI	UC	0.384	0.317	0.230	0.224	0.332	19	ab	25.25	a	24.5	a	21.25	ab
	PA	0.368	0.116	-0.027	0.014	0.247	7.75	b	8.5	cd	11.75	b	16.75	abc
	PA_R	0.436	0.304	0.172	0.191	0.326	19.75	a	23.75	a	24.5	a	24.25	a
	PA_2T	0.348	0.129	-0.031	0.001	0.201	8.5	ab	6.75	d	9.75	b	12.25	c
	PYR	0.317	0.225	0.057	0.050	0.182	14	ab	17	b	14	b	10.25	c
	PYR+PA_R	0.428	0.198	0.031	0.052	0.230	14	ab	14	bc	14.5	b	14.25	bc
	GLY	0.342	0.312	-0.043	-0.091	-0.057	18.5	ab	6.25	d	2.5	c	2.5	d
EXG	UC	0.309	0.325	0.260	0.281	0.270	21	a	26.25	a	26	a	25.75	a
	PA	0307	0211	0.138	0.176	0220	3	c	5.25	d	8.75	c	11.5	bc
	PA_R	0.315	0.312	0.230	0.261	0.253	19.25	a	22.25	a	22.75	a	23.25	a
	PA_2T	0.308	0.241	0.135	0.177	0.216	8	bc	4.75	d	9.25	c	8.75	c
	PYR	0.295	0.296	0.202	0.219	0.227	15.75	ab	17	b	17	b	15	b
	PYR+PA_R	0.323	0.277	0.174	0.208	0.227	14.25	ab	11.75	c	14.75	b	14.75	b
	GLY	0.307	0327	0.185	0.134	0108	20.25	a	14.25	bc	3	d	2.5	d
LCI	UC	0.290	0.215	0.195	0.169	0.147	14.75	a	18.25	ab	21	ab	22	a
	PA	0.292	0.217	0.192	0.164	0.123	16	a	17.75	ab	18	b	10	bc
	PA_R	0.301	0.229	0.204	0.174	0.146	19	a	22	a	24.75	a	20.75	a
	PA_2T	0.291	0.208	0.186	0.160	0.135	12.25	a	15.75	ab	15.5	bc	15.25	ab
	PYR	0.288	0.206	0.171	0.149	0.133	12.25	a	11	bc	8.75	de	13.25	ab
	PYR+PA_R	0.290	0.211	0.183	0.155	0.140	12.5	a	14.25	ab	11	cd	17.5	ab
	GLY	0.289	0.214	0.125	0.115	0.085	14.75	a	2.5	c	2.5	e	2.75	c

Letters show significant differences between treatments within each day at P<0.05 using the Kruskal-Wallis test.

Supplementary Table 6. Results of a comparison of treatments using absolute values of vegetation indices at different DAT in 2021

VI	Treatment	Mean				Mean rank					
		Before	1st_DAT	7th_DAT	14th_DAT	1st_DAT		7th_DAT		14th_DAT	
NDVI	UC	0.626	0.732	0.769	0.770	21.75	a	23.5	a	22	a
	PA	0.633	0.570	0.688	0.715	2.5	d	10.25	c	12	b
	PA_R	0.660	0.701	0.763	0.764	16	ab	21	ab	19	ab
	PA_2T	0.635	0.640	0.662	0.703	7.25	cd	7.25	cd	10.75	bc
	PYR	0.631	0.717	0.730	0.735	18.75	ab	17	b	15.75	ab
	PYR+PA_R	0.665	0.682	0.750	0.761	12.75	bc	20	ab	19.5	ab
	GLY	0.642	0.744	0.419	0.405	22.5	a	2.5	d	2.5	c
VARI	UC	0.056	0.067	0.146	0.054	23.5	a	23.75	a	21	a
	PA	0.059	-0.067	0.061	0.024	2.5	d	12.75	bc	14.75	ab
	PA_R	0.083	0.014	0.111	0.046	13.75	c	19.75	a	18.5	ab
	PA_2T	0.058	-0.025	0.015	0.010	6.5	d	6.5	cd	10.5	bc
	PYR	0.050	0.037	0.101	0.025	18.5	b	18.25	ab	15.25	ab
	PYR+PA_R	0.085	0.009	0.092	0.049	12.25	c	18	ab	19	ab
	GLY	0.070	0.068	-0.094	-0.098	24.5	a	2.5	d	2.5	c
EXG	UC	0.071	0.064	0.124	0.049	21.75	b	26.5	a	26.5	a
	PA	0.072	0.026	0.094	0.041	2.5	f	10.5	d	11	d
	PA_R	0.078	0.052	0.110	0.045	14.25	c	20.75	b	17.25	c
	PA_2T	0.072	0.039	0.085	0.037	6.5	e	6.5	e	7	d
	PYR	0.071	0.061	0.108	0.046	19.5	b	20.25	b	21.5	b
	PYR+PA_R	0.079	0.045	0.100	0.043	10.75	d	14.5	c	15.75	c
	GLY	0.072	0.073	0.067	0.004	26.25	a	2.5	f	2.5	e
LCI	UC	0.315	0.402	0.394	0.447	19.25	a	20	a	20	a
	PA	0.318	0.344	0.366	0.417	2.5	c	12	ab	13	a
	PA_R	0.330	0.398	0.403	0.452	17	ab	20.25	a	18.5	a
	PA_2T	0.320	0.371	0.360	0.413	9.5	bc	11	bc	12	ab
	PYR	0.319	0.397	0.376	0.425	18.5	ab	15.25	ab	15.25	a
	PYR+PA_R	0.332	0.393	0.397	0.450	16.5	ab	20.5	a	20.25	a
	GLY	0.326	0.399	0.233	0.270	18.25	ab	2.5	c	2.5	b

For each VI letters show significant differences between treatments within each day at P<0.05 using the Kruskal-Wallis test.

4.9 References

Antier, C., Kudsk, P., Reboud, X., Ulber, L., Baret, P. V., and Messéan, A. (2020). Glyphosate use in the European agricultural sector and a framework for its further monitoring. Sustainability, 12(14), 5682. https://doi.org/10.3390/su12145682

Balkcom, K., Schomberg, H., and Lee, R. D. (2020). Chapter 5: Cover crop management. In J. Bergtold and M. Sailus (Eds.), Conservation tillage systems in the Southeast: Production, profitability and stewardship (pp. 56-76). Sustainable Agriculture Research and Education (SARE), Washington DC, USA.

Bartels, J., Wahmhoff, W., and Heitefuss, R. (1983). So kann der Praktiker Schadschwellen feststellen. DLG-Mitteilungen, 98, 270-274.

Beckie, H. J., Flower, K. C., and Ashworth, M. B. (2020). Farming without glyphosate? Plants, 9(1), 96. https://doi.org/10.3390/plants9010096

Ciriminna, R., Fidalgo, A., Ilharco, L. M., and Pagliaro, M. (2019). Herbicides based on pelargonic acid: Herbicides of the bioeconomy. Biofuels, Bioproducts and Biorefining, 13 (6), 1476–1482. https://doi.org/10.1002/bbb.2046

Coleman, R., and Penner, D. (2008). Organic acid enhancement of pelargonic acid. Weed Technology, 22(1), 38-41. http://www.jstor.org/stable/25194989

Cordeau, S., Triolet, M., Wayman, S., Steinberg, C., and Guillemin, J. P. (2016). Bioherbicides: Dead in the water? A review of the existing products for integrated weed management. Crop Protection, 87, 44-49. https://doi.org/10.1016/j.cropro.2016.04.016

Cornelius, C. D., and Bradley, K. W. (2017). Herbicide programs for the termination of various cover crop species. Weed Technology, 31(4), 1-9. https://www.jstor.org/stable/26567280

Datt, B., McVicar, T. R., Van Niel, T. G., Jupp, D. L., and Pearlman, J. S. (2003). Preprocessing EO-1 Hyperion hyperspectral data to support the application of agricultural indexes. IEEE Transactions on Geoscience and Remote Sensing, 41(6), 1246-1259. https://doi.org/10.1109/TGRS.2003.813206

de Mendiburu, F., and Yaseen, M. (2020). agricolae: Statistical procedures for agricultural research (R package version 1.4.0). https://myaseen208.github.io/agricolae/ https://cran.r-project.org/package=agricolae

Duddu, H. S., Johnson, E. N., Willenborg, C. J., and Shirtliffe, S. J. (2019). High-throughput UAV image-based method is more precise than manual rating of herbicide tolerance. Plant Phenomics, 2019, 1-9. https://doi.org/10.34133/2019/6036453

Eash, L., Berrada, A. F., Russell, K., and Fonte, S. J. (2021). Cover crop impacts on water dynamics and yields in dryland wheat systems on the Colorado Plateau. Agronomy, 11(6), 1102. https://doi.org/10.3390/agronomy11061102

Erunova, M. G., Pisman, T. I., and Shevyrnogov, A. P. (2021). The technology for detecting weeds in agricultural crops based on vegetation index VARI (PlanetScope). Journal of Siberian Federal University. Engineering and Technologies, 14, 347–353. https://doi.org/10.17516/1999-494X-0314

Fadin, D. A., Tornisielo, V. L., Barroso, A. A. M., Ramos, S., Dos Reis, F. C., and Monquero, P. A. (2018). Absorption and translocation of glyphosate in Spermacoce verticillata and alternative herbicide control. Weed Research, 58(5), 389-396. https://doi.org/10.1111/wre.12329

Gil, H. A. P., and Pacheco, A. D. J. M. (2020). Chapter 1: RGB spectral indices for the analysis of soil protection by vegetation cover against erosive processes. In A. Vieira and S. C. Rodrigues (Eds.), Soil erosion: Current challenges and future perspectives in a changing world (pp. 3-14). IntechOpen.

Gitelson, A. A., Kaufman, Y. J., Stark, R., and Rundquist, D. (2002). Novel algorithms for remote estimation of vegetation fraction. Remote Sensing of Environment, 80, 76-87. https://doi.org/10.1016/S0034-4257(01)00289-9

Götze, C., Jung, A., Merbach, I., Wennrich, R., and Gläßer, C. (2010). Spectrometric analyses in comparison to the physiological condition of heavy metal stressed floodplain vegetation in a standardized experiment. Open Geosciences, 2(2), 132-137. https://doi.org/10.2478/v10085-010-0002-y

Guo, Z. C., Wang, T., Liu, S. L., Kang, W. P., Chen, X., Feng, K., Zhang, X. Q., and Zhi, Y. (2021). Biomass and vegetation coverage survey in the Mu Us sandy land based on unmanned aerial vehicle RGB images. International Journal of Applied Earth Observation and Geoinformation, 94, 102239. https://doi.org/10.1016/j.jag.2020.102239

Hoagland, R. E. (1996). Chemical interactions with bioherbicides to improve efficacy. Weed Technology, 10, 651-674.

Kornecki, T. S., and Balkcom, K. S. (2020). Chapter 9: Planting in cover crop residue. In J. Bergtold and M. Sailus (Eds.), Conservation tillage systems in the Southeast: Production, profitability and stewardship (pp. 119-132). Sustainable Agriculture Research and Education (SARE), Washington DC, USA.

Krauss, J., Eigenmann, M., and Keller, M. (2020). Pelargonic acid for weed control in onions: factors affecting selectivity. Julius Kühn-Archiv 464, 415–419. https://doi.org/10.5073/jka.2020.464.062

Legleiter, T., Johnson, B., Jordan, T., and Gibson, K. (2012). Successful cover crop termination with herbicides. Purdue University, Purdue Extension Bulletin WS-50-W. Available online: https://www.extension.purdue.edu/extmedia/ws/ws-50-w.pdf (Accessed on November 07, 2022).

Lu, N., Zhou, J., Han, Z., Li, D., Cao, Q., Yao, X., Tian, Y., Zhu, Y., Cao, W., and Cheng, T. (2019). Improved estimation of aboveground biomass in wheat from RGB imagery and point cloud data acquired with a low-cost unmanned aerial vehicle system. Plant Methods, 15, 1–16. https://doi.org/10.1186/s13007-019-0402-3

Marino, S., and Alvino, A. (2019). Detection of spatial and temporal variability of wheat cultivars by high-resolution vegetation indices. Agronomy, 9(5), 226. https://doi.org/10.3390/agronomy9050226

Meena, S. V., Dhaka, V. S., and Sinwar, D. (2020). Exploring the role of vegetation indices in plant diseases identification. In Proceedings of the 2020 Sixth International Conference on Parallel, Distributed and Grid Computing (PDGC) (pp. 372-377). https://doi.org/10.1109/PDGC50313.2020.9315814

Mink, R., Linn, A. I., Santel, H. J., and Gerhards, R. (2020). Sensor-based evaluation of maize (Zea mays) and weed response to post-emergence herbicide applications of Isoxaflutole and Cyprosulfamide applied as crop seed treatment or herbicide mixing partner. Pest Management Science, 76(5), 1856-1865. https://doi.org/10.1002/ps.5715

Moretti, M. L., Watkins, S., and Hanson, B. (2014). Post efficacy with Venue (pyraflufen) as a tankmix partner in orchard crops. University of California Weed Science – Davis, California, USA. Available online: https://ucanr.edu/blogs/ucdweedscience/blogfiles/24940.pdf (Accessed on November 07, 2022).

Murata, S., Yamashita, A., Kimura, Y., Motoba, K., Mabuchi, T., and Miura, Y. (2002). Herbicidal activity and characteristics of pyraflufen-ethyl for controlling broad-leaved weeds in cereals. Journal of Pesticide Science, 27(1), 39-46. https://doi.org/10.1584/jpestics.27.39

Narmilan, A., Gonzalez, F., Salgado, A. S. A., Kumarasiri, U. W. L. M., Weerasinghe, H. A. S., and Kulasekara, B. R. (2022). Predicting canopy chlorophyll content in sugarcane crops using machine learning algorithms and spectral vegetation indices derived from UAV multispectral imagery. Remote Sensing, 14(5), 1140. https://doi.org/10.3390/rs14051140

Paganelli, A., Gnazzo, V., Acosta, H., López, S. L., and Carrasco, A. E. (2010). Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. Chemical Research in Toxicology, 23(10), 1586-1595. https://doi.org/10.1021/tx1001749

Palhano, M. G., Norsworthy, J. K., and Barber, T. (2018). Evaluation of chemical termination options for cover crops. Weed Technology, 32(3), 227-235. https://doi.org/10.1017/wet.2017.113

Pittman, K. B., Cahoon, C. W., Bamber, K. W., Rector, L. S., and Flessner, M. L. (2020). Herbicide selection to terminate grass, legume, and brassica cover crop species. Weed Technology, 34(1), 48-54. https://doi.org/10.1017/wet.2019.107

Rasmussen, J., Ntakos, G., Nielsen, J., Svensgaard, J., Poulsen, R. N., and Christensen, S. (2016). Are vegetation indices derived from consumer-grade cameras mounted on UAVs sufficiently reliable for assessing experimental plots? European Journal of Agronomy, 74, 75-92. https://doi.org/10.1016/j.eja.2015.11.026

R Core Team. (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R-project.org/

Rodene, E., Xu, G., Palali Delen, S., Zhao, X., Smith, C., Ge, Y., Schnable, J., and Yang, J. (2022). A UAV-based high-throughput phenotyping approach to assess time-series nitrogen responses and identify trait-associated genetic components in maize. The Plant Phenome Journal, 5(1). https://doi.org/10.1002/ppj2.20030

Rosario-Lebron, A., Leslie, A. W., Yurchak, V. L., Chen, G., and Hooks, C. R. R. (2019). Can winter cover crop termination practices impact weed suppression, soil moisture, and yield in no-till soybean [Glycine max (L.) Merr.]? Crop Protection, 116, 132–141. https://doi.org/10.1016/j.cropro.2018.10.020

Sakamoto, T., Shibayama, M., Kimura, A., and Takada, E. (2011). Assessment of digital camera-derived vegetation indices in quantitative monitoring of seasonal rice growth. ISPRS Journal of Photogrammetry and Remote Sensing, 66, 872–882. https://doi.org/10.1016/j.isprsjprs.2011.08.005

Satchivi, N. M., Wax, L. M., Stoller, E. W., and Briskin, D. P. (2000). Absorption and translocation of glyphosate isopropylamine and trimethylsulfonium salts in Abutilon theophrasti and Setaria faberi. Weed Science, 48(6), 675-679. https://doi.org/10.1614/0043-1745(2000)048[0675:AATOGI]2.0.CO;2

Savage, S., and Zorner, P. (1996). The use of pelargonic acid as a weed management tool. In Proceedings of the California Weed Conference (Vol. 48, pp. 46-47).

Schmitt, M. B., Berti, M., Samarappuli, D., and Ransom, J. K. (2021). Factors affecting the establishment and growth of cover crops intersown into maize (Zea mays L.). Agronomy, 11(4), 712. https://doi.org/10.3390/agronomy11040712

Shapiro, S. S., Wilk, M. B., and Chen, H. J. (1968). A comparative study of various tests of normality. Journal of the American Statistical Association, 63, 1343-1372.

Sprankle, P., Meggitt, W. F., and Penner, D. (1975). Absorption, action, and translocation of glyphosate. Weed Science, 23(3), 235-240. https://doi.org/10.1017/S0043174500052930

Streibig, J. C., Rasmussen, J., Andújar, D., Andreasen, C., Berge, T. W., Chachalis, D., Dittmann, T., Gerhards, R., Giselsson, T. M., Hamouz, P., and Jaeger-Hansen, C. (2014). Sensor-based assessment of herbicide effects. Weed Research, 54(3), 223-233. https://doi.org/10.1111/wre.12079

Tataridas, A., Kanatas, P., Chatzigeorgiou, A., Zannopoulos, S., and Travlos, I. (2022). Sustainable crop and weed management in the era of the EU Green Deal: A survival guide. Agronomy, 12(3), 589. https://doi.org/10.3390/agronomy12030589

Thenkabail, P. S., Smith, R. B., and De Pauw, E. (2000). Hyperspectral vegetation indices and their relationships with agricultural crop characteristics. Remote Sensing of Environment, 71(2), 158-182. https://doi.org/10.1016/S0034-4257(99)00067-X

Torres-Sánchez, J., Peña, J. M., de Castro, A. I., and López-Granados, F. (2014). Multi-temporal mapping of the vegetation fraction in early-season wheat fields using images from UAV. Computers and Electronics in Agriculture, 103, 104-113. https://doi.org/10.1016/j.compag.2014.02.009

Travlos, I., Rapti, E., Gazoulis, I., Kanatas, P., Tataridas, A., Kakabouki, I., and Papastylianou, P. (2020). The herbicidal potential of different pelargonic acid products and essential oils against several important weed species. Agronomy, 10(11), 1687. https://doi.org/10.3390/agronomy10111687

Travlos, I., Tsekoura, A., Antonopoulos, N., Kanatas, P., and Gazoulis, I. (2021). Novel sensor-based method (quick test) for the in-season rapid evaluation of herbicide efficacy under real field conditions in durum wheat. Weed Science, 69(2), 147-160. https://doi.org/10.1017/wsc.2021.8

Ulcuango, K., Navas, M., Centurión, N., Ibañez, M.Á., Hontoria, C., and Mariscal-Sancho, I. (2021). Interaction of inherited microbiota from cover crops with cash crops. Agronomy, 11(11), 2199. https://doi.org/10.3390/agronomy11112199

Van Bruggen, A. H., He, M. M., Shin, K., Mai, V., Jeong, K. C., Finckh, M. R., and Morris, J. G., Jr. (2018). Environmental and health effects of the herbicide glyphosate. Science of the Total Environment, 616–617, 255-268. https://doi.org/10.1016/j.scitotenv.2017.10.309

Webber, C. L., Taylor, M. J., and Shrefler, J. W. (2014). Weed control in yellow squash using sequential post-directed applications of pelargonic acid. HortTechnology, 24(1), 25-29. https://doi.org/10.21273/HORTTECH.24.1.25

Weier, J., and Herring, D. (2000). Measuring vegetation (NDVI and EVI). NASA Earth Observatory. Available online: https://earthobservatory.nasa.gov/features/MeasuringVegetation/measuring_vegetation_2.php (Accessed on November 07, 2022).

Whalen, D. M., Bish, M. D., Young, B. G., Conley, S. P., Reynolds, D. B., Norsworthy, J. K., and Bradley, K. W. (2020). Herbicide programs for the termination of grass and broadleaf cover crop species. Weed Technology, 34(1), 1-10. https://doi.org/10.1017/wet.2019.73

Woebbecke, D., Meyer, G., Von Bargen, K., and Mortensen, D. (1995). Color indices for weed identification under various soil, residue, and lighting conditions. Transactions of the ASAE, 38(1), 271-281. https://doi.org/10.13031/2013.27838

Xiao, Y., Zhao, W., Zhou, D., and Gong, H. (2013). Sensitivity analysis of vegetation reflectance to biochemical and biophysical variables at leaf, canopy, and regional scales. IEEE Transactions on Geoscience and Remote Sensing, 52(7), 4014-4024. https://doi.org/10.1109/TGRS.2013.2278838

Xue, J., and Su, B. (2017). Significant remote sensing vegetation indices: A review of developments and applications. Journal of Sensors, 2017. https://doi.org/10.1155/2017/1353691

Yang, D. (2018). Gobi vegetation recognition based on low-altitude photogrammetry images of UAV. IOP Conference Series: Earth and Environmental Science, 186(5), 012053. https://doi.org/10.1088/1755-1315/186/5/012053

Yun, H. S., Park, S. H., Kim, H. J., Lee, W. D., Lee, K. D., Hong, S. Y., and Jung, G. H. (2016). Use of unmanned aerial vehicle for multi-temporal monitoring of soybean vegetation fraction. Journal of Biosystems Engineering, 41, 126–137. https://doi.org/10.5307/JBE.2016.41.2.126

Zhang, J., Virk, S., Porter, W., Kenworthy, K., Sullivan, D., and Schwartz, B. (2019). Applications of unmanned aerial vehicle-based imagery in turfgrass field trials. Frontiers in Plant Science, 10, 279. https://doi.org/10.3389/fpls.2019.00279

Chapter 5: General Discussion

In this chapter, the findings from Chapters 2 to 4 are discussed from a broader perspective. First, the results from Chapter 2 on the influence of application timing, volume, and the addition of adjuvants on the herbicidal potential of PA are addressed. Next, as explained in Chapter 3, the effects of applying PA over two consecutive years to the same pots—where each large pot contained a patch of the evaluated weed species grown from a single ramet—are discussed, considering the initial size of the ramets. Afterward, the discussion proceeds to apply PA on cover crops as a desiccant, utilizing drone techniques to evaluate the effect of herbicide application, as presented in Chapter 4. To conclude, the benefits and drawbacks of PA as a bio-herbicide and the limitations in containing perennial weeds are addressed.

5.1 The influence of application timing, volume, and the addition of adjuvants on the herbicidal potential of PA

In this study, we focused on different aspects of increasing the efficacy of PA on perennials. Since PA, as a non-selective contact herbicide, is an emulsifiable liquid and has an oily nature, its solubility in water is not high (European Food Safety Authority, 2021). Therefore, as shown in Chapter 2, we examined the technical aspects, such as increasing the surface contact of PA with the leaf by increasing the carrier volume and adding an adjuvant. For the carrier volume, we tested double the amount of water recommended by the PA product label (Beloukha®), while using a different nozzle to maintain the same concentration (16 L/ha) of PA sprayed on the plant. This approach ensured greater surface coverage. The results demonstrated that increasing the carrier volume to 400 L/ha significantly improved the efficacy of PA on *C. arvense*, specifically on root and shoot biomass at the early elongation stage. As noted by Creech et al. (2015), and Ogbangwor and Söchting (2022), there are significant differences between contact herbicides and systemic herbicides regarding increasing the carrier volume. With systemic herbicides, increasing the concentration by reducing carrier volume typically improves weed control due to reduced surface tension and enhanced cuticular penetration, especially for glyphosate (Knoche, 1994). On the other hand, some studies indicate that reducing spray volume often decreases the effectiveness of contact herbicides. Adequate coverage is essential for effective weed control with PA-based herbicides (Campos et al., 2022b). This is aligned with the results of Ogbangwor and Söchting (2022), who found that carrier volumes of 375 L/ha and 500 L/ha for PA demonstrated the highest effectiveness on certain weeds, indicating that efficacy is heavily influenced by spray retention and leaf physical characteristics.

A common practice to enhance the effectiveness of a herbicide is adding an adjuvant, which improves its ability to spread and penetrate the surface of plant leaves (Pacanoski, 2015). This is particularly beneficial under challenging conditions, such as with plants that have hairy or waxy surfaces that resist herbicide absorption (Congreve and Cameron, 2019). Since *C. arvense* has a hairy surface with fine, bristly hairs, it creates a barrier to contact herbicide. This makes the use of adjuvants, such as surfactants, crucial for improving herbicide performance against this weed. Paraffin oil, as an emulsifiable mineral oil, was an effective adjuvant that, in our study was used to decrease the PA surface tension and enhance its coverage. In this study, paraffin oil improved the coverage of PA significantly, resulting in an additional 10%-20% reduction in root and shoot biomass compared to treatments with lower volumes or no adjuvant. Our findings corroborate those of Coleman and Penner (2008) and Travlos et al. (2020), who demonstrated that adjuvants could significantly enhance the efficacy of PA on various weed species.

Because the weed growth stage at the herbicide application time plays a crucial role in increasing or decreasing the herbicide efficacy (Kieloch and Domaradzki, 2011), we tested the application of PA on different stages of *C. arvense*. Our research indicated that at the early elongation stage, the vulnerability of *C. arvense* to PA is higher. Based on the biological attributes of *C. arvense*, the increased vulnerability was expected before the flowering period due to the utilization of nutrient reserves for producing aboveground shoots and allocating resources to flowering parts. However, the so-called compensation point, which occurs at the seven-to-ten-leaf stage, was also a vulnerable stage in this study. Therefore, any aboveground disturbance applied at these two stages might show higher effectiveness than other phenological developmental stages. Previous research focusing on nutrient reserves at different developmental stages has tested the effects of aboveground disturbances and competition, demonstrating higher efficacy of their methods at these stages (Hodgson, 1968; Gustavsson, 1997; Verwijst et al., 2018; Tavaziva et al., 2019). This indicates the importance of using appropriate concentrations of PA while applying it when target weeds are at earlier growth stages. In the case of perennial weeds with creeping roots, timing the application to coincide with periods of minimal nutrient reserves in the root is crucial to prevent above- or belowground expansion. Moreover, this phenomenon has been confirmed by studies on annual weed species. Applying PA at early developmental stages, even at the lowest concentration, achieved excellent weed control, while delaying the application by just one week, significantly reduced weed control efficacy at lower concentrations (Webber and Shrefler, 2007).

Overall, when all relevant technical factors were considered, the PA effectiveness as a contact herbicide improved. Focusing on a single factor proved insufficient; however, when multiple factors were combined, the efficacy increased significantly. This indicates that to optimize the use of PA as a contact herbicide, it is important to consider various aspects, including coverage, water solubility, the timing of application relative to weather conditions and weed developmental stages, as well as the biological characteristics of the weed species.

5.2 Effects of PA application over two consecutive years on the same spot

The impact of applying PA twice to *C. arvense* and *S. arvensis* over two consecutive growing seasons in the same pot—where each large pot contained a patch of either *C. arvense* or *S. arvensis* from a single ramet—was assessed. Furthermore, the regrowth of these weeds after PA application was also investigated. In this context, two outlined strategies in the discussion section of Chapter 3, were evaluated.

As described in the Materials and Methods section of Chapter 3, 200-liter pots with a diameter of 80 cm (surface area of 0.503 m^2), with a single ramet planted in each pot were utilized. The ramet produced aboveground shoots and expanded belowground roots, all interconnected, which can be inferred as a small patch. The soil remained undisturbed throughout the entire two-year experimental period, meaning the extended creeping roots from the initial ramet were not fragmented during this time. Various ramet sizes were utilized in this study to represent an initial root fragmentation and to assess the effects of fragment size on the efficacy of the PA as the control method. However, this setup was not intended to mimic mechanical control in real on-farm conditions. In practice, root fragment sizes can vary significantly, and encountering fragments as small as 5 cm or smaller seems relatively low. Additionally, root fragmentation may occur multiple times due to the use of various machineries such as plows, harrows, or cultivators, either intentionally or unintentionally affecting the creeping roots. Despite the absence of further root fragmentation during the experimental period, PA was still able to reduce both aboveground and belowground biomass, as well as flower production and shoot density, in pots with smaller initial ramet sizes. From these findings, it can be assumed that farm activities that fragment roots, when combined with PA application, could be more effective in perennial weed management. In real farm situations, the soil is frequently disturbed, which induces regrowth from these fragments as well as seed germination. Given this, the optimal timing for PA application is after these root fragmentation practices and before crop emergence. PA should be applied when the regrown perennials and new annual weeds are in their early developmental stages and before the crop has germinated. This strategy ensures that PA can effectively suppress the weeds at their vulnerable stages while avoiding damage to crops.

The effectiveness of PA in this study was affected by the biological characteristics of both species and the variations in environmental conditions across the two experimental years. For example, the increased precipitation in the second year benefited *C. arvense*, while other unforeseen factors, such as root longevity and interspecies competition, likely impacted both species' responses to the treatments. Our research showed that PA effectiveness differs between these species, with *C. arvense* being more vulnerable to PA. This difference highlights the significance of biological traits, as noted by Liew et al.

(2013), who found that root fragments of the same size in these species do not yield the same number of shoots. Specifically, *C. arvense* produces fewer buds on equivalent root sizes compared to *S. arvensis* (Liew et al., 2013), which affects factors like shoot density, aboveground biomass, and ultimately, treatment effectiveness. These findings are consistent with prior research indicating that various weed species display different levels of susceptibility to PA (Rowley et al., 2011; Kanatas et al., 2021; Cabrera-Pérez et al., 2022; Loddo et al., 2023). The results confirm that PA may not be sufficiently effective for controlling all weed species and emphasize the need to consider several critical factors to make PA a viable alternative to non-selective, synthetic contact herbicides, especially for managing perennial weeds. These key factors include economically realistic repeated treatments of PA, and optimal integration with other tools, tailored to the specific biological attributes of each perennial weed species.

5.3 Application of PA on cover crops as a desiccant

In one respect, substituting synthetic herbicides for controlling weeds, especially perennials, can be effectively achieved through the use of cover crops (Neve et al., 2024). However, properly terminating cover crops is essential for both suppressing perennial weeds and preventing water and nutrient loss, which could adversely affect subsequent crops (Kornecki and Kichler, 2022). Since herbicides are commonly employed for terminating cover crops to enhance their weed suppression benefits, this study explored the use of PA as a desiccant for cover crops, comparing its effectiveness to that of synthetic herbicides. It's important to clarify that cover crops were not directly used as a control method for *C. arvense* and *S. arvensis* in this research. Rather, the study aimed to investigate how alternative termination methods, like bioherbicides, could effectively replace synthetic herbicides when managing perennial weeds with cover crops. Additionally, the need for a late termination to maximize weed suppression before the next crop emergence is sought. In this study, PA demonstrated potential as a desiccant tool for cover crop management, particularly at a lower dosage of 8 L/ha, with two applications repeated at a one-week interval. Regrowth was consistently observed following PA application, but with two repeated applications at the half-recommended dosage (8 L/ha) at a one-week interval, the effect was more sustained. However, the regrowth of cover crops reduced the long-term efficacy of PA.

The selection of herbicide with the purpose of terminating cover crops is significantly influenced by the region and the species being cultivated (Legleiter et al., 2012; Pittman et al., 2020). Additionally, previous research on PA has shown that it is more effective on annuals and dicotyledons than the monocotyledons and perennials (Travlos et al., 2020; Ogbangwor and Söchting, 2022; Loddo et al., 2023). In this study, the cover crop mixture includes both dicotyledonous and monocotyledonous species. This mixture was selected for several reasons: first, to observe the PA effects on a combination of both species types as cover crop; and second, to evaluate the performance of PA in comparison to other commonly used herbicides. The experiment was conducted in a moderate maritime climate with minimal winter frost. PA's efficacy was tested both alone and in a mixture with another contact herbicide at low doses in this particular region. Since winter frost is one of the methods to terminate cover crops (Legleiter et al., 2012), the results of this study are particularly useful in regions without winter frost, introducing PA as an alternative to synthetic herbicides for cover crop termination. PA has the advantage of terminating cover crops without leaving residual effects on the next crops. PA can ensure timely cover crop termination during bottleneck situations, allowing more time for biomass production and effective weed suppression. PA's burndown effect also allows for quick termination before the next crop is sown, preventing competition with the next crop for nutrients and water. For instance, termination should be timed early enough before heavy rainfall to preserve soil moisture for the subsequent crop (Balkcom et al., 2016). Given that PA is rainfast and rain does not impact its efficacy, it is an option for managing cover crop termination in such bottleneck situations. Termination may occur also earlier during dry periods to conserve moisture (Allison, 2024). PA application could also be combined with techniques like roller-crimping to prevent seed production or the regrowth of cover crop. Moreover, termination should occur before cover crop seed maturity, ideally during the early flowering stage (Allison, 2024). In this study, PA was applied around flowering time, but its efficacy is better at earlier developmental stages as also proved by other experiments on perennial plants in this study. Therefore, farming practices should align with PA's optimal application timing, potentially involving repeated applications or adjusting cover crop sowing and termination schedules specifically for each region.

The use of a lower dosage of PA could potentially provide a more cost-effective solution for real farm situations. Although, combining PA at a lower dosage with pyraflufen, was not sufficiently effective, likely due to cover crop regrowth and the low PA dosage (5 L/ha) used in the mixture. However, this outcome cannot be generalized, as other active ingredients or higher dosages of PA were not tested in this research, leaving potential combinations to be explored in future studies.

5.4 UAV Imaging for herbicide efficacy monitoring

From the drone assessment in this study, it can be inferred that vegetation indices (VIs) from drone imagery can precisely monitor herbicide desiccation effectiveness in cover crops. This research demonstrates that the Excess Green (EXG) index, which is RGB-based, accurately visualizes herbicide effects on all assessment days. It could serve as an alternative to visual observation and a cost-effective option compared to near-infrared (NIR) and red-edge-based indices, as RGB cameras are less expensive and more budget-friendly than multispectral cameras.

Drones with advanced sensors provide reliable, high-resolution, and quick data collection across large areas, reducing human error and identifying subtle changes in crop or weed infestations that are invisible to the naked eye (Duddu et al., 2019; Rodene et al., 2022; Marino and Alvino, 2019). Using them allows efficient, scalable monitoring, enabling timely data-driven decisions (Emimi et al., 2023). Vegetation indices (VIs) derived from drone images can detect early signs of herbicide efficacy and the effectiveness of other management practices (Marino and Alvino, 2019). Early detection of effectiveness allows farmers to adjust herbicide application rates or timings and minimize crop damage (Singh et al., 2020; Emimi et al., 2023). The data obtained from drones and VIs can guide precision herbicide application, targeting only the affected areas and thereby reducing the overall use of herbicides. This approach is both cost-effective and environmentally sustainable, particularly when using PA. When applied in a targeted manner (e.g., spot application), PA could be a more cost-effective solution for weed management and crop desiccation, allowing farmers to treat specific areas without impacting the entire field. This method enables the customization of PA applications based on the specific needs of different field areas and under various weather conditions while reducing environmental impact and application costs.

5.5 Benefits and drawbacks of PA as a bio-herbicide

Despite numerous studies conducted over the past decade, findings on the effectiveness of PA still vary widely and are often specific to individual cases. In this regard, some advantages, diverse functionalities, utilization purposes, problems, and drawbacks have been mentioned in previous chapters and will be explained in more detail in the forthcoming paragraphs.

As a naturally occurring substance present in both plants and animals, PA is biodegradable and decomposes into non-toxic byproducts in the environment (European Food Safety Authority, 2021; Syguda et al., 2023). Furthermore, it does not remain in the soil for long durations, minimizing residual impacts (European Food Safety Authority, 2021; Poiger et al., 2024). This reduces concerns about prolonged adverse effects on environment when compared to synthetic herbicides (Ciriminna et al., 2019). It also indicates that PA is suitable for use in terrestrial ecosystems (Webber et al., 2014). With the advantage of having extremely rapid action and being rainfast, PA acts upon contact with plant tissues, causing desiccation and necrosis (Campos et al., 2022a). This makes it a rapid, temporary solution, particularly useful during unpredictable weather conditions and when facing time limitations, workforce, or equipment which is advantageous in agricultural practices. Being non-selective, PA exhibits a broad spectrum of activity against various plant species meaning it can influence both weeds and crop plants and so far no resistant plant species have been reported (Ciriminna et al.,2019; Campos et al., 2022a). Its mode of action gives PA numerous practical applications. It also allows for effective use in non-crop areas and before crop planting (Barker and Prostak, 2009; Kanatas et al., 2020). It can be applied for seedbed cleaning or stale seedbed technique for spring-summer or autumn sown crops

(Kanatas et al., 2020; Loddo et al., 2023). It can be employed for spot weeding, edging, lining (Savage and Zorner, 1996; Ciriminna et al.,2019), blossom thinning (Fallahi,1997; Byers, 1999), crop desiccation (Kardasz et al., 2019; Klauk et al., 2023) and sucker control (Short et al., 2020). It effectively eliminates weeds in container-grown woody ornamentals, under greenhouse benches, and in areas where systemic herbicides might inadvertently harm desired plants (Savage and Zorner, 1996; Poiger et al., 2024). This versatility makes it a suitable tool for integrated weed management strategies (Loddo et al., 2023).

When considering the drawbacks of PA, several critical issues are revealed. One significant concern is the occurrence of temporary phytotoxic effects and a risk of regrowth following treatment, indicating that PA may not provide a lasting effect. It does not translocate within the plant, requiring a higher rate or frequent reapplication in some cases (Campos et al., 2022a, 2022b). The timing of application and weather conditions are critical factors that require meticulous planning, as improper conditions can diminish herbicidal efficacy. Its efficacy is reduced against well-established weeds, posing a challenge for comprehensive weed management. It also exhibited inconsistent herbicidal efficacy across tested weed species, with grass weeds demonstrating higher tolerance, no mortality, and minimal biomass reduction even at high application rates (Jagarapu, 2022; Loddo et al., 2023). Among dicot weeds, some species were able to withstand PA application, influenced by leaf characteristics such as angle, hairiness, and the presence of cuticle wax (Jagarapu, 2022). Weather conditions such as light, temperature, and humidity significantly influence the success of weed control (Jagarapu, 2022; Krauss et al., 2020; Campos et al., 2022b; Loddo et al., 2023). High relative humidity improves the efficacy of herbicides by enhancing spray deposit hydration and penetration across the cuticle (Campos et al., 2022b). Despite the label suggestions for applying PA herbicides on sunny days, another research indicates that PA action is not light-dependent (Lederer et al., 2004). However, light might still play a role through transpiration, as higher transpiration rates with open stomata contribute to the mode of action for PA (Campos et al., 2022b; Jagarapu, 2022). It was also reported by Jagarapu (2022) and Loddo et al., (2023) that hot and dry conditions can enhance weed leaf traits that reduce sensitivity to the herbicide and decrease spray droplet persistence on leaf surfaces, thereby limiting its penetration and efficacy. Technical aspects of the application like carrier volume, boom height, and water temperature can also affect the PA efficacy, for instance raising the water temperature used for application from 5°C to 25°C led to a threefold increase in the initial penetration of PA (Loddo et al., 2023). One of the most important concerns about PA is that the production cost and application tend to be higher compared to synthetic herbicides, which greatly impacts its economic feasibility for farmers, potentially limiting its widespread adoption (Crmaric et al., 2018; Ciriminna et al., 2024).

The current research aimed to leverage the beneficial aspects of PA application while addressing the drawbacks mentioned above. Our goal was to identify potential solutions to enhance PA efficacy and demonstrate its full potential. This research advances the current knowledge base by illustrating how its rapid burndown effect, non-selective contact herbicidal action, minimal residual activity, and lack of detrimental effects on subsequent crops can be leveraged for crop desiccation and introduces a novel application in cover crop termination.

For the first time, to our knowledge, PA was tested in these studies for its effectiveness against two challenging perennial weed species, which are typically difficult to control even with more aggressive and intensive methods. The outcomes indicated the potential of PA to be integrated with other management tools, as discussed in detail in Section 5.2. Notably, certain characteristics of PA, such as its moderate and non-persistent phytotoxic effects, can be considered benefits rather than drawbacks. These characteristics may make PA a suitable supplement to other moderate, environmentally friendly control methods, such as mechanical techniques, in perennial weeds management.

This research reaffirmed the factors that influence the efficacy of PA, particularly in the context of perennial weed management. It highlighted the impact of biological variability among species regarding their susceptibility to herbicides, and the importance of application timing and weed growth stage on the PA efficacy.

The study also explored the feasibility of PA application by investigating repeated applications on the same patch over two consecutive years, taking into account the initial ramet size. In this research, PA was applied once in the spring at the four-to-eight-leaf stage, always targeting the same patch. This highlights the importance of spot application on specific patches rather than treating the entire field. This method could be particularly useful during or even after crop establishment and for the management of the weed population in the field edges in combination with hoeing or mowing before the flowering time of the weed species. While these results provide valuable insights into cost reduction strategies—such as increasing carrier volume or decreasing the frequency of applications through combination with other methods—they also highlight the need for evaluating the economic aspects of these approaches to determine their cost-effectiveness for farmers. Additionally, this research addressed another drawback of PA through technical improvements that considered its low solubility and emulsifiable nature. By incorporating a mineral oil adjuvant and testing increased carrier volumes while adjusting nozzle size to maintain the desired concentration, the study identified methods to enhance the efficacy of PA application.

Overall, the findings of this study not only addressed existing knowledge gaps but also identified new approaches for future research, providing valuable insights for possible optimizing PA application in weed management.

5.6 The limitations of containing perennial weeds

There are certain limitations in containing perennial weeds such as *C. arvense* and *S. arvensis* despite various management options available, primarily due to their capability to propagate from a vast network of adventitious buds on an extensive creeping root system (Moore, 1975; Donald, 1990; Vanhala et al., 2006). Research has shown that different ecotypes of *C. arvense* can reduce the effectiveness of control methods, especially herbicides (Frank and Tworkoski, 1994; Cripps et al., 2019). In this study, the ramets of both species were obtained from a single plant to exclude the effect of various colons or genotypes. However, in real farm situations, another influencing factor on control method efficacy (e.g., PA efficacy) could be the variation in genotypes of these weed species. The dormancy of *S. arvensis* at the end of summer or the beginning of autumn inhibits sprouting and consequently preserves its nutrient reserves in the creeping roots (Håkansson, 1982; Håkansson and Wallgren, 1972). Therefore, mechanical control in autumn has been deemed less effective (Brandsæter et al., 2010; Tørresen et al., 2010). These factors bring difficulties and complications in control of these perennials. Thus, a combination of various methods is necessary because relying on a single approach often results in less than optimal control (Vanhala et al., 2006; Leathwick and Bourdôt, 2012; Thomsen et al, 2011). Several of these approaches are not feasible or economically viable (Leathwick and Bourdôt, 2012). Considering the diversity of agricultural systems, no single control strategy proves effective in all systems (Vanhala et al., 2006). Therefore, it is crucial to continually improve the current control methods and enhance their efficacy. For instance, to manage perennial weeds, it is recommended that disturbance occur during the developmental stage when the plant is most vulnerable, typically coinciding with its minimum belowground nutrient reserve (Graglia et al., 2006; Tavaziva, 2012). It is a complex matter because the time required to reach the minimum dry weight depends on environmental factors such as seasonality, temperature, and competition from other plants (Tavaziva, 2012; Leathwick and Bourdôt, 2012). Another challenge in perennial weed control arises from the EU's Farm to Fork strategy, which aims to reduce pesticide reliance by 50%. Compounding this issue is the uncertainty surrounding glyphosate's future regulatory status in Europe, necessitating the exploration of alternative tools. In this regard, current research using PA on creeping perennial weeds provides information on how PA can be integrated as a bio-herbicide into control strategies considering the biological attributes of perennial weed species with creeping root systems. As outlined in previous sections, PA can be incorporated at various stages of crop production for weed management. For instance, prior to sowing, PA may be applied in conjunction with agronomic practices that fragment weed root systems, such as tillage or stubble cultivation. Additionally, PA can be employed during or post-crop establishment or for managing weed populations along field margins, particularly when combined with mechanical methods such as hoeing or mowing as post-regrowth treatment. Additionally, PA is effective for targeted spot weeding when paired with drone technology and mechanical disturbance techniques. It is crucial to consider both technical and environmental factors in all these approaches. The strategy should be optimized based on the specific crop, weed species, annual weather conditions, and the conditions of the region.

5.7 Further research

To reduce perennial weed infestations, combining PA application with mechanical root fragmentation during intercropping could be an option that was not included in this research. Therefore, field testing is recommended to validate the findings for practical farming situations.

Bio-herbicides containing PA are currently costly in the market and require higher application rates per hectare than synthetic herbicides, which may limit their cost-effectiveness in large-scale arable farming. Currently, PA is approved in Europe for weed control in non-cultivated areas, desiccating plants in potatoes, and controlling suckers in perennial crops like hops and grapevines. Further research is required to compare the financial aspects of PA versus synthetic herbicides and evaluate the economic implications of various scenarios. Furthermore, to explore cost-effective application methods, field trials should investigate the efficacy of over-the-top application of PA or spot weeding and precision agriculture techniques. Spot weeding involves targeting weed infestations in specific, small areas of a field. This approach reduces herbicide usage and minimizes the impact on surrounding crops and the environment. By utilizing precision agriculture techniques like Variable Rate Application (VRA) and remote sensing, the timing and dosage of PA application can be optimized, improving efficacy and reducing costs. According to He (2023), VRA allows inputs, such as herbicides, to be applied at varying rates across the field based on location-specific data. This precision method leverages real-time data collection and advanced mapping techniques (He, 2023). Adhering to label instructions is essential for maintaining effective PA application while establishing a sustainable market share over time. VRA can be integrated with label guidelines by adjusting application rates according to the label-specified maximum. Labels may also outline specific conditions, such as weather or infestation levels, that dictate when and where to apply herbicides. VRA enables precise applications by targeting areas that meet these conditions, preventing over-application in other zones. When labels provide a range of rates, The VRA can implement a lower rate in areas with reduced demand and a higher rate in areas exhibiting greater need.

The research setup used in this study for drone assessments can be further enhanced by integrating emerging technologies like AI and machine learning to predict plant responses to herbicides under varying conditions, thereby improving the precision and efficiency of agricultural practices. In this regard, drones can gather real-time data on weed distribution, and AI and machine learning can analyze the collected data to identify weed growth patterns, density, and species. This information can then be used to guide variable-rate sprayers or autonomous drones to apply herbicides only in affected areas. The system can continuously evolve through feedback loops, where post-application evaluations refine the AI models, optimizing future weed detection and herbicide applications for greater efficiency and sustainability.

Supplementary research is also essential to enhance technical aspects such as the use of adjuvants and the adjustment of carrier volumes to achieve better plant coverage and improve weed control effectiveness. Given the potential of adjuvants to enhance the herbicidal activity of PA, additional adjuvants should be evaluated by conducting further trials across various locations and time periods.

5.8 References

Allison, K. (2024). Cover crop termination for small farms and gardens. Marion County Soil and Water Conservation District & Indiana's Natural Resources Conservation Service. https://marionswcd.org/wp-content/uploads/USDA-NRCS-Indiana-Small-Farms-and-Gardens-Cover-Crop-Termination.pdf (Accessed on October 03, 2024).

Balkcom, K. S., Duzy, L. M., Kornecki, T. S., and Price, A. J. (2016). Timing of cover crop termination: Management considerations for the Southeast. Crop, Forage and Turfgrass Management, 2(1). https://doi.org/10.2134/cftm2015.0161

Barker, A.V. and Prostak, R.G. (2009). Alternative management of roadside vegetation. HortTechnology. 19(2), 346-352. https://doi.org/10.21273/HORTTECH.19.2.346

Brandsæter, L.O., Fogelfors, H., Fykse, H., Graglia, E., Jensen, R.K., Melander, B., Salonen, J., and Vanhala, P. (2010). Seasonal restrictions of bud growth on roots of Cirsium arvense and Sonchus arvensis and rhizomes of Elymus repens. Weed Research, 50(2), 102-109. https://doi.org/10.1111/j.1365-3180.2009.00756.x

Byers, R. E. (1999). Effects of bloom-thinning chemicals on peach fruit set. Journal of tree fruit production, 2(2), 59-78.

Cabrera-Pérez, C., Royo-Esnal, A., and Recasens, J. (2022). Herbicidal effect of different alternative compounds to control Conyza bonariensis in vineyards. Agronomy, 12(4), 960.

Campos, J., Bodelon, L., Verdeguer, M., and Baur, P. (2022a). Mechanistic aspects and effects of selected tank-mix partners on herbicidal activity of a novel fatty acid ester. Plants, 11(3), 279. https://doi.org/10.3390/plants11030279

Campos, J., Mansour, P., Verdeguer, M., and Baur, P. (2022b). Contact herbicidal activity optimization of methyl capped polyethylene glycol ester of pelargonic acid. Journal of Plant Diseases and Protection, 130(1), 93-103. https://doi.org/10.1007/s41348-022-00661-0

Ciriminna, R., Fidalgo, A., Ilharco, L. M., and Pagliaro, M. (2019). Herbicides based on pelargonic acid: Herbicides of the bioeconomy. Biofuels, Bioproducts and Biorefining, 13 (6), 1476–1482. https://doi.org/10.1002/bbb.2046

Ciriminna, R., Angellotti, G., Luque, R., and Pagliaro, M. (2024). Green chemistry and the bioeconomy: a necessary nexus. Biofuels, Bioproducts and Biorefining, 18(2), 347-355.

Coleman, R., and Penner, D. (2006). Desiccant activity of short chain fatty acids. Weed Technology, 20, 410-415. https://doi.org/10.1614/WT-06-195.1

Congreve, M. and Cameron, J. (2019) Adjuvants oils, surfactants and other additives for farm chemicals used in grain production a national reference manual. Australia: GRDC Publication.

Creech, C., Henry, R., Werle, R., Sandell, L., Hewitt, A., and Kruger, G. (2015). Performance of postemergence herbicides applied at different carrier volume rates. Weed Technology, 29(3), 611–624. https://doi.org/10.1614/WT-D-14-00101.1

Crmaric, I., Keller, M., Krauss, J., and Delabays, N. (2018). Efficacy of natural fatty acid based herbicides on mixed weed stands. Julius-kühn-Archiv 458, 328–333. https://doi.org/10.5073/jka.2018.458.048

Cripps, M. G., Dowsett, C. A., Jackman, S. D., Van Koten, C., Goeke, D. F., and Houliston, G. J. (2019). Genetic variation in tolerance to defoliation in Cirsium arvense. Weed research, 60(1), 78-84.

Donald, W. W. (1990). Management and control of Canada thistle (Cirsium arvense). Reviews of weed Science, 5, 193-249.

Duddu, H. S., Johnson, E. N., Willenborg, C. J., and Shirtliffe, S. J. (2019). High-throughput UAV image-based method is more precise than manual rating of herbicide tolerance. Plant Phenomics, 2019, 1-9. https://doi.org/10.34133/2019/6036453

Emimi , M., Khaleel, M., and Alkrash , A. (2023). The Current Opportunities and Challenges in Drone Technology. International Journal of Electrical Engineeringand Sustainability, 1(3), 74–89. Retrieved from https://ijees.org/index.php/ijees/article/view/47

European Food Safety Authority (EFSA), Alvarez, F., Arena, M., Auteri, D., Borroto, J., Brancato, A., Carrasco Cabrera, L., Castoldi, A.F., Chiusolo, A., Colagiorgi, A., Colas, M., Crivellente, F., De Lentdecker, C., Egsmose, M., Fait, G., Gouliarmou, V., Ferilli, F., Greco, L., Ippolito, A., Istace, F., Jarrah, S., Kardassi, D., Kienzler, A., Leuschner, R., Lava, R., Linguadoca, A., Lythgo, C., Magrans, O., Mangas, I., Miron, I., Molnar, T., Padovani, L., Parra Morte, J.M., Pedersen, R., Reich, H., Santos, M., Sharp, R., Szentes, C., Terron, A., Tiramani, M., Vagenende, B., and Villamar-Bouza, L. (2021). Conclusion on the peer review of the pesticide risk assessment of the active substance pelargonic acid (nonanoic acid). EFSA Journal, 19(8): 6813. https://doi.org/10.2903/j.efsa.2021.6813

Fallahi, E. (1997). Blossom thinning effects of pelargonic acid, endothalic acid, and hydrogen cyanamide in apple and peach. HortScience, 32(3), 524E-525.

Frank, J. R., and Tworkoski, T. J. (1994). Response of Canada Thistle (Cirsium arvense) and Leafy Spurge (Euphorbia esula) Clones to Chlorsulfuron, Clopyralid, and Glyphosate. Weed Technology, 8(3), 565-571. https://doi.org/10.1017/S0890037X00039695

Graglia, E., Melander, B., and Jensen, R. K. (2006). Mechanical and cultural strategies to control Cirsium arvense in organic arable cropping systems. Weed Research, 46(4), 304-312.

Gustavsson, A. M. D. (1997). Growth and regenerative capacity of plants of Cirsium arvense. Weed Research. 37(4), 229-236. https://doi.org/10.1046/j.1365-3180.1997.d01-37.x

Håkansson, S. and Wallgren, B. (1972). Experiments with Sonchus arvensis L. II. Reproduction, plant development and response to mechanical disturbance. Swed. J. Agric. Res. 2, 3-14.

Håkansson, S. (1982). Multiplication, growth and persistence of perennial weeds. In: Holzner, W., Numata, M. (eds) Biology and ecology of weeds. Geobotany, vol 2. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-0916-3_11

He, L. (2023). Variable Rate Technologies for Precision Agriculture. In: Zhang, Q. (eds) Encyclopedia of Digital Agricultural Technologies. Springer, Cham. https://doi.org/10.1007/978-3-031-24861-0_34

Hodgson, J. M. (1968). The nature, ecology, and control of Canada thistle. U.S. Dep. Agric.Tech. Bull. 1386. 32 pp.

Jagarapu, K. K. (2022). Evaluate the efficacy of pelargonic acid and lemongrass essential oil for the control of different weed species (Second Cycle Degree (MSc) thesis). Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), Università degli Studi di Padova. Available at :https://thesis.unipd.it/handle/20.500.12608/42988

Kanatas, P., Travlos, I., Papastylianou, P., Gazoulis, I., Kakabouki, I., and Tsekoura, A. (2020). Yield, quality and weed control in soybean crop as affected by several cultural and weed management practices. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 48(1), 329-341. https://doi.org/10.15835/nbha48111823

Kanatas, P., Antonopoulos, N., Gazoulis, I., and Travlos, I. S. (2021). Screening glyphosate-alternative weed control options in important perennial crops. Weed Science, 69(6), 704-718. https://doi.org/10.1017/wsc.2021.55

Kardasz, P., Miziniak, W., Bombrys, M., and Kowalczyk, A. (2019). Desiccant activity of nonanoic acid on potato foliage in Poland. Journal of Plant Protection Research, 12-18.

Kieloch, R., and Domaradzki, K. (2011). The role of the growth stage of weeds in their response to reduced herbicide doses. Acta Agrobotanica, 64(4). https://doi.org/10.5586/aa.2011.068

Klauk, B., Rosenhauer, M., and Petersen, J. (2023). Experiences with electrophysical desiccation in early potatoes from Rhineland-Palatinate. Journal of Cultivated Plants/Journal für Kulturpflanzen, 75.

Knoche, M. (1994). Effect of droplet size and carrier volume on performance of foliage-applied herbicides. Crop Protection, 13(3), 163–178. https://doi.org/10.1016/0261-2194(94)90075-2

Krauss, J., Eigenmann, M., and Keller, M. (2020). Pelargonic acid for weed control in onions: factors affecting selectivity. Julius Kühn-Archiv 464, 415–419. https://doi.org/10.5073/jka.2020.464.062

Leathwick, D. M. and Bourdôt, G.W. (2012). A conceptual model for the population dynamics of Cirsium arvense in a New Zealand pasture. New Zealand Journal of Agricultural Research, 55(4), 371-384. https://doi.org/10.1080/00288233.2012.728532

Lederer, B., Fujimori, T., Tsujino, Y., Wakabayashi, K., and Böger, P. (2004). Phytotoxic activity of middle-chain fatty acids II: peroxidation and membrane effects. Pesticide Biochemistry and Physiology, 80(3), 151-156.

Legleiter, T., Johnson, B., Jordan, T., and Gibson, K. (2012). Successful cover crop termination with herbicides. Purdue University, Purdue Extension Bulletin WS-50-W. Available online: https://www.extension.purdue.edu/extmedia/ws/ws-50-w.pdf (Accessed on November 07, 2022).

Liew, J., Andersson, L., Boström, U., Forkman, J., Hakman, I., and Magnuski, E. (2013). Regeneration capacity from buds on roots and rhizomes in five herbaceous perennials as affected by time of fragmentation. Plant ecology, 214, 1199-1209. https://doi.org/10.1007/s11258-013-0244-4

Loddo, D., Jagarapu, K. K., Strati, E., Trespidi, G., Nikolić, N., Masin, R., Berti, A., and Otto, S. (2023). Assessing Herbicide Efficacy of Pelargonic Acid on Several Weed Species. Agronomy, 13(6): 1511. https://doi.org/10.3390/agronomy13061511

Marino, S., and Alvino, A. (2019). Detection of spatial and temporal variability of wheat cultivars by high-resolution vegetation indices. Agronomy, 9(5), 226. https://doi.org/10.3390/agronomy9050226

Moore, R.J. (1975). The biology of Canadian weeds.: 13. Cirsium arvense (L.) Scop. Canadian Journal of Plant Science, 55(4), 1033-1048. https://doi.org/10.4141/cjps75-163

Ogbangwor, N., and Söchting, H.P. (2022). Studies on the efficacy of pelargonic acid for weed control Julius-kühn-Archiv 468, 424–431. https://doi.org/10.5073/20220124-074954

Pacanoski, Z. (2015). Herbicides and adjuvants. In A. Price, J. Kelton, & L. Sarunaite (Eds.), Herbicides: Physiology of action and safety. IntechOpen. https://doi.org/10.5772/60842

Pittman, K. B., Cahoon, C. W., Bamber, K. W., Rector, L. S., and Flessner, M. L. (2020). Herbicide selection to terminate grass, legume, and brassica cover crop species. Weed Technology, 34(1), 48-54. https://doi.org/10.1017/wet.2019.107

Poiger, T., Müller, J., Kasteel, R., and Buerge, I. J. (2024). Degradation and sorption of the herbicide pelargonic acid in subsoils below railway tracks compared to a range of topsoils. Environmental Sciences Europe, 36(1), 4. https://doi.org/10.1186/s12302-023-00825-1

Rodene, E., Xu, G., Palali Delen, S., Zhao, X., Smith, C., Ge, Y., Schnable, J., and Yang, J. (2022). A UAV-based high-throughput phenotyping approach to assess time-series nitrogen responses and identify trait-associated genetic components in maize. The Plant Phenome Journal, 5(1). https://doi.org/10.1002/ppj2.20030

Rowley, M. A., Ransom, C. V., Reeve, J. R., and Black, B. L. (2011). Mulch and organic herbicide combinations for in-row orchard weed suppression. International Journal of Fruit Science, 11(4), 316–331.

Savage, S., and Zorner, P. (1996). The use of pelargonic acid as a weed management tool. In Proc. Calif. Weed Conf (Vol. 48, pp. 46-47).

Short, M. M., Vann, M. C., and Suchoff, D. H. (2020). Organic sucker control: screening different active ingredients for commercial application. Tobacco Science, 57(1), 17-20. https://doi.org/10.3381/TOBSCI-D-20-00008

Singh, V., Rana, A., Bishop, M., Filippi, A. M., Cope, D., Rajan, N., & Bagavathiannan, M. (2020). Unmanned aircraft systems for precision weed detection and management: Prospects and challenges. Advances in Agronomy, 159, 93–134. https://doi.org/10.1016/bs.agron.2019.08.004

Syguda, A., Ławniczak, Ł., Wróbel, P., Walkiewicz, F., Framski, G., Parus, A., Woźniak-Karczewska, M., Niemczak, M., Gierka, A., & Chrzanowski, Ł. (2023). Biodegradable amidequats, derivatives of caprylic and pelargonic acids as cationic surfactants for agricultural applications. Journal of Molecular Liquids, 391, Part A, 123221. https://doi.org/10.1016/j.molliq.2023.123221

Tavaziva, V. J. (2012). Effects of competition on compensation point and phenological development in Sonchus arvensis L. [Master thesis]. [Uppsala, Sweden]: Swedish University of Agricultural Sciences. https://stud.epsilon.slu.se/4572/

Tavaziva, V. J., Lundkvist, A., and Verwijst, T. (2019). Effects of selective cutting and timing of herbicide application on growth and development of Cirsium arvense in spring barley. Weed research, 59(5), 349-356. https://doi.org/10.1111/wre.12371

Thomsen, M. G., Brandsæter, L. O., and Fykse, H. (2011). Sensitivity of Cirsium arvense to simulated tillage and competition. Acta Agriculturae Scandinavica, Section B–Soil and Plant Science, 61(8), 693-700.

Tørresen, K. S., Fykse, H., and Rafoss, T. (2010). Autumn growth of Elytrigia repens, Cirsium arvense and Sonchus arvensis at high latitudes in an outdoor pot experiment. Weed Research, 50(4), 353-363.

Travlos, I., Rapti, E., Gazoulis, I., Kanatas, P., Tataridas, A., Kakabouki, I., and Papastylianou, P. (2020). The herbicidal potential of different pelargonic acid products and essential oils against several important weed species. Agronomy, 10(11), 1687. https://doi.org/10.3390/agronomy10111687

Vanhala, P., Lötjönen, T., and Hurme, T. (2006). Managing Sonchus arvensis using mechanical and cultural methods. Agricultural and Food Science, 15(4), 444-458. https://doi.org/10.2137/145960606780061498

Verwijst, T., Tavaziva, V. J., and Lundkvist, A. (2018). Assessment of the compensation point of Cirsium arvense and effects of competition, root weight and burial depth on below-ground dry weight–leaf stage trajectories. Weed Research, 58(4), 292-303. https://doi.org/10.1111/wre.12312

Webber, C. L., and Shrefler, J. W. (2007). Pelargonic acid weed control: Concentrations, adjuvants, and application timing. Proceedings of the 26th Oklahoma-Arkansas Horticultural Industry Show, 145-148.

Webber, C. L., Shrefler, J. W., and Taylor, M. J. (2014). Adjuvants Affect Duckweed (Lemna minor) Control with Pelargonic Acid. Journal of Agricultural Science, 6(12), 1. https://doi.org/10.5539/jas.v6n12p1

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