Leaf-specific weed control for vegetable crops in the UK

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Declaration of original authorship

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

Nikolaos Koukiasas

This thesis is dedicated to my father

Keep Ithaca always in your mind.
Arriving there is what you're destined for.
But don't hurry the journey at all.
Better if it lasts for years,
so that you're old by the time you reach the island,
wealthy with all you've gained on the way,
not expecting Ithaca to make you rich.
Ithaca gave you the marvellous journey.
Without her you would have not set out.
She has nothing left to give you now.

And if you find her poor, Ithaca won't have fooled you.
Wise as you will have become, so full of experience,
you'll have understood by then what these Ithacas mean.

C. P. Cavafy

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Abstract

Weed control in field vegetables in the UK is becoming increasingly challenging due to the loss of herbicide actives and demands by policy makers and consumers for lower pesticide use. Research at University of Reading in conjunction with Concurrent Solutions LLC in the USA, is developing a robotic weeder for field vegetables using image analysis to locate weed leaves and a novel Drop-on-Demand (DoD) applicator to apply droplets of herbicides to these leaves. No chemical is applied to the crop and none directly to the soil. Leaf-specific application of herbicide droplets is an alternative to selective chemistry or biotechnology while potentially reducing herbicide use. Although targeted micro-rates of herbicides have been studied, little is known about the exact rates needed to control weeds when microdoses are applied as one droplet to a single leaf or plant.

In glasshouse trials, individual weed seedlings were controlled by applying a single droplet of herbicide and dose-response relationships were quantified. As a general recommendation, weeds that are up to the 4-leaf stage can be controlled with a dose of 32 μg of glyphosate and 28 μg of glufosinate-ammonium when they are applied as a single droplet per seedling. In order to answer the question if the efficacy is reproducible in the field, manually applied droplets of glyphosate and glufosinate-ammonium were made to the naturally occurring weed population in transplanted cabbage and leek crop. Droplet applications made on three and ten occasions after transplanting the cabbages and leeks, respectively reduced residual weed biomass at harvest by over 90% compared to the weedy control. Also, droplet treatments gave a crop yield, which did not differ significantly from the weed-free control. At the same time, the total amount of herbicide active ingredient applied was up to 82% and 94% lower than currents spraying methods for the leeks and cabbages, respectively. Because of the high value of the crop and the higher yields associated with ultra-precise droplet application, it would appear to be economical to apply these droplets using a robotic weeder. The applicator which was developed by Concurrent Solutions LLC in the USA for Drop-on-Demand droplet applications was tested under indoor conditions. The effect of pressure, distance from the target, wind direction and motion of the applicator was tested on the targeting accuracy of the applicator. Recommendations for future field applications suggested that the applicator should operate at 138 kPa pressure and set at 15 cm height from weeds.

Contents

List of publications

Results from Chapters 2 and 3 are presented in the following publications, of which the full versions are included in the Appendix.

1. Koukiasas N., Yu T. and Murdoch A. (2016). Dose response relationship of droplet applications for the leaf-specific weed control in vegetable crops. *Aspects of Applied Biology,* **132,** 343-348.
2. Murdoch A., Koukiasas N., De La Warr P. and Price-Jones F. (2017). Precision targeting of herbicide droplets potentially reduces herbicide inputs by at least 90%. *Aspects of Applied Biology,* **135,** 39-44.
3. Koukiasas N., Martinez-Perez J., Pilgrim R.A., Sanford S. and Murdoch A.J. (2019). Implications of dose-response relationships of herbicide droplet applications for leaf-specific weed control in leeks. *In*: Proceedings of The 12th European Conference On Precision Agriculture, 2019. (accepted)

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1. Koukiasas, N., Murdoch, AJ. Annual Weeds Review, Rothamsted, November 2015. Poster.
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4. Koukiasas, N. SCI Young Researchers in Agri-Food, Reading, May 2016. Poster.
5. Koukiasas, N. SCI Young Researchers in Crop Sciences, July 2016. Oral.
6. Koukiasas, N., Murdoch, AJ. Annual Weeds Review, Rothamsted, November 2016. Oral & Poster.
7. Koukiasas, N. Crops Production Symposium, Reading, November 2016. Oral & Poster.
8. Koukiasas, N. AHDB Crops PhD Conference, Stratford-upon-Avon, November 2016. Oral & Poster.
9. Murdoch, AJ, Koukiasas, N. et al. FLRC Conference, New Zealand, February 2017. Oral.
10. Koukiasas, N. AgriFood Charities Partnership Student Forum, Hatfield, April 2017. Oral.
11. Murdoch, AJ, Koukiasas, N. et al. Precision Systems AAB Conference, Pershore College, October 2017. Oral.
12. Murdoch, AJ, Koukiasas, N. et al. British Onion Growers & British Leek Growers Meeting. Cranfield University, October 2017. Oral.
13. Koukiasas, N. Crops Production Symposium, Reading, November 2017. Oral & Poster.
14. Koukiasas, N. AHDB Crops PhD Conference, Stratford-upon-Avon, November 2017. Oral.
15. Koukiasas, N. Doctoral Research Conference, Reading, June 2018. Oral.
16. Koukiasas, N., Murdoch, AJ., et al. Precsion Spraying Event, Diss, November 2018. Oral

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Chapter 1
Introduction

Global population is rising rapidly at a rate of approximately 1.1% each year and is expected to reach 9.7 billion by 2050 (Alexandratos & Bruinsma, 2012). A year which is considered a landmark by government and industry bodies as the agricultural production will need to increase correspondingly, in order to meet the demands for more food, feed and fibre (Westwood *et al*., 2018). Today's levels of crop production are not enough to feed the increasing population and meeting the expected demands is considered a major task. A situation which might get worse if one considers the challenges agriculture is facing, which involve the impact of climate change, lack of resources (i.e. water), human labour shortages, reductions in arable land, crop losses from pests, weeds and diseases and pressures from the public for more sustainable agricultural production (Bogue, 2016).

According to FAO (2009), weeds should be considered by farmers as the No.1 enemy, causing $95 billion losses per year in food production which can be even higher if the time and effort devoted to weed control is considered. Weeds are the most important among other pest groups accounting for the highest potential yield loss (34%) with other pests and pathogens being less important (18% and 16% yield losses, respectively) (Oerke, 2006). Depending on the infestation level, these unwanted plants can cause actual yield losses from 30% to 100% if weed control is not implemented (Singh *et al*., 2014). Several studies are quantifying yield losses as a result of weed competition. In a field study in the UK, Roberts *et al.* (1977) found that grass and broadleaf weeds at a density of 65 m^{-2} resulted in total loss of marketable lettuces. For the same crop, in three field studies in California, Shrefler *et al.* (1996) reported that competition from *Amaranthus spinosus* reduced lettuce head quality and weight. Hodgson (1968) found that two shoots m^{-2} of *Cirsium arvense* caused 15% reduction in wheat yield. In fields with seeded tomato, Monaco *et al.* (1981) observed yield losses up to 71% when 11 weed plants (*Datura stramonium*) m^{-2} were growing within the crop row.

Some of the most common agricultural practices for controlling weeds include cultural, chemical, physical and biological methods with the use of herbicides being the principal tool for weed control (Harker & O'Donovan, 2013). It is worth mentioning that herbicides account for the 45% of the total pesticide market (50% in the UK) (Zimdahl, 2013). Although herbicide-based weed control can be both highly efficacious and economical, there are situations where improper use can have adverse environmental impacts (Slaughter *et al*., 2008a). There is a need of critical importance to balance effective weed control, maintaining and increasing productivity while minimising the negative effects of herbicides (Power *et al*., 2013)

1.1 Challenges of herbicide-based weed control

In a world where agriculture must feed two billion more people in the next 30 years, the scale of crop losses due to weeds is not acceptable. Widespread use of herbicides as a tool of weed control is a common practice in most developed nations and even more in developing countries where they face shortages for labour-intensive tasks, like hand-weeding (Gianessi, 2013).

Lack of new herbicide actives

It was the loss of labour in agriculture that stimulated the introduction of herbicides in the 1940s and the 1950s which then led to the widely adoption of conventional herbicides during the 1960s and 1970s (Duke, 2012). A characteristic example of the herbicide revolution is that from the 1950s to 1980s, a new herbicide mode of action (MOA) was commercialised approximately every three years (Duke, 2012). However, since 1990 no new modes of action have been discovered and from 2010 to 2014 only four new herbicides were introduced in the market (Rüegg *et al*., 2007). Today the 80% of the herbicide market is dominated by six MOAs (Jeschke, 2016). Contributing to the lack of available herbicide actives, to develop a new crop protection product can cost up to $286 million and take up to 11 years (Duke, 2012). With only few big agrochemical companies left and the downward trend of introducing new herbicide chemistries, it is likely that will continue. A reason for that could also be that there are no new good sites in the metabolic pathway for a herbicide to target so that a new MOA can be discovered (Westwood *et al*., 2018). Looking at things from a pessimistic point of view the lack of new MOAs alongside with the problems caused by herbicide resistance will make almost all existing herbicides unusable (Westwood *et al*., 2018).

Legislations introducing reductions in herbicide use

Typical weed control methods for field vegetables include use of pre-planting tillage followed by an application of a pre-emergence herbicide, spraying a post-emergence selective herbicide (where available), inter-row cultivation and hand-weeding (Slaughter *et al*., 2008). The most common method of herbicide application is with nozzles, mounted on a conventional boom sprayer at 50 cm from the soil surface or the crop canopy, spraying a uniform dose over an entire field. Although this method has been an effective weed control tool, it does not consider the variability and uneven distribution of weed species. Therefore, it can be wasteful and inefficient, especially in the case of foliar-acting herbicides (Jensen *et al*., 2013). If one considers that weed seedlings at an early growth stage may only cover a soil surface of 1 cm^2 and with numbers of weeds ranging from 100 to 400 per m^2 this corresponds to 1%-4% soil coverage by the weeds. So, if a foliar-acting herbicide is applied by broadcast spraying, 96% or more will be applied directly to the soil surface or the crop, having potential environmental impact (Zijlstra *et al*., 2011).

Trying to respond to concerns about pesticide use and potential effects on human health and adverse impacts on the environment, UK and EU legislation is promoting reductions in pesticide inputs (Hillocks, 2012). Legislations like the Regulation EC no. 1107/2009, the EU Water Framework Directive and the Sustainable Use of Pesticides (SUP) Directive along with UK's National Action Plan for SUP have resulted in actual losses of approval for some herbicide actives and have decreased the likelihood of new compounds gaining approval (Baker & Knight, 2017). One example is that in 2008 the European Commission removed the approval of herbicides that contained the active ingredient propachlor, which, for cabbage growers, was their most effective herbicide (Utstumo *et al*., 2018). The herbicide was regarded as a risk to human health because it was contaminating ground water and the overall aquatic life. The challenges will only get worse for vegetable growers as they rely on a limited and old spectrum of available herbicide chemistries with the first commercialised during the 1960s and 1970s, which require a lot of effort and funding to keep them in the market (Fennimore *et al*., 2014). An example of an old herbicide is cycloate, an active ingredient used for weed control in fresh spinach, which was first introduced approximately 50 years ago (Lati *et al*., 2015).

The lack of available herbicide chemistry for vegetable growers and legislations from policy makers have resulted in more physical and mechanical weed control methods being used (Garthwaite *et al*., 2017), which can often be expensive and time consuming especially in some cases of vegetable crops which are known to be weak competitors against weeds (e.g. leeks, carrots). In Sweden and Denmark vegetable growers can spend up to 300 h ha^{-1} to hand-weed onions and carrots (Melander, 1998) and in particular, weedy infestations in direct-sown leek crops, hand-weeding times can go up to 450 h ha^{-1} (Melander & Rasmussen, 2001). In the USA, typical hand-weeding times in broccoli and tomato are 50 and 94 h ha^{-1}, respectively (Gianessi & Reigner, 2007). Currently the cost of manual labour in the UK is £10.16 h^{-1} (Redman, 2017) therefore, based on the times mentioned earlier means that a leek and an onion grower might end up spending £3,048 and £4,572 ha^{-1} for hand-weeding, respectively which makes this method the most expensive weed control tool.

Pressures from policy makers and lack of new herbicide actives call for a paradigm shift in weed control for vegetable crops.

1.2 Potential paradigm shifts in weed control

Herbicide-tolerant (HT) vegetable crops

It has been proven that breeding vegetable crops to be herbicide resistant is feasible (Pinthus *et al*., 1972). Such an examples is the use of CRISPR/Cas technology to develop potato resistant to the herbicide imazamox (Butler *et al.*, 2016). Also, results from field testing glyphosate-tolerant lettuce demonstrated complete weed control when glyphosate treatments were applied with no effects on lettuce (Fennimore & Umeda, 2003). Regardless of the success of the glyphosate-tolerant lettuce, the horticultural industry was reluctant to adopt this technology because of fears that consumers will reject the crop and possibly even boycott products of the companies which will use this technology (Bradford & Alston, 2004). Furthermore, the vegetable seed market is small compared to broad-acre crops and it is very unlikely that adding a trait for herbicide resistance will be economically viable (Clark *et al*., 2004). Fennimore and Cutulle

(2019) suggest the use of CRISPR technology to edit plant genomes so that they emit spectral reflectance at a wavelength not found in weeds. Breeding a vegetable crop with these characteristics would facilitate crop and weed classification by a machine vision system for robotic weed control. It is also hypothesized since this approach does not involve breeding a crop for herbicide resistance, and it will be less cumbersome to develop. However, at the moment in the European Union, breeding crops using CRISPR technology, falls in the same category as with genetically modified organisms (GMOs) (Callaway, 2018).

Although the use of herbicide tolerant crops is a paradigm-shift to weed control, it may still involve spraying on a whole-field basis. An evaluation in the UK demonstrated that environmental impacts were not different between GM-HT and conventional crops (Hawes *et al*., 2003). So far, using CRISPR, genetic modification or conventional breeding does not have an impact on weed control programs for vegetable crops (Fennimore & Cutulle, 2019).

It is becoming evident that the challenges vegetable crop growers are currently facing is unlikely that they will be solved with registration of new herbicides or development of herbicide resistant cultivars. With limited time available, in order to develop successful weed management programmes, it is advocated in this thesis that research should focus on developing new ways of herbicide application, which will utilise existing chemistry while reducing inputs. Robotic platforms which will use real-time weed recognition and targeted application of micro-rates of herbicides, holds great promise as a method of weed control.

Automated systems for weed control

The technologies that are required for an autonomous machine to perform real time weed control are: vision guidance system, weed detection and mapping which then passes the information to a decision algorithm that manages the micro-sprayer system (Slaughter *et al.*, 2008b). Herbicide application is carried out using microlitre volume droplets which are directed to areas where weeds are located. This kind of application has been described as Drop-on-Demand (DoD) and delivery of the micro-doses has been attempted with inkjet printer heads, arrangements of solenoid valves, pneumatic valves and needles (Utstumo *et al.*, 2018). In order to be effective, these systems need accurate identification of weed and crop plants, high level of precision in herbicide application and control of droplets deviating from the target (Fennimore *et al.*, 2016). The concept of these systems is to avoid direct herbicide applications to the soil and the crop and therefore, the potential reductions in herbicide use can be over 95% (Christensen *et al.*, 2009).

One of the first automated micro-spray systems was developed by Lee *et al.* (1999) and it was designed to identify and spray weeds between the rows of tomato crop. This prototype real-time, micro-spray system was equipped with eight independent solenoid valves (two series of four), which would deliver the herbicide to the spray ports and then to the weed leaf. Using an image analysis algorithm, the machine vision would distinguish the crop from the weeds and would divide each image into a grid consisting of eight rows and 18 columns, giving a cell size of 0.64 cm by 1.27 cm (0.81 cm^2). A weed leaf corresponded to each cell for which the valves would operate for 10 ms delivering a flow rate of 0.98 L min^{-1} through each port. In field conditions when travelling at a speed of 0.8 km h^{-1} the droplets' deviation from the centre of the target was 7 mm with a standard deviation of 5 mm. Although the concept of the system was that no spray mixture would be applied to the crop or the soil and applications would be made only to the weeds, when going through the field, it recognized correctly 76% of the tomato plants (the rest were sprayed) and spayed only 48% of the weeds, leaving many untreated. Trying to improve on the system's plant recognition, Lamm *et al.* (2002) adopted the precision spraying system from Lee *et al.* (1999) and used it was for robotic weed control in cotton. When tested in 14 cotton fields, the new machine vision technique resulted in 11% of the weeds untreated and 21% of the cotton plants sprayed, when travelling at 1.62 km h^{-1} speed. No herbicide was applied and evaluation of the system's performance was conducted after spraying, based on the amount of blue dye deposited onto the plant's surface.

Giles *et al.* (2004) conducted a field trial to evaluate the biological performance of a micro-dosing system in processing tomatoes. The spraying system was the same as with the one described in the two previous studies and was spraying dose rates of 37 µl per cell of 0.63 cm x 1.25 cm. Three concentrations of glyphosate were applied (0.25%, 0.375% and 0.5%) in conjunction with a surfactant (0.25%) and a polyethylene oxide polymer to reduce drift (0% and 0.03%). However, the system's plant recognition was not tested and applications were carried out manually by holding the spray tube 5 cm above the weed and pulsing the control valve for 6 ms per cell. Efficacy of weed control ranged from 80% to 100% while all glyphosate treatments achieved satisfactory yields. However, phytotoxic symptoms and crop damage were reported because of "micro-drift" (splash effect) when the polyethylene oxide polymer was not used (Giles *et al*., 2004). Trying to minimize the splashing effect from a micro-dosing applicator, Downey *et al.* (2004) carried out laboratory studies which highlighted the need for oxide polymers in formulations with glyphosate. From these studies it is becoming evident the need for a better crop/weed classification system and control of any droplets drifting which might end up damaging to the crop.

The application rate and accuracy of a machine vision-controlled, micro-dosing system was investigated by Søgaard and Lund (2007). Under indoor conditions the micro-dosing system was able to target cells of 25 mm^2 in a 110 mm^2 circle (being the average size of a small weed seedling) delivering 2.5 µl per cell. Although four shots were fired per circle, on average one shot was off-target. When the same system was tested in field conditions with *Brassica napus* L. (oilseed rape) as a test weed, it sprayed 82% of the plants with the precision of the system being insufficient when targeting weeds smaller than 100 mm^2. On average, the micro dosing system was delivering droplet volumes of 11.3 µl per seedling which contained 22.6 µg of glyphosate. Although efficacy of weed control was only observed visually, it is reported that 22.6 µg of glyphosate was enough to control individual seedlings in outdoor conditions (Søgaard *et al*., 2006). Assuming a weed density of 100 plants m^{-2}, it was argued that weed control could be achieved with as little as 22.6 g glyphosate ha^{-1} which corresponds to a 96% reduction of the minimum label recommendation for broadcast application of glyphosate (540 g ha^{-1}).

A tractor-pulled, automated system for control of volunteer potatoes in sugar-beet fields was tested by Nieuwenhuizen *et al*. (2010). Droplet application was carried out using five needles covering 20 cm width, fixed at 30 cm above the soil. The image analysis system was able to distinguish the volunteer potatoes from the sugar beet plants and divide each image into grid cells of 10 x 40 mm size. However, plants smaller than 1200 mm^2 were rejected by the detection system. One droplet of 20 (±5) µl was applied per grid cell containing 5% of glyphosate (450 µg of glyphosate per droplet). This resulted in 77% of the volunteer potato plants being controlled and 1% of crop plants dying when travelling at 2.9 km h^{-1}. According to the authors, damage to the sugar beet plants was caused by satellite droplets drifting or by droplets which were correctly applied to nearby volunteer potatoes ending up on the leaves of the crop. Furthermore, the results from the targeting accuracy test reported droplets deviating on average from the target 7.5 mm perpendicular to and 14 mm in the direction of travel. Although based on the size of the grid cell this resulted droplets ending up very close to the border, however, no droplets were reported outside of the targets/cells (Nieuwenhuizen *et al*., 2010). Although results from these studies are encouraging, Fennimore *et al*. (2016) suspects that weeds with ground cover areas lower than 400 mm^2 (i.e. 10 x 40 mm) will need to be accurately targeted, if these systems are to be acceptable for weed control in commercial vegetable crops.

Trying to improve on the weed control efficacy and the detection accuracy, the micro-sprayer developed by Midtiby *et al.* (2011) was able to control 94% of the weeds which were as small as 121 mm^2 (11 x 11 mm) without damaging the crop (maize) plants. The inkjet printer heads were delivering droplets of 0.2 μl, containing 1 μg of glyphosate. However, all tests were carried out under indoor conditions with pots and with the system travelling at steady speed of 1.8 km h^{-1}. Another limitation was that the vision system was ineffective against weeds smaller than 11 x 11 mm, an example being the 37% weed control achieved for small mayweed seedlings.

Miller, Tillet, Hague & Lane (2011) developed and evaluated an automated system for spot herbicide application in horticultural row crops, to control volunteer potatoes. This engineering solution comprised a novel image analysis system that detected the position of the crop rows and weed plants and specialised nozzles ("Alternator" by Hypro EU Ltd). The system applied herbicide sprays targeted directly to individual detected weeds. When it was field-tested in onion, carrot and parsnip crops, using spot applications of glyphosate, the system achieved high levels of weed control (90-95%). Although some crop damage occurred, the yield penalty was deemed "commercially acceptable". When it was tested in leeks, control of volunteer potatoes, mugwort and wild mint was 95% with no detectable residues found in crop plants adjacent to weeds treated (Miller, Tillet & Hague, 2013).

In a more recent study, Utstumo *et al.* (2018) tested the efficacy of the DoD system developed by Urdal *et al*. (2014) which, was part of the Asterix robot (Adigo

AS) (Figure 1.1 (C)). A major advantage of this approach over that proposed by Miller *et al.* (2011, 2013) is the use of individual droplets of herbicide solution rather than a spray with the potential for collateral damage to the crop. In indoor conditions, it was demonstrated that *Chenopodium album*, *Tripleurospermum inodorum*, *Poa annua* and *Stellaria media* were controlled with 7.56 μg of glyphosate per seedling, applied as three droplets each with a volume of 1.16 μl. When tested in the field with a carrot crop, for intra-row weed control, weeds were controlled when droplets containing 5.3 μg of glyphosate (2.1 μl) were used. However, the authors did not mention how many droplets were applied per plant. Furthermore, although a crop/weed detection system is described, when the robot was tested in the field it treated all weed and carrot plants. Also, it was calculated that the equivalent rate of 191 g of glyphosate ha^{-1} was applied in the field which represents reduction from 73% to 95% when compared with the range of recommended rates for spraying glyphosate. However, the authors assume that two to three treatments with the robot would be needed for the crop to remain weed-free which, will increase the total application rate over the growing season and reduce the herbicide savings compared to just one robotic treatment.

An autonomous vehicle (HortiBot) was able to navigate itself using the crop rows as a guide and perform weeding operations (Sørensen *et al.*, 2007). The team developing HortiBot has stated their intention of fitting a precision herbicide sprayer tool (e.g. cell sprayer) which will selectively spray weeds based on their leaf patterns (Graham-Rowe, 2007) (Figure 1.1 (A)). An autonomous robot for plant phenotyping of maize (BoniRob) was described by Ruckelshausen *et al.* (2009) and was fitted with an application module, consisting of eight nozzles for precision spraying ("Precision Spraying App") of individual weeds growing inter-row, intra-row and close-to-crop (Scholz *et al.*, 2014) (Figure 1.1 (B)). An autonomous robotic system (AgBot II) is described by Bawden *et al.* (2017) which can perform both mechanical weeding and precision herbicide application (Figure 1.1 (D)). Once the machine vision system has detected and classified the weeds, the most appropriate weed control tool is deployed based on particular species. The Australian Centre for Field Robotics at the University of Sydney developed an autonomous robotic platform (Ladybird) for real-time detection and precision spraying of fertilisers and herbicides for vegetable crops (Underwood *et al.*, 2015). A smaller version of the Ladybird robot has also been developed for Intelligent Perception and Precision Application (RIPPA) which, like its predecessor, is used for precision application of agrochemicals (Bogue, 2016) (Figure 1.1 (E)). One of the most recent robotic weeders was developed by the Swiss company ecoRobotix Ltd (https://www.ecorobotix.com/en/) (Figure 1.1 (F)). The lightweight (~130 kg) ecoRobotix platform uses a camera system to distinguish the crop from the weed and two robotic arms carry out precision herbicide spraying. The company claims that the platform uses 20-times less herbicide when compared with broadcast spraying and that it can cover an area of three hectares per day on the basis that it takes one second to spray two weeds. However, since it is solar powered, usage of the platform is limited only during daytime with sunny weather.

From the micro-dosing systems described earlier it is becoming evident that although targeted micro-rates of herbicides have been studied, little is known about the exact amounts needed to control weeds when doses are applied as one droplet to a single leaf or plant. According to Graglia (2004) and Young and Giles (2013) more research is needed is to test the susceptibility of individual weed species to micro-dose herbicide application. Only the study from Mathiassen *et al*. (2016) tested the efficacy of known amounts of glyphosate (from 0.22 to 7 μg per weed) applied as a single droplet to a single leaf of four weed species. However, there is no attempt in that study to link the six doses applied to the recommended rate for spraying as doses were applied irrespective of the size of the weeds and only glyphosate was tested.

Furthermore, from the studies with the automated systems described earlier, although efficacy of weed control using micro-doses of herbicides is reported, there are no indications of how crop yield was affected and efficacy is not compared with current spraying methods. A reason could be that the main objective of these studies was to test the crop/weed classification system and the precision of the micro-dosing system. Even when efficacy of weed control is reported no actual percentages are given and words like “sufficient” (Søgaard *et al*., 2006) and “successful” (Utstumo *et al*., 2018) are used. Although, Søgaard *et al*. (2006) reported efficacies of 82% this relates to the average percentage of plants treated assuming that the average dose of 22.6 μg of glyphosate per plant was controlling the test weed treated in outdoor conditions.

Predictions on potential herbicide savings when weeds are treated leaf-specifically ranges from 95% to 99% (Christensen *et al*., 2009; Zijlstra *et al.*, 2011; Blackmore, 2014). In the studies described earlier, droplets emitted from the micro-dosing systems were not counted and therefore the equivalent application rate per unit land area was not calculated. Only in the study by Utstumo *et al.* (2018) the application rate was estimated (191 g of glyphosate ha^{-1}) when the Asterix robotic platform was tested in field with a carrot crop. However according to the authors two to three treatments will be needed for effective weed control which increases the applications and reduces further the potential herbicide savings of 73% to 95%, based on conventional spraying of glyphosate. Furthermore, in other studies where doses applied per weed were estimated for weed control, to demonstrate the potential on herbicide savings, weed densities from 100 to 300 m^{-2} were assumed (Søgaard *et al*., 2006; Mathiassen *et al*., 2016)

Figure 1.1. Examples of robotic platforms performing precision weed control. (A) HortiBot (Sørensen *et al.*, 2007), (B) BoniRob (Ruckelshausen *et al.*, 2009), (C) Asterix (Utstumo *et al.*, 2018), (D) AgBot II (Bawden *et al.*, 2017), (E) RIPPA (Bogue, 2016) and (F) ecoRototix (https://www.ecorobotix.com/en/).

1.3 Objectives

The scope of the study presented in this thesis is to support the development of an autonomous platform for leaf-specific weed control in UK vegetable crops. The platform is being jointly developed by the University of Reading and Concurrent Solutions LLC in the USA. It will use a machine vision system to distinguish the crop from the weed and identify individual weed leaves as targets for herbicide droplets. A novel applicator module will then apply an appropriate dose as a single droplet to identified targets. The PhD research which is part of the overall project has the following four main objectives:

1. To test the efficacy of herbicide droplet application for leaf-specific weed control.
2. To investigate alternatives to glyphosate for leaf-specific weed control in order to reduce the risks associated with dependence on a single herbicide.
3. To compare yields, quality and profitability of cabbage and leek field crops where weeds are controlled (a) leaf-specifically, (b) with conventional herbicide sprays or (c) by hand. After the end of the project, further research will be needed to fulfil this objective from a commercial perspective.
4. To assess the targeting accuracy of a prototype applicator system.

In order to meet these objectives, the following hypotheses are tested:

1.4 Hypotheses

1. Leaf-specific applications of droplets of a systemic, translocated herbicide at the recommended dose for conventional spraying achieves at least 90% efficacy of weed control in both glasshouse and field.
2. The yield, quality and economic value of cabbage and leek crops, where weeds are controlled leaf-specifically, are not significantly lower than for either conventional pre- and post-emergence herbicide treatment or for hand-weeding.
3. In the field, herbicide inputs per unit land area are significantly lower when droplet applications are compared with spray applications of commercial herbicides and do not exceed the label recommendations for the products used.

1.5 Thesis structure

In order to fulfil the objectives and test the hypotheses a series of glasshouse and field experiments are reported in Chapters 2 and 3, respectively. In addition to the field experiments a preliminary economic analysis is carried out in Chapter 3. The targeting accuracy of a prototype applicator is then tested in Chapter 4. These three experimental chapters have been written up for publication as refereed journal articles. Each chapter, therefore, includes a brief literature review and discussion.

Literature cited in each of these chapters is listed at the end of the thesis. Supplementary information for each chapter is included at the end of each chapter. Although they could be included in an appendix, photographs of experimental materials have been included to facilitate better understanding of the research for readers of the thesis. They may not, however, be included in papers for publication.

Chapter 2
Dose-response relationships of herbicide droplet applications for the leaf-specific weed control

2.1 Summary

Leaf-specific application of herbicide droplets is an alternative to selective chemistry or biotechnology while potentially reducing herbicide use. Although targeted micro-rates of herbicides have been studied, little is known about the exact rates needed to control weeds when microdoses are applied as one droplet to a single leaf or plant. In this study herbicides were applied as droplets (1-3 µl) with doses applied ranging from 1/256th to six-times the recommended rates for spray applications. The recommended rates of the herbicides (L ha^{-1}) were estimated in µg of a.i based on the mean ground cover of the seedlings and droplets were applied by a micropipette. Three weeks after application biomass data were recorded. *Senevio vulagris* was controlled with 0.24 µg of glyphosate per seedling making it the most susceptible, among the weeds tested, to glyphosate with the least being the perennial *Rumex crispus*. For 90% control of *Chenopodium album* the equivalent of 90 g ha^{-1} and 75 g ha^{-1} were needed when glyphosate and glufosinate-ammonium were used, respectively. Red amaranth was more susceptible to glyphosate than glufosinate-ammonium. It was concluded that weeds can be controlled leaf-specifically by droplet application provided that a broad-spectrum, translocated herbicide is used. As a general recommendation, weeds that are up to the 4-leaf stage can be controlled with a dose of 32 µg of glyphosate and 28 µg of glufosinate-ammonium per seedling. Dose-response trials in glasshouse conditions have, therefore, shown the efficacy of glyphosate, glufosinate-ammonium and 2,4-D droplet application to annual and perennial weeds.

2.2 Introduction

Weeds are one of the major threats to crop yield and quality worldwide, accounting for highest potential loss (34%) with pathogens and pests being less important (16% and 18% yield loss) (Oerke, 2006). These unwanted plants are farmers' No.1 enemy as they can cause total yield loss if no weed control is applied (Singh *et al*., 2014). Herbicide-based weed control remains today the most effective method in agriculture with herbicides accounting for the 45% of the total pesticide market (50% in the UK) (Zimdahl, 2013). However, applying herbicides as a uniform single dose by spraying the entire field, can be wasteful and inefficient, especially in the case of post-emergence herbicides (Jensen *et al.*, 2013). It has also been argued that the over-reliance on some of these chemicals can (i) have an adverse effect on the ecosystem, (ii) lead to herbicide-resistant weeds, (iii) cause pollution of aquatic environments, including ground water, raising concerns about public health and safety (Power *et al*., 2013). Evidently chemical weed control is receiving a lot of negative attention while, there are pressures from policy makers and consumers to lower use of pesticides in general. Furthermore, UK and EU pesticide reviews have resulted in actual and potential losses of crop protection products. Such examples include actual losses of approvals under the Regulation (EC) No. 1107/2009, potential losses of herbicides under the directives related to water quality (Water Framework Directive (2000/60/EC) and Drinking Water Directive (1998/83/EC)), reduction of pesticide use under the Sustainable Use Directive (2009/128/EC) and the UK National Action Plan (NAP) (Hillocks, 2012; Baker & Knight, 2017). Therefore, there is a need to balance controlling weeds, maintaining and increasing productivity while minimising the negative effects of herbicides.

As a result, from these pressures much research has been focused on new technologies, which aim to reduce herbicide use. Christensen *et al*. (2009) reviewed different application methods of herbicides using variable doses according to the weed species, distribution and density. These techniques range from spraying weed patches using broadcast application methods (Gutjahr & Gerhards, 2010; Berge *et al*., 2012), spot spraying weeds after they have been divided to cells of decimetre and centimetre-size using image analysis (Lee *et al*., 1999; Lund *et al*., 2008)) to treating individual plants using microdoses of herbicides (Giles *et al*., 2004; Søgaard *et al*., 2006;

Nieuwenhuizen *et al*., 2010; Midtiby *et al*., 2011; Utstumo *et al*., 2018). The latter method has been also described as Drop-on-Demand (DoD) technology, which emits very low volume droplets (1-5 μl) of a translocated herbicide. Blackmore (2013) speculated that such methods could reduce herbicide inputs by up to 99%. Although some research has been carried out using targeted applications of herbicides, little is known about the exact dose rates needed to control weed seedlings. Therefore, there is a need to evaluate the efficacy of micro-rate applications when they are applied as droplets directly on leaves of weeds (Graglia, 2004; Young & Giles, 2013).

Dose-response trials have been widely used in weed science to evaluate the efficacy of herbicides (Waite *et al*., 2013), for testing herbicide resistant weeds (Bell *et al.*, 2013) and also for studying the absorption and translocation of herbicides (Goggin *et al*., 2016). Doses for all of these experiments are applied in g or kg per ha using sprayers with nozzles calibrated to deliver several L per ha. Therefore the actual amount of active ingredient applied per plant is not known and it can only be based on an average rate per unit land area or estimated using chemical analysis.

The study from Graglia, (2004) tested the biological efficacy of manually applied droplets of glyphosate on leaves of *Solanum nigrum* L. (black nightshade) in outdoor conditions. The way the doses applied were calculated was by combining different concentrations of glyphosate (0.06 μg μL^{-1} to 1 μg μL^{-1}), with volume of droplets (0.25 to 4 μL) and number of droplets applied per plant (1, 2 and 4 per plant), which was irrespective of the ground cover of the seedlings (2-leaf stage). It is worth mentioning that in this study was found that a single droplet of 0.8 μg of glyphosate per plant can provide a 95% control of *S. nigrum*. However, no other growth stages, no other weed species and no other herbicides were tested. Also, the study from Mathiassen *et al*. (2016) tested the efficacy of known amounts of herbicide applied as a single droplet to a single leaf of *Chenopodium album*, *Silene noctiflora*, *Echinochloa crus-galli* and *Brassica napus*. However, the main purpose of this study was to test the effect of different adjuvants on the retention and efficacy of droplets generated by a DoD system described by Søgaard and Lund (2007). The six doses that were applied ranged from 0.22 to 7 μg per weed seedling (0.27 to 8.75 g of glyphosate L^{-1}) they were irrespective of the size of the seedlings and there is no evidence how they were calculated. In a more recent study by Utstumo *et al*. (2018) the efficacy of single herbicide droplets emmited by a DoD system was tested. Glyphosate and iodosulfuron were applied to pot trials using *C. album*, *Tripleurospermum inodorum*, *Poa annua* and *Stellaria media* weed seedlings (2 to 5-leaf stage). Two doses of each herbicide were applied (high and low) as three droplets of 1.16 μL volume each and it was proven that 7.56 μg of glyphosate per plant controlled the four weeds. For all the above studies it is not mentioned how the doses applied relate to the recommended rate for spraying the commercial formulation and no other broad-spectrum herbicides than glyphosate were tested.

The study presented in this paper is part of a project which is developing an automated system for herbicide droplet applications to weed leaves in field vegetables. The objective is to model dose-response relationships of herbicide droplet application for leaf-specific weed control for principal weeds of field vegetables (Roberts & Stokes, 1966). In addition to weed testing, the susceptibility of a vegetable crop to droplet application will be tested. Also, in order to minimize the likelihood of herbicide resistance and alleviate concerns about the overreliance on a single herbicide active (i.e. glyphosate), mixtures of 2,4-D with glyphosate and 2,4-D with glufosinate-ammonium need to be tested. The hypotheses tested in this study were that: (i) the dose-response model used to describe spray applications of herbicides would also be appropriate for droplet applications, (ii) the susceptibility of the weeds tested to droplet application would vary between species, (iii) application of the recommended doses of herbicides for conventional spraying as droplet to a single leaf would provide at least 90% efficacy, Glufosinate-ammonium could provide an alternative to glyphosate for droplet application, because of the acropetal movement and the limited translocation of glufosinate-ammonium (Pline *et al*., 1999) the herbicide will be less potent than glyphosate.

2.3 Materials and Methods

Dose-response trials were carried out in glasshouse conditions during summer months (July & August) at Reading UK (51°26'19.7" N, 0°56'05.4"W) (2015-2018) in order to test the efficacy of glyphosate, glufosinate-ammonium and 2,4-D droplet applications according to the ground cover of the weed and crop seedlings (Figure 2.1 (A)). *Amaranthus cruentus* was tested in Benton, Kentucky, USA (36°46'50.7"N, 88°24'45.7"W) (2017) (Figure 2.1 (B)). Mean temperatures (day/night) during summers of 2015, 2016 and 2018 were: 24/16 °C, 26/17 °C and 29/19 °C respectively. All trials were randomized complete blocks. Deionized water was used for the trials in Reading UK and distilled water for the trials in Benton USA, to prepare all the solutions.

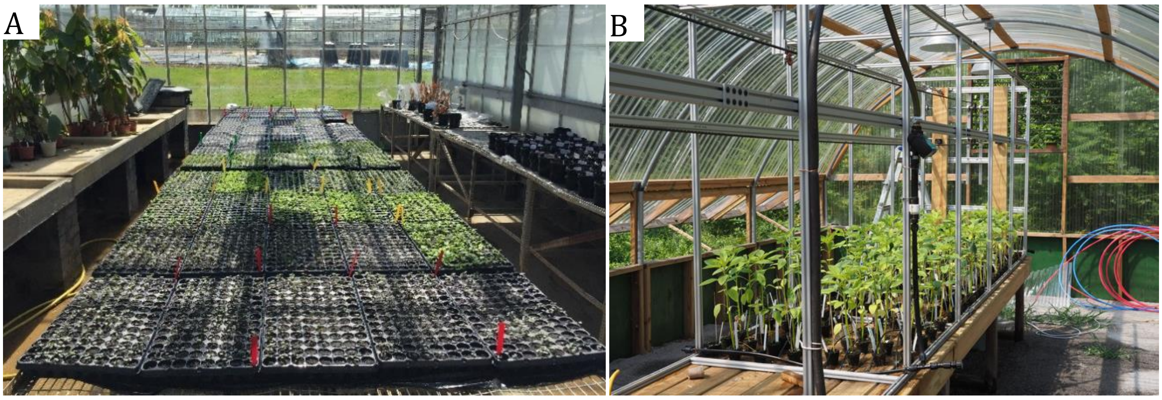


Figure 2.1. (A) Glasshouse in Reading UK where weeds and cabbages seedlings were grown during May 2016. (B) Glasshouse in Benton, Kentucky, USA where the dose response trial with *A. cruentus* took place, during July 2018

2.3.1 Plant material

Trials applying herbicide droplets were carried out in this study using the annual weed species: *Chenopodium album* L. (fat-hen), *Urtica urens* L. (small nettle), *Stellaria media* L. Vill. (common chickweed), *Galium aparine* L. (cleavers), *Matricaria recutita* L. (German chamomile), *Poa annua* L. (annual meadow grass), *Senecio vulgaris* L. (common groundsel) and *A*. *cruentus* L. (red amaranth) and the perennial weed *Rumex crispus* L. (curly dock). In addition to the weeds, herbicide droplets were applied to crop seedlings. Cabbages, savoy variety (Famosa F1), were provided from Hammond Produce (Hammond Produce Ltd, Nottingham, UK) and were transplanted into flowerpots (9 cm diameter) at BBCH 12-13 (Feller *et al*., 1995). Seeds of *C. album* and *R. crispus* were collected by hand at the Reading University Farm in 1974 and 1988, respectively and stored at 2-4°C. *Urtica urens*, *S*. *media*, *G. aparine*, *M*. *recutita*, *P*. *annua* and *S*. *vulgaris* seeds were provided by Herbiseed Ltd. and were sown on the surface of J. Arthur Bowers multi-purpose compost in multi cell plastic trays. Seeds of *A. cruentus* were provided by the Two Willies Nursery (Lucedale, Mississippi, USA) and the Moisture Control Potting Mix from Miracle-Gro (The Scotts Company LLC, Marysville, Ohio, USA) was used as a potting soil. The trays used in this study consisted of 84 cells with individual cell size being 35 mm x 35 mm and 45 mm deep. Five to seven weed seeds from each species were sown in each cell and after germination they were thinned to one seeding per cell. Temperature and humidity were recorded using loggers (Thermochron-Hygrochron, iButton®, iButtonLink, LLC.). All trials were carried out under natural sunlight.

2.3.2 Ground cover assessment

In order to estimate the amount (µg) of active ingredient, volume (µL) and number of droplets needed to apply the recommended rate of the herbicides (L ha^{-1}) individual images of weed seedlings were taken using a Nikon D90 Digital SLR Camera with an 18-105 mm VR Lens Kit, mounted on a tripod (ManFrotto Compact Action). Seedlings assessed (10-30 per trial) were selected randomly and came from a destructive treatment which was included in design of the trial. The images taken were analysed using the WinDIAS Leaf Image Analysis System (Delta-T Devices Ltd, Cambridge, UK) (Webb & Jenkins, 2000) and ground cover was estimated in cm^2 by the proportion of green pixels in an image of known area (Table 2.2). Following that the exact amount of herbicide needed to apply the minimum label recommendation, was calculated using Equation 2.1:

$$A = gc*(rd/100) \qquad 2.1$$

where A is the amount of herbicide in µg per seedling, gc is the mean ground cover of one seedling in cm^2 and rd refers to the recommended dose of the herbicide in g of a.i. ha^{-1}.

2.3.3 Glyphosate, glufosinate-ammonium and 2,4-D dose-response trials

In order to apply the herbicides' label recommendations as single droplets of 1-2 µl (i.e. the volumes that will be applied by the automated system), the a.i. concentrations prepared ranged from 2.5% to 20%. However, when glyphosate was applied to *U. urens* and for both the dose-response trials of *A. cruentus* more than one droplet was applied and higher volumes had to be used due to large seedling size (Table 2.2). Herbicide dose rates ranged from 1/256 to 6x the minimum label recommendation. Control treatments involved the use of droplets containing water, adjuvant with water (1%) and undiluted herbicide. For the dose-response curves the biomass data which corresponds to the 0 dose, includes the response of treatments containing only water. In addition to the herbicides described in Table 2.1 a combined treatment of 2,4-D and glufosinate-ammonium was also tested with *P. annua*, *S. vulgaris* and *C. album* seedlings. When droplets of 2,4-D were applied to *P. annua* seedlings the maximum label recommendation was used (3.3 L ha^{-1}). All herbicide treatments included the adjuvant AS 500 SL (Z.P.H Agromix, Niepołomice, Poland) which comprises non-ionic surfactants, ammonium salts, organic acid, pH buffer and humectant and was applied at the rate of 1 L 100 L^{-1} water (Woznica *et al*., 2015). When droplets of glyphosate and glufosinate-ammonium were applied to *A. cruentus* seedlings, USA formulations of the herbicides were used (Table 2.1). When Liberty® 280 SL was applied, the adjuvant Verimax Ams Dry (ammonium sulfate, polyacrylamide, dimethylpolysiloxane, 100%, Innvictis Crop Care, LLC™) was used at 1% concentration for every solution of the herbicide.

Two pipettes were used for the droplet application, one with a volume range from 0.1 to 2.5 µl (ErgoOne® Single-Channel, STARLAB (UK), Ltd) and one to apply droplets in the 2.5-10 µl range (Micropipette Single Channel, Scilogex llc). Droplets were applied to the adaxial side of the youngest fully expanded leaf and when two droplets of the same herbicide were needed to be used, they were applied on the same spot of the same leaf. In the case of the combined treatment of 2,4-D and glufosinate-ammonium the two droplets of the different herbicides were applied on either side of the central vein of the same leaf. Twenty days after herbicide applications fresh and dry biomass data were recorded and treatments were scored using the European Weed Research Council (EWRC) scoring system (Ciba-Geigy, 1975). Dry weights were estimated after oven-drying fresh seedlings for 48h at 80°C using an analytical balance (weighing to the nearest 0.0001 g).

Table 2.1. Details of herbicides used for the dose-response trials.

Commercial name	Supplier	Active ingredient	Concentration (g L^{-1})	Recommended dose (L ha^{-1})
Roundup® Biactive GL	Monsanto (UK) Ltd.	glyphosate	360	1.5
Harvest®	Bayer CropScience Ltd.	glu-amm	150	3
Depitox®	Nufarm (UK) Ltd.	2,4-D	500	1.4
Kyleo®	Nufarm (UK) Ltd.	2,4-D & glyphosate	160 & 240	3
Envy™ Six Max*	Innvictis Crop Care, LLC™	glyphosate	540	1.18
Liberty® 280 SL*	Bayer CropScience LP	glu-amm	280	2.25

glu-amm = glufosinate-ammonium
*applied only to *A. cruentus* seedlings in the U.S.A

Table 2.2. Details of the leaf-specific herbicide application including the mean ground cover for each of the weed species and savoy cabbage tested with their respective growth stages, number and volume of droplets and concentration (% a.i.) and amount of active ingredient per seedling in order to apply the minimum label recommendation. Herbicide formulations are specified in Table 2.1. Trials with the same number were carried out simultaneously.

					Application of recommended dose (1x)		
Trial No.	**Block No.**	**Plant Species**	**BBCH Growth Stage**	**Ground cover (cm²)**	**No. x Volume (µL) of droplets**	**a.i. (%)**	**Amount of a.i. (µg)**
			Roundup® Biactive GL (360 g L⁻¹ glyphosate)				
1	30	(a) *C. album*	12-14	1.08 (0.44)	1 x 0.648	2.5	5.83
2	10	(b) *C. album*	14-16	7.21 (1.07)	1 x 1.082	10	38.9
3	11	(c) *S. media*	14-16	9.03 (3.63)	1 x 1.354	10	48.8
4	17	(d) *M. recutita*	12-14	3.13 (1.26)	1 x 0.940	5	16.9
5	15	(e) *G. aparine*	12-14	1.56 (0.42)	1 x 0.936	2.5	8.42
6	15	(f) *U. urens*	16-18	25.4 (3.21)	2 x 1.905	10	137.2
7	10	(g) *P. annua*	23-24	10.54 (1.65)	1 x 1.580	10	56.9
8	10	(h) *S. vulgaris*	12-13	2.23 (0.31)	1 x 1.340	2.5	12.1
9	25	*(i) R. crispus*	13-14	3.28 (0.90)	1 x 0.984	5	17.7
10	29	(j) *B. oleracea*	14-15	20.8 (3.4)	2 x 1.560	10	112.2
			Envy™ Six Max (540 g L⁻¹ glyphosate)				
11	10	(k) *A. cruentus*	16-18	51.04 (13.2)	1 x 2.986	20	322.5
			Harvest® (150 g L⁻¹ glufosinate-ammonium)				
12	13	(l) *C. album*	14-16	4.84 (1.56)	1 x 1.452	10	21.8
2	10	(m) *C. album*	14-16	7.21 (1.07)	1 x 2.163	10	32.5
13	12	(n) *U. urens*	14-16	6.25 (2.11)	1 x 1.876	10	28.1
8	10	(o) *S. vulgaris*	12-13	2.23 (0.31)	1 x 1.340	5	10.0
7	10	(p) *P. annua*	23-24	10.54 (1.65)	1 x 1.580	20	47.5
			Liberty® 280 SL (280 g L⁻¹ glufosinate-ammonium)				
11	10	(q) *A. cruentus*	16-18	51.04 (13.2)	2 x 2.870	20	321.6
			Kyleo® (160 g L⁻¹ 2,4-D and 240 g L⁻¹ Glyphosate)				
2	10	(r) *C. album*	14-16	7.21 (1.07)	1 x 2.163	10	86.5
8	10	(s) *S. vulgaris*	12-13	2.23 (0.31)	1 x 1.339	5	26.8
7	10	(t) *P. annua*	23-24	10.54 (1.65)	1 x 1.580	20	126.5
			2,4-D (500 g L⁻¹) + Glufosinate-ammonium (150 g L⁻¹)				
2	10	(u) *C. album*	14-16	7.21 (1.07)	1x1.01 + 1x2.163	10+10	50.5+32.4
8	10	(v) *S. vulgaris*	12-13	2.23 (0.31)	1x1.25 + 1x1.340	2.5+5	15.6+10
7	10	(w) *P. annua*	23-24	10.54 (1.65)	1x1.74 + 1x1.580	20+20	174+47.5
			Depitox® (500 g L⁻¹ 2,4-D)				
2	10	(x) *C. album*	14-16	7.21 (1.07)	1 x 1.010	10	50.5
8	10	(y) *S. vulgaris*	12-13	2.23 (0.31)	1 x 1.250	2.5	15.6
7	10	(z) *P. annua*	23-24	10.54 (1.65)	1 x 1.74	20	174

2.3.4 Statistical analysis

In order to generate the dose-response curves, dry weight data were analysed with non-linear regression analysis, using the four-parameter log-logistic model (Equation 2.2) as described by Streibig (1988):

$$y = c + (d - c) / [1+\exp(b(\log(x) - \log(ED_{50})))] \qquad 2.2$$

where y is the biomass, c and d are the lower and upper limits of y, respectively, b is the relative slope of the curve around ED_{50}, x is the herbicide dose and ED_{50} is the dose corresponding to 50% reduction of y. Weed dry matter is the most common and most objective measurement for plotting herbicide dose-response curves and estimating effective doses (Streibig, 1993). However, for the glyphosate dose-response curve of *R. crispus* fresh weight data are provided because dry weight data did not give a satisfactory fit to the dose-response model. Additionally, in order to be able to compare results in this study with similar research in the literature, fresh biomass data of *C. album* were plotted against doses of glyphosate and glufosinate-ammonium. Analyses were carried out using the open source statistical software R, version 3.2.1 (R Development Core Team, 2014) and the add-on package "drc" (Ritz *et al*., 2015a). The effective dose (ED) which corresponds to 90% (ED_{90}) reduction in biomass was calculated after data were fitted to the dose-response model (Ritz, 2010). Because treatments were replicated a lack-of-fit test was carried out using the modelFit() function which compares the residual sum of squares from fitting the equation 2.2 with the residual some of squares of the one-way ANOVA model (Ritz & Streibig, 2012). Where appropriate dry weight data were expressed as % reduction of growth and were transformed using the equation 2.3:

$$\% \text{ Reduction of growth} = [1 - (W_t - W_0) / (W_c - W_0)] \times 100 \qquad 2.3$$

where W_t corresponds to the dry weight at harvest, W_c is the dry weight of the water control treatment and W_0 is the mean dry weight of the seedlings on the day of droplet application. To plot the % reduction of growth dose-response curves a three-parameter model was used (in equation 2.2, parameter c = 0). Reduction of growth dose-response curves were plotted against the dose relative the recommended rate of spraying the herbicides (1x). Because of the difference in the concentration of glyphosate when it was applied alone (540 g ha^{-1}) and as the mixture (720 g ha^{-1}) with 2,4-D, the two curves were plotted against the doses relative the recommended rate of when the herbicide was applied alone (i.e. for mixture 1x=1.33x the recommended rate of 540 g ha^{-1})

When fitting multiple dose-response curves the hypotheses that the curves are parallel (common b), that they have common upper and lower limits (parameters c and d) and that there is a common ED_{50} (parameter e) were tested (Seefeldt *et al.*, 1995). Model comparison was carried out with ANOVA by an *F*-test based on the residual sum of squares of the dose-response curves with and without similar parameters using R. When comparing two dose-response curves it is important to take into account the biological exchange rate between the two herbicides, especially when developing new products or assessing the combined effect of mixtures (Streibig & Jensen, 2000). This can be performed by comparing the ED_x values and is known as relative potency (R). In the case of similar regressions which are only different at the ED_{50} values, the relative potency is expressed as the displacement of the curve along the x-axis (Ritz *et al.*, 2006). The built-in function in the drc package EDcomp() was used to obtain the relative potency (Ritz *et al.*, 2015a). In order to assess the effect of the adjuvants used in this study, biomass data were subjected to one-way ANOVA using GenStat 16th Edition (VSN International Ltd, Hemel Hempstead, UK).

2.4 Results

In Reading, no significant effect was observed on weed biomass after the application of droplets containing the adjuvant AS 500 SL (1%) when compared to the treatments with droplets of water. Similarly, in the USA, no significant effect of the adjuvant Verimax Ams Dry occurred to *A. cruentus* seedlings (Supplementary table 2.1.). A lack-of-fit test was significant only when droplets of glufosinate-ammonium were applied to *P. annua* seedlings (P=0.04), suggesting that for all the other dose-response curves, data can be described by Equation 2.2 (Supplementary table 2.2).

After fitting the data to Equation 2.2, it was estimated that fresh weight of *C. album* can be reduced by 50% and 90% with 3.5 μg and 32 μg of glyphosate respectively. For the same effects, doses of 9.4 μg and 60 μg of glufosinate-ammonium were needed (Table 2.3). Increasing phytotoxic effects were observed with increase of dose to the *Chenopodium album* seedlings for trial number two when the recommended rates of the herbicides used in this study were applied as a single droplet to a single leaf (Figure 2.2). Similar phytotoxic effects were observed for all the other species tested in this study (Supplementary figure 2.1). From the annual weed species tested in this study, which had 2 to 6 leaves, the most susceptible to glyphosate was *S. vulgaris*, a dose of 0.24 µg of glyphosate per seedling (~1/50 of the recommended) reducing weed dry weight by 90% (Table 2.4 (h)). Among the weed species treated with glufosinate-ammonium droplets, *U. urens* was the most susceptible with a dose which was close to 1/8th of the recommended needed for 90% weed control. For the savoy cabbage, 36 µg of glyphosate applied as a single droplet, reduced the biomass of the crop by 50% (Table 2.4 (j))

Table 2.3. Parameter estimates (±SE) of the log-logistic dose-response model (Eqn 2.2) and ED_{90} value for the fresh weight of *C. album* seedlings, size of 1.08 cm^2 (Trial No.1) and 4.84 cm^2 (Trial No.12). Recommended doses per seedling were 5.83 µg of glyphosate and 21.8 µg of glufosinate-ammonium.

Parameters	Estimates (±SE)	
	Glyphosate	**Glufosinate-ammonium**
b (slope)	1.00 (0.24)	1.19 (0.43)
c (lower limit) (g)	0.04 (0.01)	0.07 (0.04)
d (upper limit) (g)	0.15 (0.01)	0.50 (0.03)
e (ED50) (µg)	3.54 (1.01)	9.38 (3.03)
ED90 (µg)	31.8 (18.6)	59.7 (47.7)

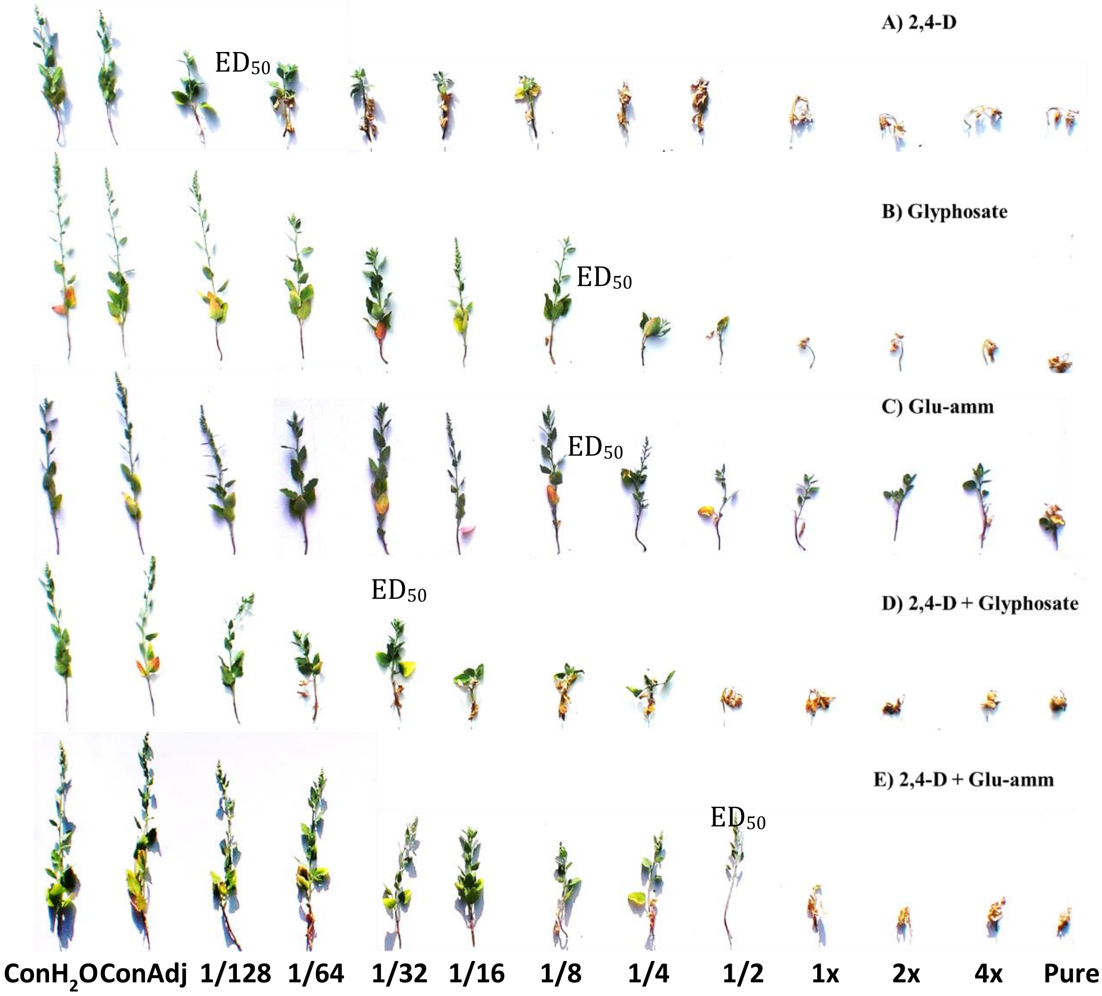


Figure 2.2. Chenopodium album seedlings (Trial No. 2) three weeks after application of droplets containing recommended amounts of active ingredients (Table 2.2). For the combined treatment (E), one droplet containing 50.5 μg 2,4-D and one with 32.5 μg of glufosinate-ammonium were applied on the same leaf. For the control treatments, one droplet of deionized water (ConH2O) or containing 1% adjuvant (ConAdj) or with undiluted herbicide (Pure) was applied. To estimate the 50% reduction in the biomass of the seedlings (ED_{50}) dry weight data were fitted to Equation 2.2.

When herbicide droplets were applied to different sizes of *C. album* seedlings at different times (trial numbers 1, 2 and 12), it was demonstrated that approximately $1/6^{th}$ of the recommended doses of the two herbicides (glyphosate and glufosinate-ammonium) was required for a 50% reduction in the biomass with the ED_{90} value being 1.5-times the recommended rate (Table 2.5). Furthermore, little attrition was observed when droplets of glyphosate were applied to the *C. album* seedlings measuring 1.08 cm^2 (Trial No. 1) (Figure 2.4). A common dose-response curve was fitted when glufosinate-ammonium was applied to *C. album* with ground cover 4.84 cm^2 (trial no. 12) and 7.21 cm^2 (trial no. 2). The model used to plot the curves of different size *C. album* seedlings, had a common slope, ED_{50} and ED_{90} values. The increase in the residual sum of squares was not statistically significant from the separate lines model (P=0.26) (Supplementary table 2.3), meaning that the model can be used to describe the effects of herbicide droplet application to *C. album* seedlings.

A model with two separate dose-response curves was fitted when doses relative to the recommended rates of glyphosate and glufosinate-ammonium (1x) were plotted against the dry weights (% Control) of *U. urens and A. cruentus* (Figure 2.4). When the recommended rate of glufosinate-ammonium was applied to *U. urens* seedlings the weed was completely controlled whereas the ED_{90} value for glyphosate was 3-times the recommended rate (Table 2.5). For 90% control of *A. cruentus*, 1/10th of glyphosate's recommended rate was needed while the same relative dose for glufosinate-ammonium reduced the dry weights of the seedlings by 50% (Table 2.5).

When the herbicides tested in this study were applied for the same dose-response trial to *C. album* seedlings it was demonstrated that the recommended rate of glyphosate and glufosinate-ammonium reduced the growth of the weed by 90% (Table 2.6). For the same effect, 1/4th of the recommended rate was needed when the proprietary mix of 2,4-D and glyphosate was applied as single droplet to a single leaf. The reduction of growth recorded from 2,4-D and the combined treatment of 2,4-D and glufosinate-ammonium was not significant for both of the effective doses (ED_{50} and ED_{90}) (Table 2.6).

Application of droplets containing recommended amounts of herbicides reduced the growth of *S. vulgaris* from 90% to 100% (Figure 2.5). Although no common parameters were fitted to the dose-response models of the weed, approximately the same levels of efficacy were observed when glyphosate and the mix of 2,4-D and glyphosate were applied to *S. vulagris* (Table 2.6).

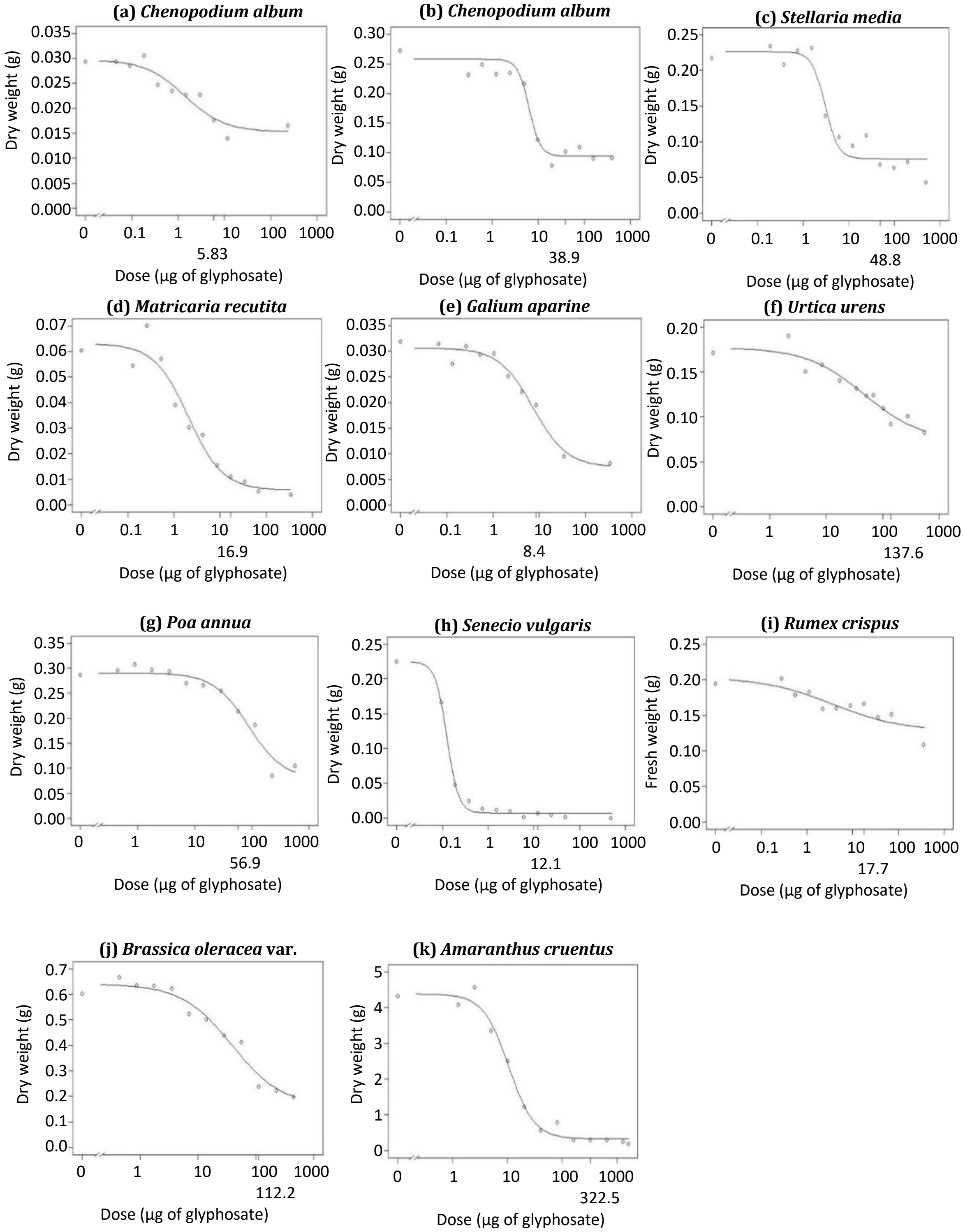
Common dose-response curves were fitted to plot the reduction of the growth of *P. annua* caused by glyphosate and glufosinate-ammonium and when they were mixed with 2,4-D (Figure 2.5). There was no significant increase in the residual sum of squares from the model with four separate lines (P=0.33) (Supplementary table 2.4). A dose which was equivalent to 1.5-times the recommended dose of glyphosate and glufosinate-ammonium reduced the growth of *P. annua* by 50%. Higher doses than the label recommendations for both herbicides were needed for 90% weed control. Lower effective doses were recorded for both ED levels when the two herbicides were mixed with 2,4-D (Table 2.6). The recommended rates of the herbicide mixtures with 2,4-D reduced the growth of *P. annua* by approximately 70% (Figure 2.5).

No complete weed control was recorded when droplets of glyphosate were applied to the perennial *R. crispus* and only when the control treatment of undiluted product was applied (20x the recommended) it caused a 90% reduction in the growth of the weed (Figure 2.5). Droplets of glyphosate containing the recommended rate of glyphosate reduced the growth of the annual weeds *G. aparine*, *S. media* and *M. recutita* by approximately 80% 100% and 120% respectively.

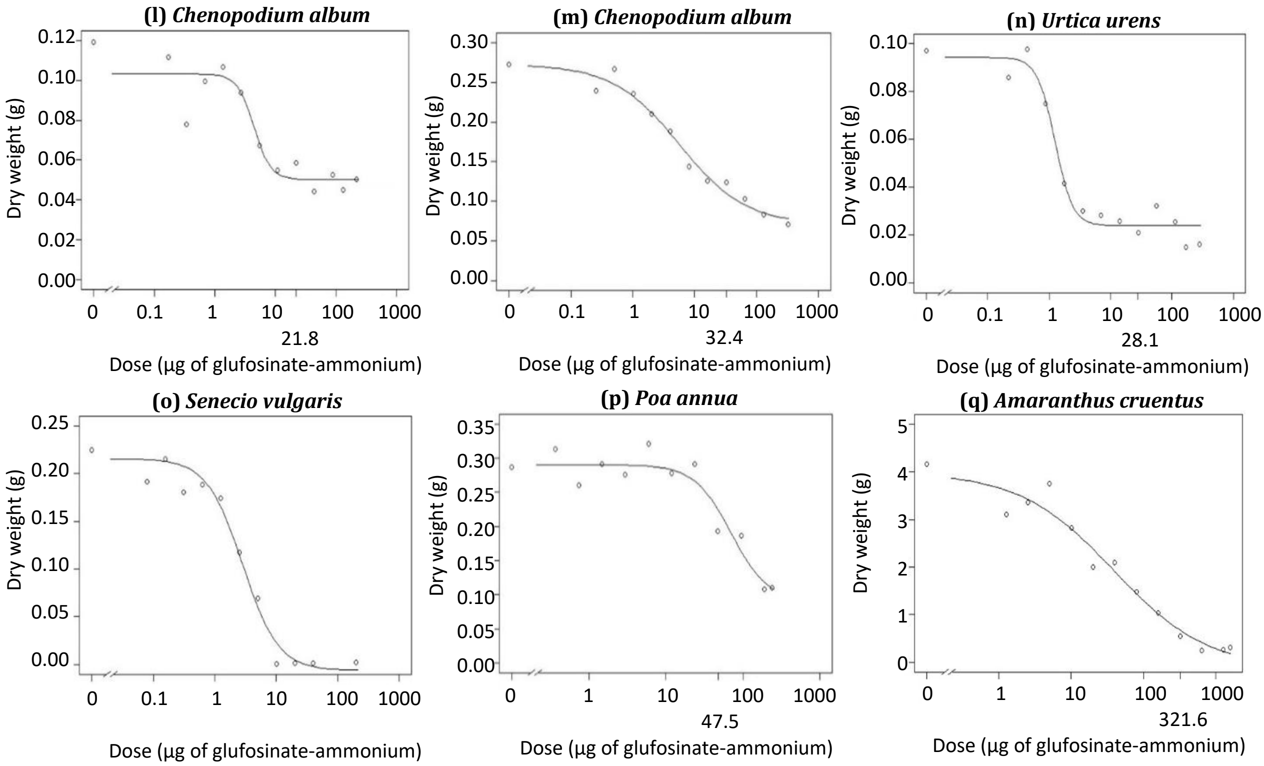
When the dose-response curves of glyphosate and its mixture with 2,4-D where plotted together against the doses relative to the 540 g ha^{-1} (1x), common slope and upper limit of the curves were fitted, allowing for the relative potency to be calculated (Table 2.7). Because of the commonality in the parameters (lower limit fixed at 0) the relative potency was independent of the response level. For *C. album* it was calculated that the relative potency ($R=ED_{50(glyphosate)}/ED_{50(mixture)}$) is 3.32 (±SE: 0.8) whereas for the *S. vulagris* is 0.53 (±SE: 0.06). For dose-response curves of *P. annua* a common line was fitted which did not differ significantly from the separate line model (P=0.07) (Table 2.7).

Increase in the dry weight of *C. album*, *M. recutita*, *S. media*, *U. urens*, *P. annua* and *A. cruentus* was observed from low doses of glyphosate ranging from 1/256 to 1/32 of the recommended rate. Furthermore, doses up to 1/16th of the recommended dose of glyphosate appeared to be increasing the growth of *P. annua* seedlings (Figure 2.4 and Figure 2.5). Application of low doses (from 1/256 to 1/64 of the recommended) appeared to be promoting the growth of *S. media*, *R. crispus* and *M. recutita* (Figure 2.5).

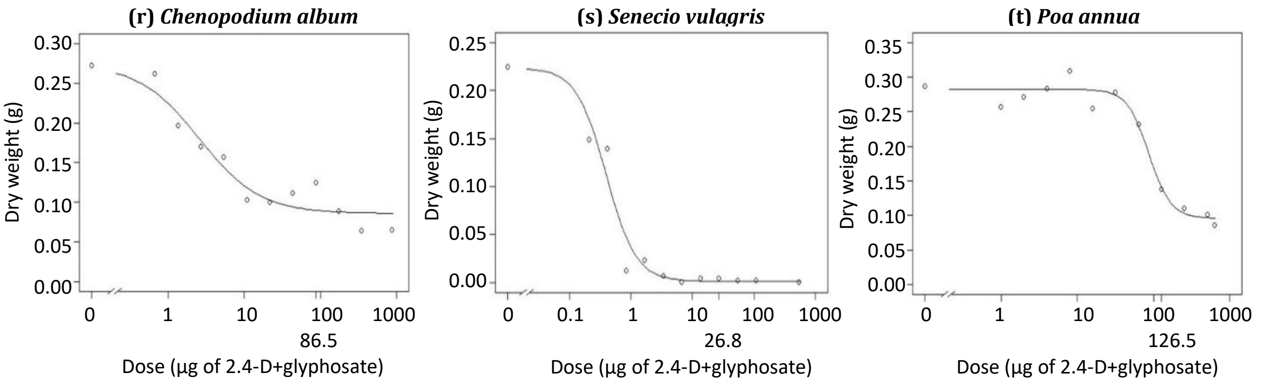
Glyphosate dose-response curves



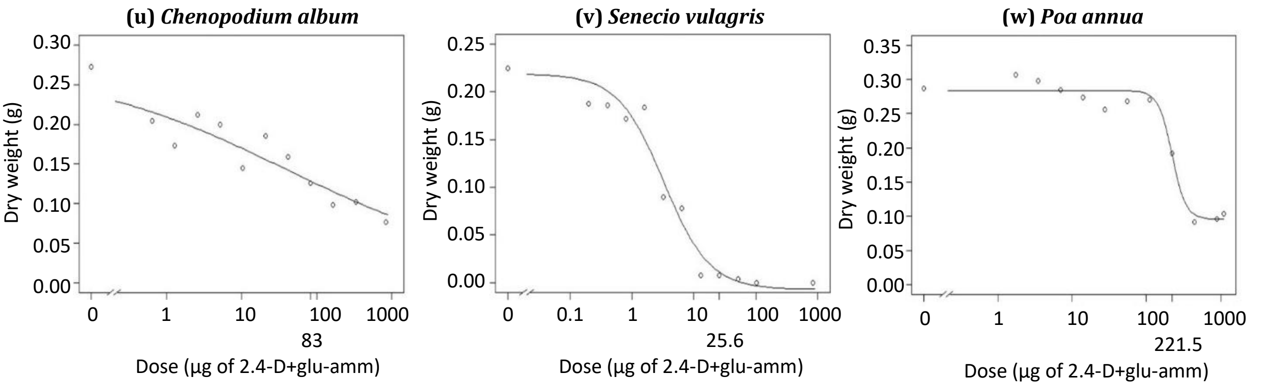
Glufosinate-ammonium dose-response curves



2,4-D + Glyphosate dose-response curves



2,4-D + Glufosinate-ammonium dose-response curves



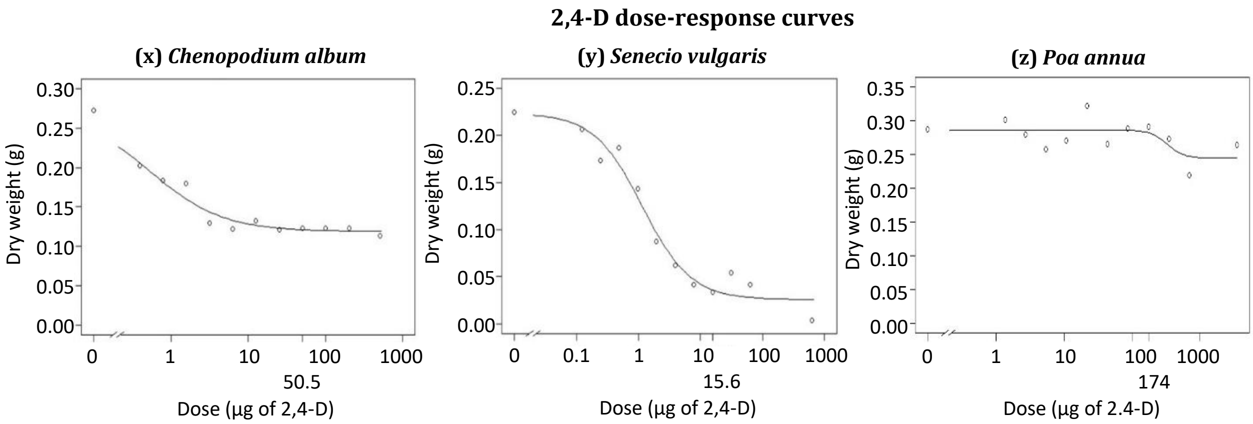


Figure 2.3. Dose-response curves after droplets of glyphosate were applied to *C. album* (a & b), *S. media* (c), *M. recutita* (d), *G. aparine* (e), *U. urens* (f), *P. annua* (g), *S. vulgaris* (h), *R. crispus* (i), *B. oleracea* var. *sabauda* (j) and *A. cruentus* (k) seedlings. Glufosinate-ammonium droplets were applied to *C. album* (l & m), *U. urens* (n), *S. vulgaris* (o), *P. annua* (p) and *A. cruentus* (q) seedlings. Droplets of the proprietary mixture of 2,4-D and glyphosate and the combined treatments of 2,4-D and glufosinate ammonium were applied to C. *album* (r & u), *S. vulgaris* (s & v) and *P. annua* (t & w). Droplets containing 2,4-D were applied to *C. album* (x), *S. vulgaris* (y) and *P. annua* (z). Biomass data were harvested 20 days after droplet application. Equation 2.2 was used to plot herbicide dose and biomass data. Parameter values are presented in Table 2.4. Results from the lack-of-fit test for each of the curves are presented in Supplementary table 2.2.

Table 2.4. Regression parameters (±SE) of the log-logistic dose-response model (Equation 2.2) and glyphosate, glufosinate-ammonium, 2,4-D, 2,4-D + glyphosate and 2,4-D + glufosinate-ammonium doses (μg) which provided 90% reduction in weeds' biomass (ED_{90}) 20 days after droplet application, for each of the weed species tested. Application details and calculation of recommended doses applied (1x) is described in Table 2.2. Plotted dose-response curves are presented in Figure 2.3.

		Parameter estimates (±SE)				
Dose-response curves	**b**	**c (g)**	**d (g)**	**ED_{50} (μg)**	**ED_{90} (μg)**	**1x (μg)**
			Glyphosate			
(a) *C. album*	1.01 (0.27)	0.02 (0.001)	0.03 (0.001)	1.39 (0.56)	12.1 (8.06)	5.83
(b) *C. album*	3.54 (1.58)	0.09 (0.01)	0.26 (0.01)	6.27 (0.83)	11.7 (3.44)	38.9
(c) *S. media*	3.00 (3.86)	0.08 (0.02)	0.23 (0.01)	3.04 (1.10)	6.33 (7.83)	48.8
(d) *M. recuita*	1.17 (0.32)	0.006 (0.004)	0.06 (0.004)	2.07 (0.63)	13.6 (8.9)	16.9
(e) *G. aparine*	1.61 (0.71)	0.01 (0.003)	0.03 (0.001)	6.89 (2.15)	27.1 (20.8)	8.44
(f) *U. urens*	0.96 (0.28)	0.08 (0.01)	0.18 (0.01)	46.5 (15.9)	460 (389)	137.2
(g) *P. annua*	1.41 (0.43)	0.08 (0.03)	0.29 (0.01)	87.4 (23.2)	413 (266)	56.9
(h) *S. vulgaris*	3.39 (0.75)	0.007 (0.005)	0.22 (0.01)	0.13 (0.01)	0.24 (0.04)	12.1
(i) *R. crispus*	0.58 (0.25)	0.13 (0.01)	0.20 (0.01)	3.70 (3.27)	168 (340)	17.7
(j) *B. oleracea*	0.97 (0.17)	0.17 (0.03)	0.66 (0.02)	35.9 (7.99)	346 (171)	112.2
(k) *A. cruentus*	1.95 (0.32)	0.33 (0.09)	4.30 (0.12)	10.9 (1.10)	33.5 (7.1)	322.5
			Glufosinate-ammonium			
(l) *C. album*	3.14 (2.61)	0.05 (0.01)	0.10 (0.01)	4.43 (1.21)	8.91 (5.93)	21.8
(m) *C. album*	0.81 (0.22)	0.07 (0.02)	0.27 (0.01)	5.66 (2.39)	84.7 (89.6)	32.4
(n) *U. urens*	3.01 (1.06)	0.02 (0.003)	0.09 (0.004)	1.25 (0.17)	2.60 (0.78)	28.1
(o) *S. vulgaris*	1.45 (0.28)	-0.01 (0.01)	0.22 (0.01)	2.9 (0.37)	13.2 (4.3)	10.0
(p) *P. annua*	1.82 (0.61)	0.09 (0.03)	0.29 (0.01)	70.4 (24.7)	235 (166)	47.5
(q) *A. cruentus*	0.60 (0.12)	-0.25 (0.38)	4.15 (0.16)	37.5 (14.5)	1453 (1466)	321.6
			2,4-D + Glyphosate			
(r) *C. album*	1.09 (0.33	0.09 (0.01)	0.27 (0.01)	2.61 (0.68)	19.7 (14.8)	86.5
(s) *S. vulgaris*	1.81 (0.34)	0.002 (0.005)	0.22 (0.01)	0.40 (0.04)	1.33 (0.30)	26.8
(t) *P. annua*	3.01 (1.10)	0.09 (0.01)	0.28 (0.01)	86.7 (11.6)	180 (58.8)	126.5
			2,4-D + Glufosinate-ammonium			
(u) *C. album*	0.31 (0.07)	0.02 (0.04)	0.27 (0.01)	35 (40)	39209 (93409)	82.9
(v) *S. vulgaris*	1.20 (0.20)	-0.01 (0.01)	0.22 (0.01)	3.26 (0.46)	20.4 (7.15)	25.7
(w) *P. annua*	4.83 (3.89)	0.09 (0.01)	0.28 (0.01)	222 (18.7)	349 (126)	221.4
			2,4-D			
(x) *C. album*	1.05 (0.41)	0.12 (0.01)	0.27 (0.01)	0.57 (0.19)	4.60 (3.68)	50.5
(y) *S. vulgaris*	1.12 (0.23)	0.03 (0.01)	0.22 (0.01)	1.16 (0.19)	8.21 (3.71)	15.6
(z) *P. annua*	3.56 (3.27)	0.24 (0.02)	0.28 (0.01)	329 (167)	610 (337)	174

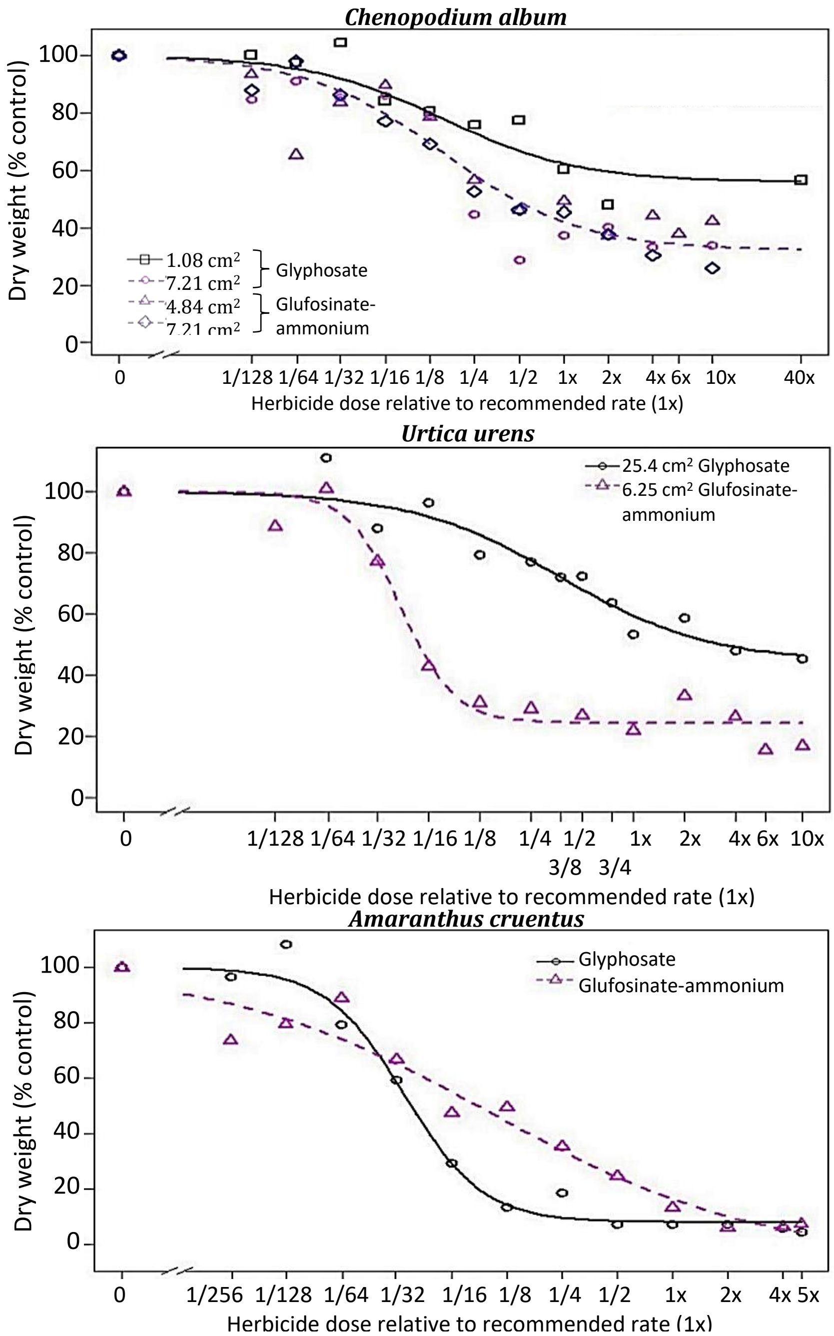


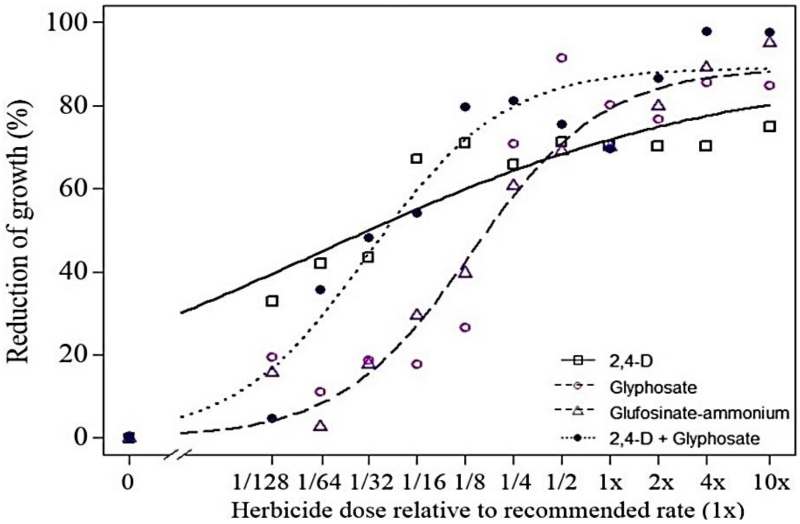
Figure 2.4. Dose response curves of *C. album U. urens* and *A. cruentus*. *Chenopodium album* seedlings with ground cover of 1.08 cm², 7.21 cm² and 4.84 cm² were tested at the trials with numbers 1, 2 and 12, respectively. *Uritca urens* seedlings with ground covers of 6.25 cm² and 25.4 cm² were tested at the trials with numbers 6 and 13, respectively. *Amaranthus cruentus* seedlings were tested for the trial number 11. Dry weight data are expressed as % of control and were plotted against the doses relative to the recommended rate of glyphosate and glufosinate-ammonium. The upper limit for all the curves was fixed at 100%. Model comparison between the fitted and the separate line models is presented in Supplementary table 2.3. The separate lines model for *C. album* is presented in Supplementary figure 2.2. Parameter estimates are presented in Table 2.5.

Table 2.5. Regression parameters (±SE) of the log-logistic model for the *Chenopodium album*, *Urtica urens* and A. *cruentus* dose-response curves presented in Figure 2.4. The upper limit of the curves (parameter d) was fixed at 100%. Model using common parameter estimates for the *C. album* dose-response curves is based on non-significant differences in the residual sum of squares from the separate line model (P=0.26). Commonality between the parameters of the *U. urens* and *A. cruentus* dose-response models significantly increased the residual sum of squares (P<0.001) (Supplementary table 2.3).

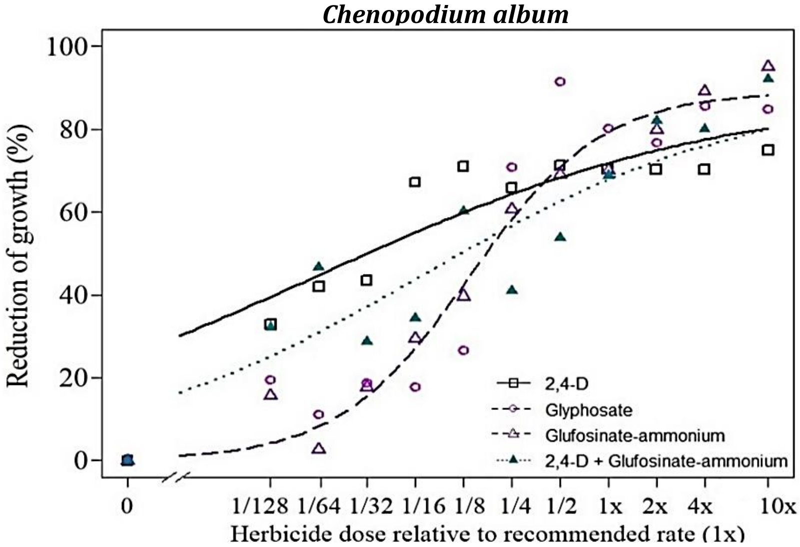
			Parameter Estimates (±SE)			
Species	**Herbicide**	**Ground cover (cm^2)**	**b:**	**c (%):**	**e: ED_{50}**	**ED_{90}**
C. album	glyphosate	1.08	0.98 (0.16)	55.5 (3.21)	0.16	1.55
		7.21		31.8 (3.61)		
	glu-amm	4.84				
		7.21				
U. urens	glyphosate	25.4	0.99 (0.25)	44.5 (6.1)	0.37 (0.12)	3.40 (2.69)
	glu-amm	6.25	2.75 (0.81)	24.5 (2.42)	0.04 (0.005)	0.09 (0.03)
A. cruentus	glyphosate	51.04	2.03 (0.31)	7.97 (2.28)	0.03 (0.003)	0.10 (0.02)
	glu-amm		0.60 (0.09)	-5.04 (7.61)	0.10 (0.03)	4.04 (3.42)

glu-amm=glufosinate-ammonium

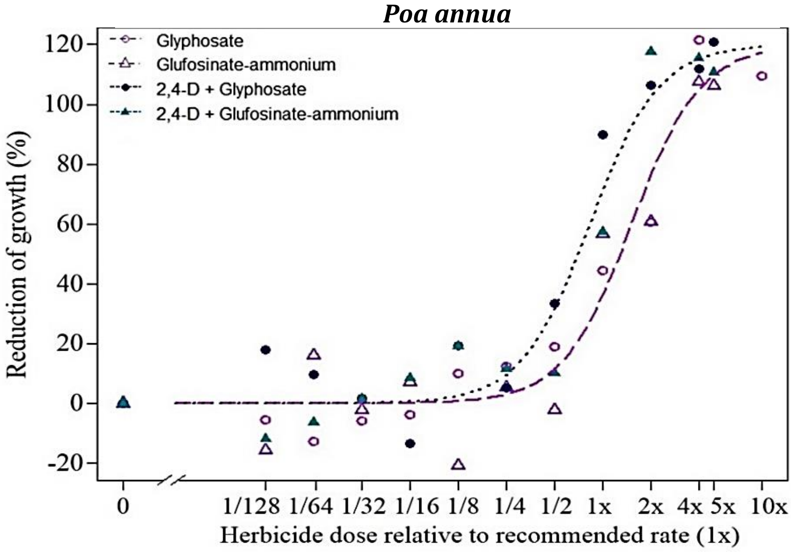
Chenopodium album



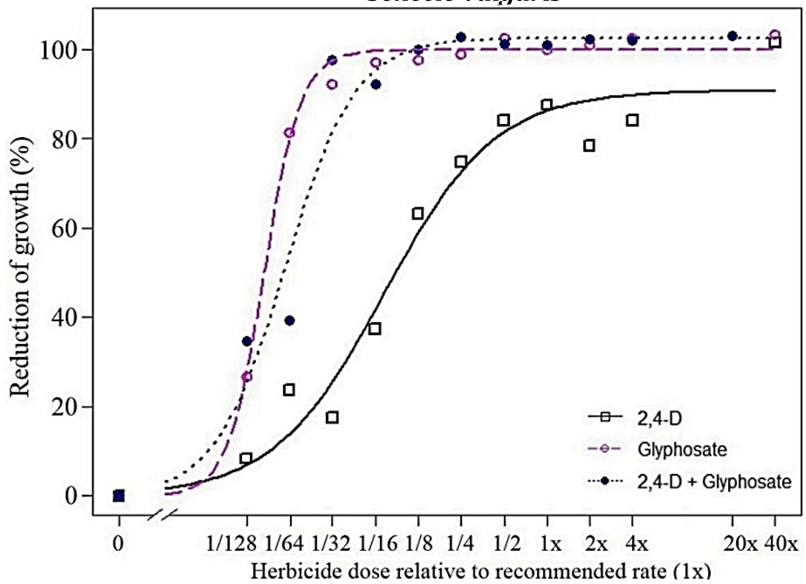
Chenopodium album



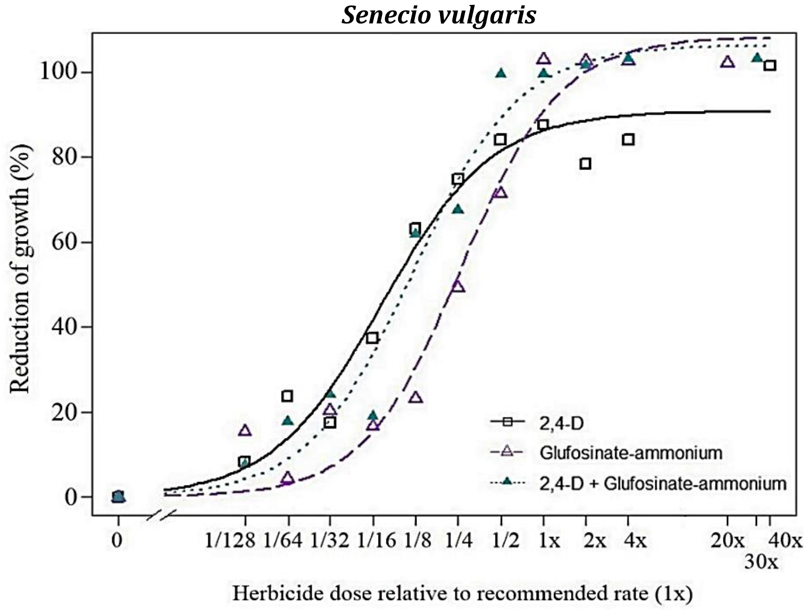
Poa annua



Senecio vulgaris



Senecio vulgaris



M. recutita, G. aparine, R. crispus, S. media

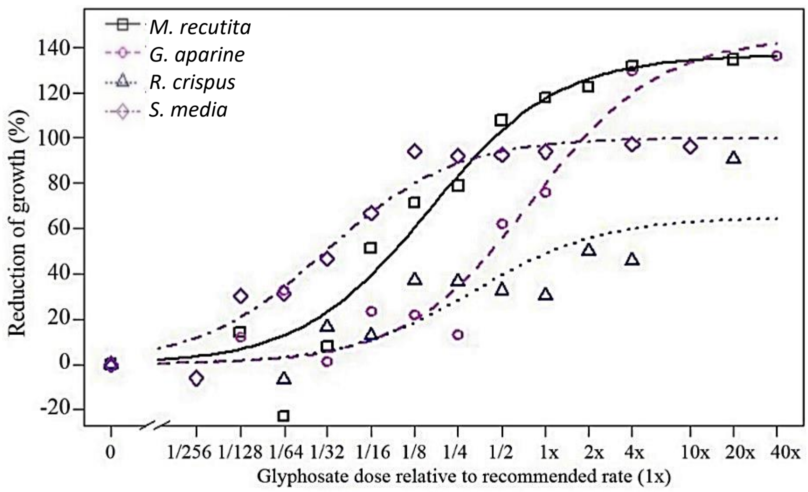


Figure 2.5. Dose-response curves of *C. album*, *P. annua* and *S. vulagris* after droplets of 2,4-D, glyphosate, glufosinate-ammonium, a mixture of glyphosate and 2,4-D and a combined treatment of 2,4-D and glufosinate-ammonium were applied during summer 2018. Droplets of glyphosate were applied only to *M. recutita*, *G. aparine*, *R. crispus* and *S. media* during summer 2015. Dry weight data were transformed to % Reduction of growth using equation 2.3 and were plotted against the doses relative to the minimum label recommendations (1x) using the three-parameter log-logistic model (Equation 2.2 with parameter c=0). Parameters of the fitted models are presented in Table 2.6. Dose-response curves of the separate lines model is presented in Supplementary figure 2.3.

Table 2.6. Parameter estimates for the regressions of reduction of growth as a function of dose of different herbicides (Figure 2.5**)**. For *C. album*, *P. annua*, *M. recutita*, *G. aparine*, *R. crispus* and *S. media* analyses to fit common parameter estimates are presented in Supplementary table 2.4. Parameter c was fixed at 0 in Equation 2.2.

		Parameters Estimates (±SE)			
Weed species	**Herbicides**	**b**	**d (%)**	**ED_{50}**	**ED_{90}**
	2,4-D	-0.34 (0.10)		0.01 (0.01)	9.43 (15.7)
	glyphosate		89.1 (3.31)		
C. album	glu-amm	-1.05 (0.19)		0.14 (0.02)	1.11 (0.5)
	2,4-D + glyphosate	-1.04 (0.28)		0.03 (0.007)	0.26 (0.17)
	2,4-D + glu-amm	-0.43 (0.08)		0.06 (0.03)	11.2 (10.5)
	2,4-D	-1.12 (0.20)	90.8 (3.66)	0.07 (0.01)	0.51 (0.22)
	glyphosate	-3.40 (0.75)	99.9 (2.07)	0.01 (0.001)	0.02 (0.003)
S. vulgaris	glu-amm	-1.24 (0.19)	108.2 (4.37)	0.26 (0.03)	1.54 (0.51)
	2,4-D + glyphosate	-1.78 (0.32)	102.6 (2.32)	0.02 (0.001)	0.05 (0.01)
	2,4-D + glu-amm	-1.17 (0.17)	106.4 (3.87)	0.12 (0.02)	0.80 (0.26)
	glyphosate			1.53 (0.19)	4.68 (1.14)
	glu-amm				
P. annua	2,4-D + glyphosate	-1.97 (0.30)	120 (5.98)	0.84	2.56 (0.60)
	2,4-D + glu-amm				
M. recutita			136.5 (11)	0.16 (0.05)	1.48 (1.05)
G. aparine	glyphosate	-0.98 (0.21)	144.7 (15)	0.81 (0.28)	7.66 (5.34)
R. crispus			65 (16.5)	0.32 (0.39)	3.02 (4.75)
S. media			100 (9.7)	0.03 (0.01)	0.29 (0.21)

glu-amm = glufosinate-ammonium

Table 2.7. Parameter estimates of dose-response curves after (%) reduction of growth data were fitted against the doses relative to recommended rate of glyphosate (1x = 540 g ha^{-1}). Increase in the residual sum of squares from the common parameter model was not statistically significant from the separate line model for the *C. album* (P=0.62), *S. vulagris* (P=0.08) and *P. annua* (P=0.07). Dose-response curves are presented in Supplementary figure 2.4.

<table>
<tr><th></th><th></th><th colspan="4">Parameters Estimates (±SE)</th></tr>
<tr><th>Weed species</th><th>Herbicides</th><th>b</th><th>d (%)</th><th>ED_{50}</th><th>ED_{90}</th></tr>
<tr><td rowspan="2">C. album</td><td>glyphosate</td><td rowspan="2">-1.29 (0.27)</td><td rowspan="2">86.7 (3.17)</td><td>0.13 (0.02)</td><td>0.71 (0.28)</td></tr>
<tr><td>2,4-D + glyphosate</td><td>0.04 (0.01)</td><td>0.21 (0.1)</td></tr>
<tr><td rowspan="2">S. vulgaris</td><td>glyphosate</td><td rowspan="2">-2.27 (0.34)</td><td rowspan="2">101 (1.48)</td><td>0.01 (0.001)</td><td>0.03 (0.004)</td></tr>
<tr><td>2,4-D + glyphosate</td><td>0.02 (0.002)</td><td>0.05 (0.01)</td></tr>
<tr><td rowspan="2">P. annua</td><td>glyphosate</td><td rowspan="2">-1.77 (0.37)</td><td rowspan="2">120 (8.03)</td><td rowspan="2">1.15 (0.17)</td><td rowspan="2">4 (1.40)</td></tr>
<tr><td>2,4-D + glyphosate</td></tr>
</table>

2.5 Discussion

Results presented in this paper demonstrate the efficacy of weed control using herbicide droplets which are applied leaf specifically in glasshouse conditions. All herbicide droplets contained 1% concentration of the adjuvant AS 500 SL. Although other adjuvants could have been used, this was not an objective of this study and the AS 500 SL proved to be effective. A different adjuvant was used for the trials which took place in the USA (Verimax Ams Dry) in order to simulate glufosinate-ammonium spraying practices. It has been well documented that absorption and translocation of glyphosate is influenced by herbicide concentration and droplet size ((Liu *et al.*, 1996; Dill *et al.*, 2010). Although a range of concentrations (2.5% to 20%) and droplet volumes (0.7 μl to 3 μl) were used, the absorption and translocation were not studied as this was not an objective of this study.

The log-logistic model, which is normally used to describe effects of herbicide spray applications, fitted the biomass data reported in this study, so the hypothesis of the model's adequacy for droplet treatments was accepted for most datasets. Although, in the case of modelling the effect of glufosinate-ammonium droplet application to *P.*

annua the test for lack of fit was significant however, according to Ritz *et al.* (2015b) and Streibig and Green (2017) when the relative slope of the curve (parameter b) exceeds 8 is an indication that the data may not fit the dose-response model (parameter b here is 1.82 (±0.61)). With regards to the glyphosate dose-response trials the values of the slope observed (1 to 3.5) were similar with those of Streibig & Green (2017) who in their studies with the dose-response model, are finding that the relative slope of biomass data for conventionally-sprayed glyphosate ranged between 2 and 4.

For 50% control of *S. media*, 1/16 of the recommended dose of the Roundup® Biactive GL formulation was needed. When the same formulation of the herbicide was used for dose-response studies in the glasshouse, the most susceptible species when it was compared to *G. aparine* (Madsen & Jensen, 1995) and *C. album* (Madsen & Streibig, 2000) was *S. media*, with similar ED_{50} values observed for all three weed species. The perennial weed *R. crispus* was the least susceptible among the species tested, requiring a dose almost 10-times the glyphosate recommended rate. Findings from a glasshouse study reported that a dose of 7200 g of glyphosate ha^{-1} was having an effect before 100% mortality of *R. crispus* seedlings (Boutin *et al.*, 2004). From all the above it becomes evident the difference in the susceptibility of the weeds to herbicide droplet application between species thus accepting the second hypothesis in this paper.

The hypothesis that when the recommended doses of herbicides for conventional spraying are applied as droplet to a single leaf would provide at least 90% efficacy was accepted for *C. album*, *S. vulgaris*, *S. media*, *M. recutita*, *U. urens* (glufosinate-ammonium only) and *A. cruentus* (glyphosate only). The doses to achieve a 90% level of weed control for *P. annua* exceeded (4.7-times) the label recommendations for spraying glyphosate and glufosinate-ammonium and therefore, rejecting the hypothesis. The recommended rates of both glyphosate and glufosinate-ammonium achieved the same level of weed control (90%) when applied to the same size *C. album* seedlings. The ED_{50} values were $1/6^{th}$ of the 540 g ha^{-1} and 450 g ha^{-1} recommended rates for glyphosate and glufosinate-ammonium, respectively (90 g glyphosate ha^{-1} and 75 g glufosinate-ammonium ha^{-1}) suggesting that the weed is more susceptible to glufosinate-ammonium than glyphosate at the 4 to 6-leaf stage. These results agree with those of Tharp *et al*. (1999) who, in their glasshouse dose-response studies with *C. album* reported that the weed was more sensitive to glufosinate than glyphosate when sprayed at the 4 to 6-leaf stage ($ED_{50\ Glufosinate}$ = 69 g ha^{-1} and $ED_{50\ Glyphosate}$ = 120 g ha^{-1}). However, for larger weeds (8 to 10-leaf stage) the same ED_{50} values were observed (200 g ha^{-1}). Furthermore, Tharp *et al*. 1999 observed no significant differences among the ED_{50} values when doses of glyphosate were sprayed to three different growth stages of *C. album*. The same is reported here when glyphosate is applied as droplets to two different growth stages of the weed. Regardless of the size of *C. album*, 1.5-times (810 g ha^{-1}) the minimum label recommendation of glyphosate was required to control the weed. In previous studies recommended rates of 840 g ha^{-1} have been proven to control the weed in the field and in greenhouse trials with the ED_{50} values of biotypes sensitive to glyphosate ranging from 100 to 430 g ha^{-1} (the equivalent of 90 g ha^{-1} is reported here) (Lich *et al.*, 1997; Westhoven *et al*., 2008; Schafer *et al*., 2012; DeGreeff *et al*., 2018).

In greenhouse dose-response studies *P. annua* is controlled with 780 g of glyphosate ha^{-1} (Binkholder *et al.*, 2011) whereas half of the recommended dose of glufosinate-ammonium is needed for 50% control (Riemens *et al.*, 2008). Droplet application was carried out at the growing point of the weed which can explain the reduced efficacy reported here as a result from the limited translocation of the herbicides. To test that hypothesis the trial needs to be repeated alongside with a dose-response trial where the same doses of the herbicides are applied as an overall spray.

Also, similar to the efficacy when spraying the herbicides with that of droplet application reported here, hormetic effects were observed when sublethal doses of glyphosate were applied. Although the increase in the growth of some weeds when low doses were applied can be an experimental variability. However, the equivalent doses of glyphosate which appeared to be causing hormetic effect range from 2 to 17 g ha^{-1}, literature suggests that low doses of glyphosate ranging from 1.8 to 36 g ha^{-1} can stimulate the growth of plant and weed species (Velini *et al.*, 2008; Brito *et al.*, 2018). Especially in the case of *C. album* (2 to 4 leaves) where doses of glyphosate in the range of 16 - 32 g ha^{-1} appeared to be causing hormetic effects (Nadeem *et al.*, 2017). When droplets of glyphosate were applied to the same growth stage of the weed an increase in the dry weight of the weed was observed for doses up to 17 g ha^{-1}. However, no such effects were observed for bigger growth stages of *C. album*.

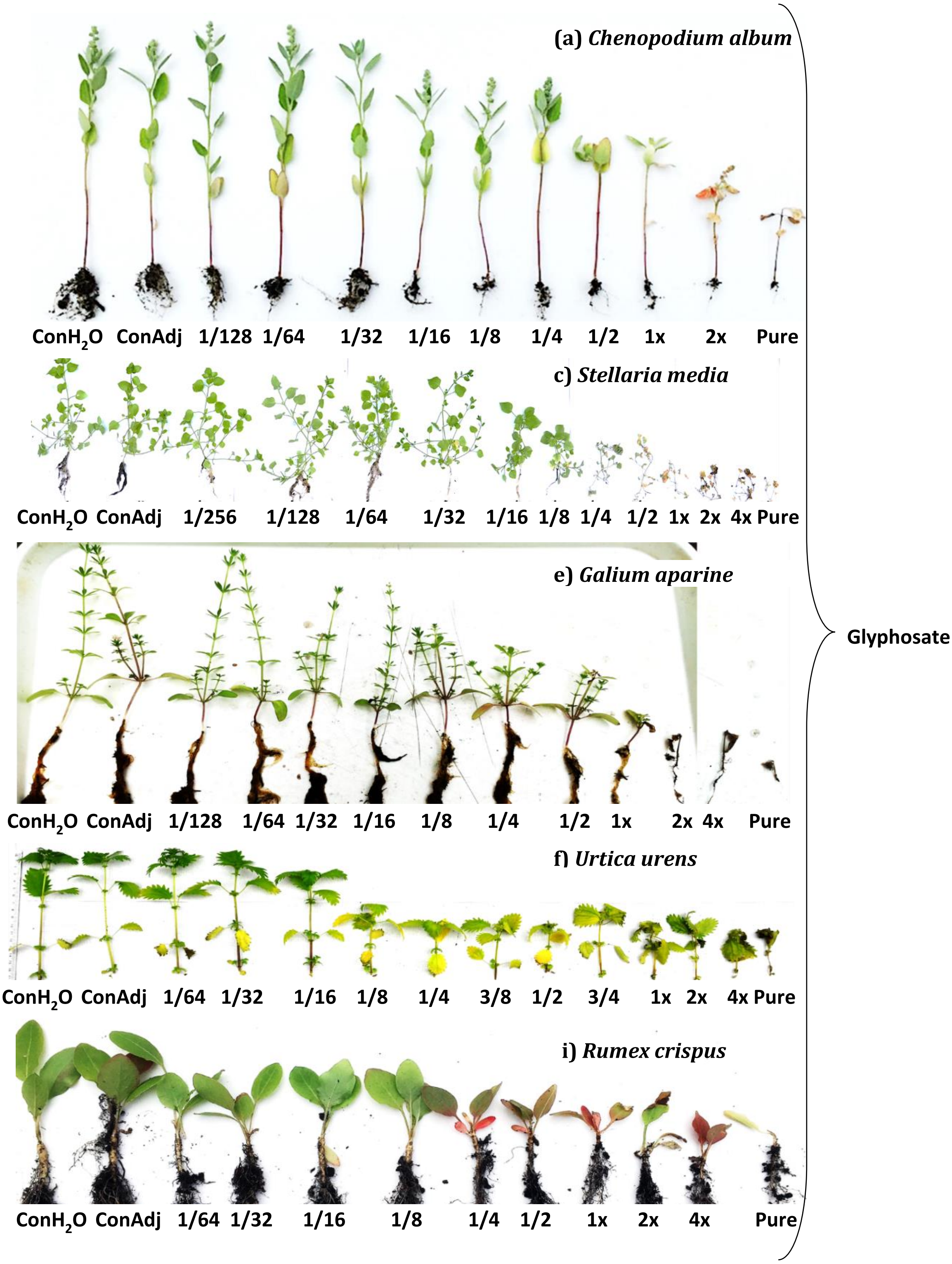
The hypothesis of glyphosate being more potent than glufosinate-ammonium was accepted for groundsel and the red amaranth. Groundsel was controlled with ED_{90} being 1/50th (0.24 μg of glyphosate) of the recommended whereas for glufosinate-ammonium a higher dose was required (1.5x the recommended). Also, the 1/32 relative dose of glyphosate, controlled *A. cruentus* whereas the same dose for glufosinate-ammonium reduced the growth of the seedlings by 50%. Although no work has been reported with relation to dose-response spray applications of glyphosate for *A. cruentus*, efficacy achieved here is similar to what was reported with wild types of *Amaranthus tuberculatus* species which have never been exposed to glyphosate (ED_{50}=24 g ha^{-1}) (Zelaya & Owen, 2005). However, when droplets of the two herbicides were applied to *U. urens* seedlings the weed was more susceptible to glufosinate-ammonium than to glyphosate and therefore, rejecting the hypothesis mentioned earlier. Droplets containing 2.6 μg of glufosinate-ammonium were needed for weed control, a dose which was 1/10th of the recommended. For glyphosate, although it was imprecisely estimated, approximately 3-times the label recommendation was needed for 90% control. This matches the recommendation of Roundup® Biactive GL which suggests doses from 1080 to 1800 g ha^{-1} for the control of nettle weeds. However, this hypothesis needs to be tested for the same growth stage weed seedlings where dose-response trials of the two herbicides will be carried out simultaneously.

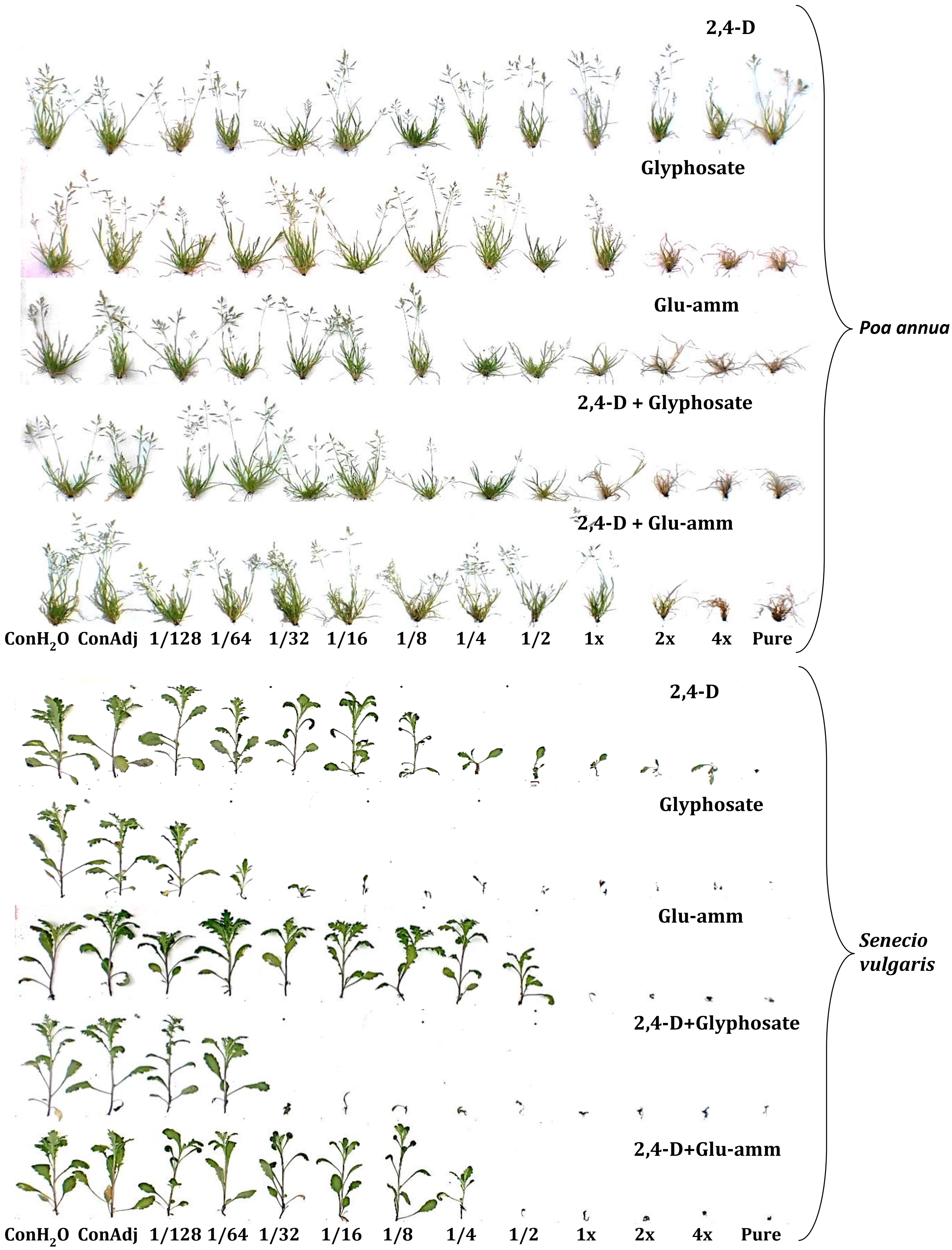
The specific objective of this study to model the effects of 2,4-D mixtures with glyphosate and glufosinate-ammonium, when doses are applied as single droplets, was fulfilled for *C. album*, *S. vulgaris* and *P. annua*. When glyphosate and the proprietary mix of 2,4-D and glyphosate were tested, it was proven that for *C. album* the mixture was 3.3-times more potent than glyphosate. However, for *S. vulgaris* glyphosate was approximately two times (1/0.53) potent than the mixture, whereas for *P. annua* there were no significant differences between the two. Antagonism has been reported when glyphosate is mixed with synthetic auxin herbicides like 2,4-D (Flint & Barrett, 1989) and dicamba (Meyer *et al.*, 2015) when applied to *Sorghum halepense* and *Echinochloa crus-galli* weeds, respectively. However, this antagonism, especially in the case of glyphosate and 2,4-D is dependent upon dose rates (O'Donovan & O'Sullivan, 1982). Loss in herbicidal activity was observed when lower rates of 2,4-D (280 g ha^{-1}) were mixed with glyphosate (560 g ha^{-1}) however, no antagonism was observed for higher doses (Flint & Barrett, 1989). Similar effects were observed here especially at low doses (1/64) whereas there were no differences when doses of 1/8th of the recommended and higher were used for the control of *S. vulgaris*. It is also worth mentioning that antagonism is only observed when the herbicides are mixed and not when commercially available pre-packed mixtures are used (Flint & Barrett, 1989). Although there is limited research testing the efficacy of auxin herbicides when mixed with glufosinate, an increased control of *C. album* is reported when 2,4-D is mixed with glufosinate (Chahal & Johnson, 2012; Merchant *et al.*, 2013). However no such claim can be made here cause of the impresicely estimates for both of the effective doses when the combined treatment of the two herbicides was applied to *C. album*. Also higher levels of grass weeds control are reported when 2,4-D is sprayed with glufosinate compared to glufosinate alone in field with soybean (Craigmyle *et al.*, 2013). Similar to this finding, control of *P. annua* was enhanced with the application of the combined treatment of 2,4-D and glufosinate-ammonium.

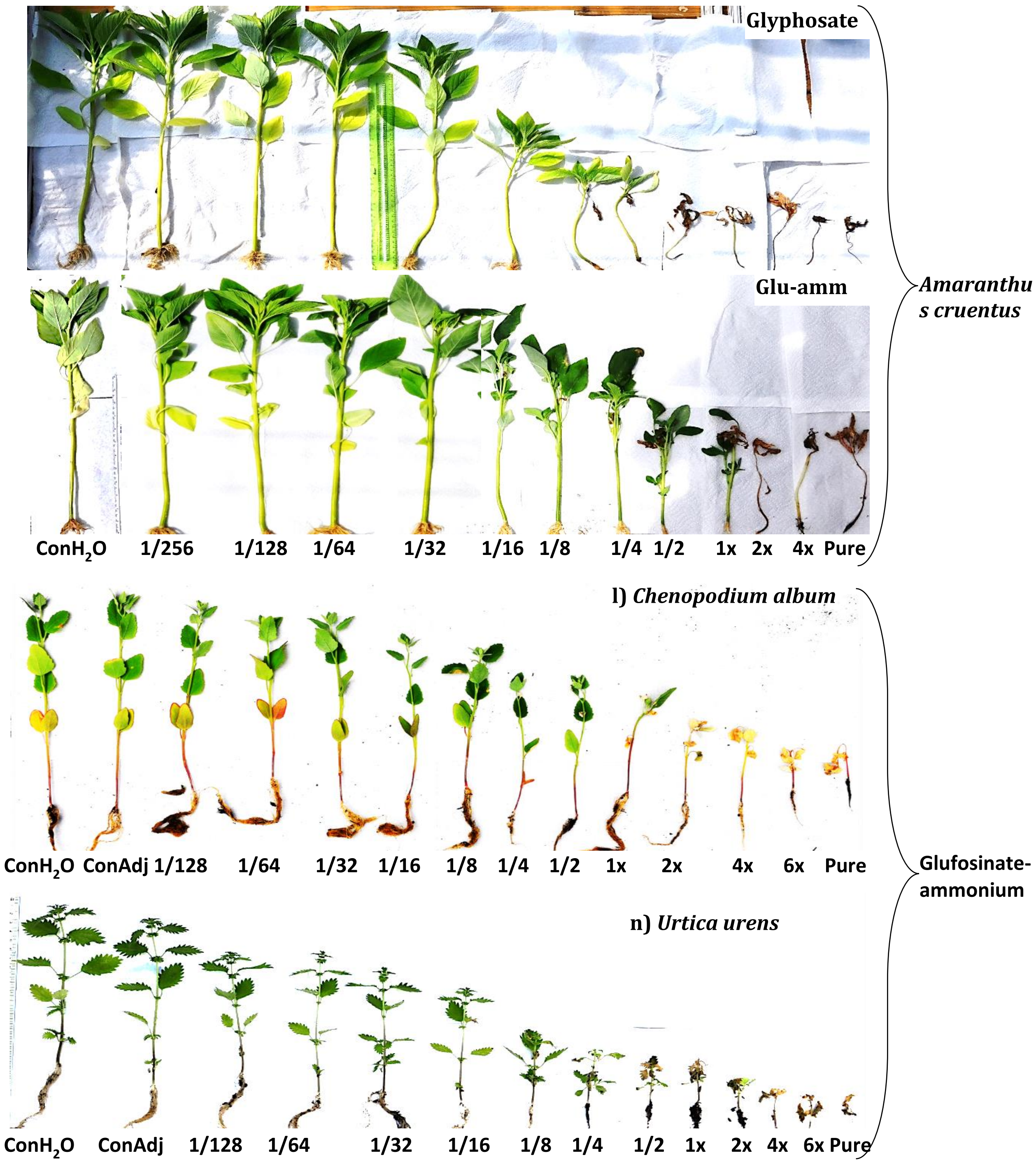
With regards to the amount of active ingredient required to control individual seedlings using droplets of glyphosate, the same ED_{50} values were observed with Mathiassen *et al*. (2016), who reported that 3.7 μg of glyphosate per seedling reduced the fresh weight of *C. album* seedlings (2-leaf stage) by 50% (3.5 μg of glyphosate achieved here). Although no dose-response trial was carried out, Utstumo *et al.* 2018 demonstrated that *C. album* and *P. annua* were controlled in glasshouse conditions with doses per plant of 7.56 μg of glyphosate. However, this is a level of uncertainty because when the trial was replicated the same doses did not control the weeds.

In conclusion it became evident that weeds can be controlled leaf-specifically by droplet application to one leaf providing that non-selective and broad-spectrum herbicides like glyphosate and glufosinate-ammonium were used. The efficacy of weed control reported here is, if not better, at least as good as what is expected if the herbicides were applied using a conventional spray. Moreover, a major improvement in application technology is that the amount of herbicide applied to each plant is known whereas, when spraying, only the average per unit land area is known. Also, the detrimental effect of accidentally misplacing glyphosate droplets on leaves of a cabbage crop is equally evident. Applying herbicides as a single droplet per weed has the potential of herbicide savings when compared to broadcast spraying over an entire field. When Søgaard *et al.* 2006 tested an automatic micro-spray system in field conditions they achieved 82% control of *Brassica napus* with averagely 22.6 µg of glyphosate per plant at the 2-leaf stage. Assuming a weed density of 100 seedlings per m^2 the system is applying 96% less glyphosate than the label recommendation of 540 g ha^{-1}. Herbicide savings from 73% to 95% were achieved when droplets of glyphosate were applied using a robotic weeder in a field with carrot crop (Utstumo *et al.*, 2018). The ED_{90} values observed here for weeds at the two to four-leaf stage ranged from 0.24 to 32 µg of glyphosate. Assuming the 100 weeds density if they were applied from robotic applicator, they would achieve herbicide reductions ranging from 94% to 99%.

2.6 Supplementary material




2,4-D
Glyphosate
Glu-amm
2,4-D + Glyphosate
2,4-D + Glu-amm
ConH$_2$O ConAdj 1/128 1/64 1/32 1/16 1/8 1/4 1/2 1x 2x 4x Pure
Poa annua
2,4-D
Glyphosate
Glu-amm
2,4-D+Glyphosate
2,4-D+Glu-amm
ConH$_2$O ConAdj 1/128 1/64 1/32 1/16 1/8 1/4 1/2 1x 2x 4x Pure
Senecio vulgaris



Supplementary figure 2.1. Seedlings of weed species tested in this study, three weeks after applying droplets containing different concentrations of 2,4-D, glyphosate, glufosinate-ammonium, 2,4-D plus glyphosate and 2,4-D plus glufosinate-ammonium. For the trials 1, 3, 5, 6 and 9 droplets of glyphosate were applied to *C. album*, *S. media*, *G. aparine*, *U. urens* and *R. crispus* seedlings, respectively. Trials 7, 8 and 11 were carried out to *P. annua*, *S. vulgaris* and *A. cruentus* seedlings. For the trials 12 and 13 droplets containing glufosinate-ammonium were applied to *C. album* and *U. urens*, respectively. Pictures of seedlings from the trials 4 and 10 are not available.

Supplementary table 2.1. Mean values of biomass data 20 days after application of droplets containing 1% of the adjuvant AS 500 SL and droplets containing only distilled water for all the weed species tested. When glufosinate-ammonium was applied to *A.cruentus* seedlings, the adjuvant Verimax Ams Dry was used whereas no adjuvant was used when glyphosate was applied to the same seedlings.

Weed Species	Adjuvant (1%)	Water	LSD	*P*-value
	Glyphosate			
Chenopodium album	0.17	0.15	0.02	0.09
Rumex crispus	0.23	0.20	0.04	0.11
Galium aparine	0.04	0.03	0.01	0.36
Matricaria recutita	0.06	0.06	0.02	0.76
Stellaria media	0.17	0.22	0.07	0.17
Urtica urens	0.19	0.18	0.03	0.24
Senecio vulgaris	0.22	0.22	0.02	0.63
Poa annua	0.28	0.29	0.02	0.47
Brassica oleracea	0.68	0.63	0.01	0.31
	Glufosinate-ammonium			
Amaranthus cruentus	4.16	4.10	0.57	0.81

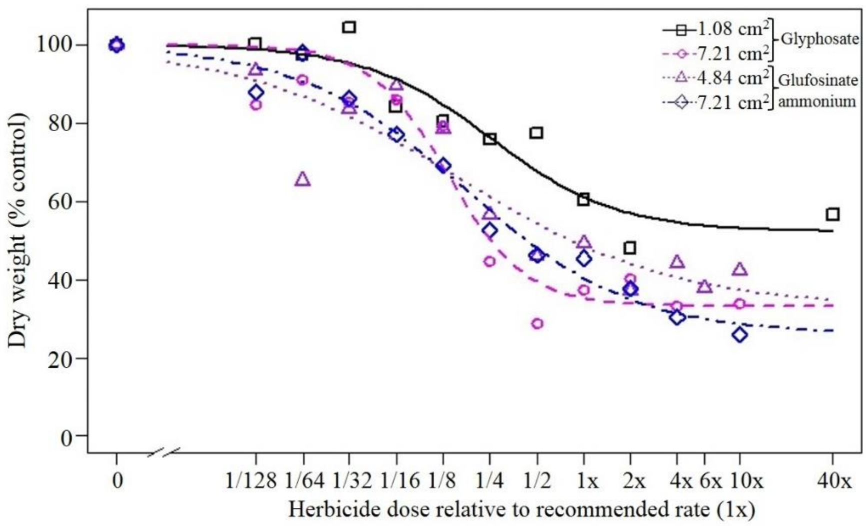
Supplementary table 2.2. The lack-of-fit test after using the modelFit() function in R which compares the residual sum of squares from the non-linear regression analysis (DRM model) with the residual some of squares of the one-way ANOVA. Dose-response curves are presented in Figure 2.3.

	Sum of Squares			
Dose response curves	**ANOVA**	**DRC model**	**df**	***P*-value**
	Glyphosate			
(a) *Chenopodium album*	0.04	0.04	7	0.12
(b) *Chenopodium album*	0.42	0.46	8	0.17
(c) *Stellaria media*	1.09	1.13	9	0.81
(d) *Matricaria recutita*	0.10	0.10	8	0.39
(e) *Galium aparine*	0.02	0.02	7	0.65
(f) *Urtica urens*	0.18	0.19	9	0.14
(g) *Poa annua*	0.53	0.56	8	0.57
(h) *Senecio vulgaris*	0.11	0.11	8	0.88
(i) *Rumex crispus*	1.07	1.11	7	0.25
(j) *Brassica oleracea*	9.66	9.93	9	0.32
(k) *Amaranthus cruentus*	24.6	27.9	9	0.39

Supplementary table 2.2 continued overleaf

Supplementary table 2.2 continued

Dose response curves	Sum of Squares ANOVA	DRC model	df	*P*-value
	Glufosinate-ammonium			
(l) *Chenopodium album*	0.25	0.26	9	0.42
(m) *Chenopodium album*	0.53	0.54	8	0.90
(n) *Urtica urens*	0.08	0.08	9	0.59
(o) *Senecio vulgaris*	0.23	0.25	8	0.09
(p) *Poa annua*	0.42	0.47	8	**0.04**
(q) *Amaranthus cruentus*	19.2	24.4	9	0.07
	2,4-D + Glyphosate			
(r) *Chenopodium album*	0.35	0.38	8	0.07
(s) *Senecio vulgaris*	0.28	0.30	8	0.15
(t) *Poa annua*	0.43	0.46	8	0.48
	2,4-D + Glufosinate-ammonium			
(u) *Chenopodium album*	0.56	0.60	8	0.31
(v) *Senecio vulgaris*	0.29	0.32	8	0.08
(w) *Poa annua*	0.39	0.41	8	0.49
	2,4-D			
(x) *Chenopodium album*	0.44	0.45	8	0.94
(y) *Senecio vulgaris*	0.19	0.21	8	0.08
(z) *Poa annua*	0.63	0.67	8	0.27



Supplementary figure 2.2. Dose-response curves of the *C. album* separate lines model presented in Figure 2.4.

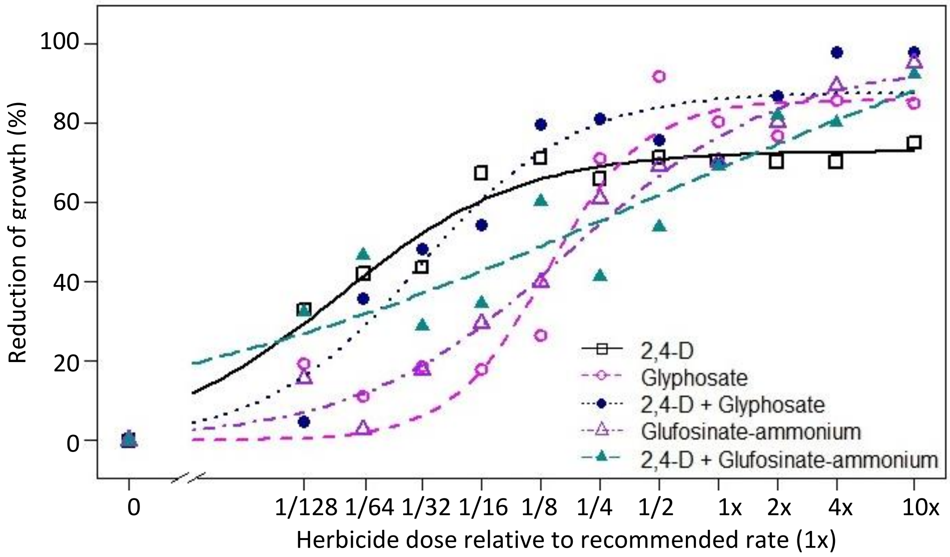
Supplementary table 2.3. Results from testing for similarity of parameters between the separate line model and common line or the fitted model for the dose-response curves presented in Figure 2.4.

Species	Models	Sum of Squares	d.f.	*P*-value
C. album	common line	1283570		
	separate lines	796744	9	<0.001
	common slope & ED_{50} (fitted)	805813		
	separate lines	796744	8	0.26
U. urens	common ED_{50}	171352		
	separate lines	154513	1	<0.001
	common line	251391		
	separate line	154513	2	<0.001
A. cruentus	common ED_{50}	33701		
	separate line	30797	1	
	common lower limit	31610		
	separate line	30797	1	0.04
	common line	40943		
	separate line	30797	3	<0.001

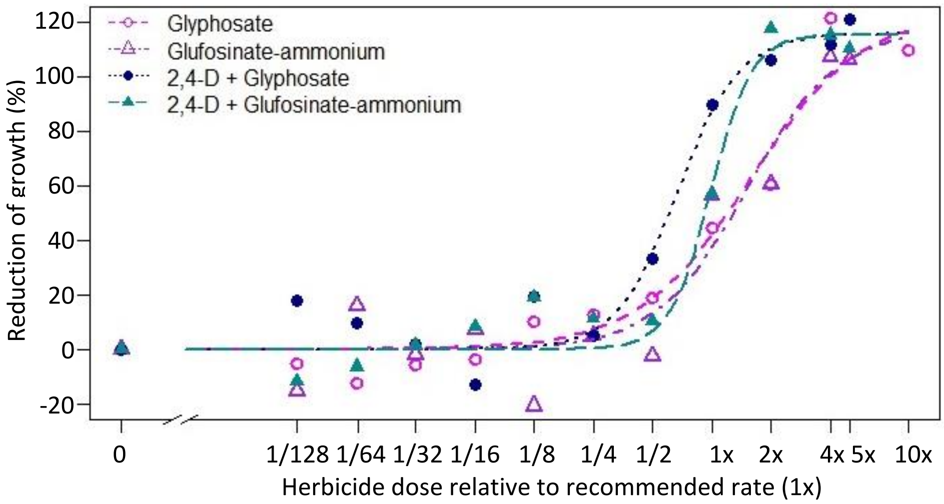
Supplementary table 2.4. Results from testing for similarity of parameters between the separate line model and common line or the fitted model for the dose-response curves presented in Figure 2.5.

Species	Models	Sum of Squares	d.f.	*P*-value
C. album	fitted	547147		
	separate line	539409	6	0.09
	common line	599094		
	separate line	539409	7	<0.001
P. annua	fitted	698840		
	separate line	688502	8	0.33
	common line	722669		
	separate line	688502	9	<0.001
S. vulgaris	common ED_{50}	309559		
	separate line	246446	4	<0.001
	common upper limit	250181		
	separate line	246446	4	0.03
	common slope	252582		
	separate line	246446	4	0.001
M. recutita	common slope (fitted)	2558416		
G. aparine	separate line	2538739	3	0.13
R. crispus	common ED_{50}	2610887		
S. media	separate line	2538739	3	<0.001

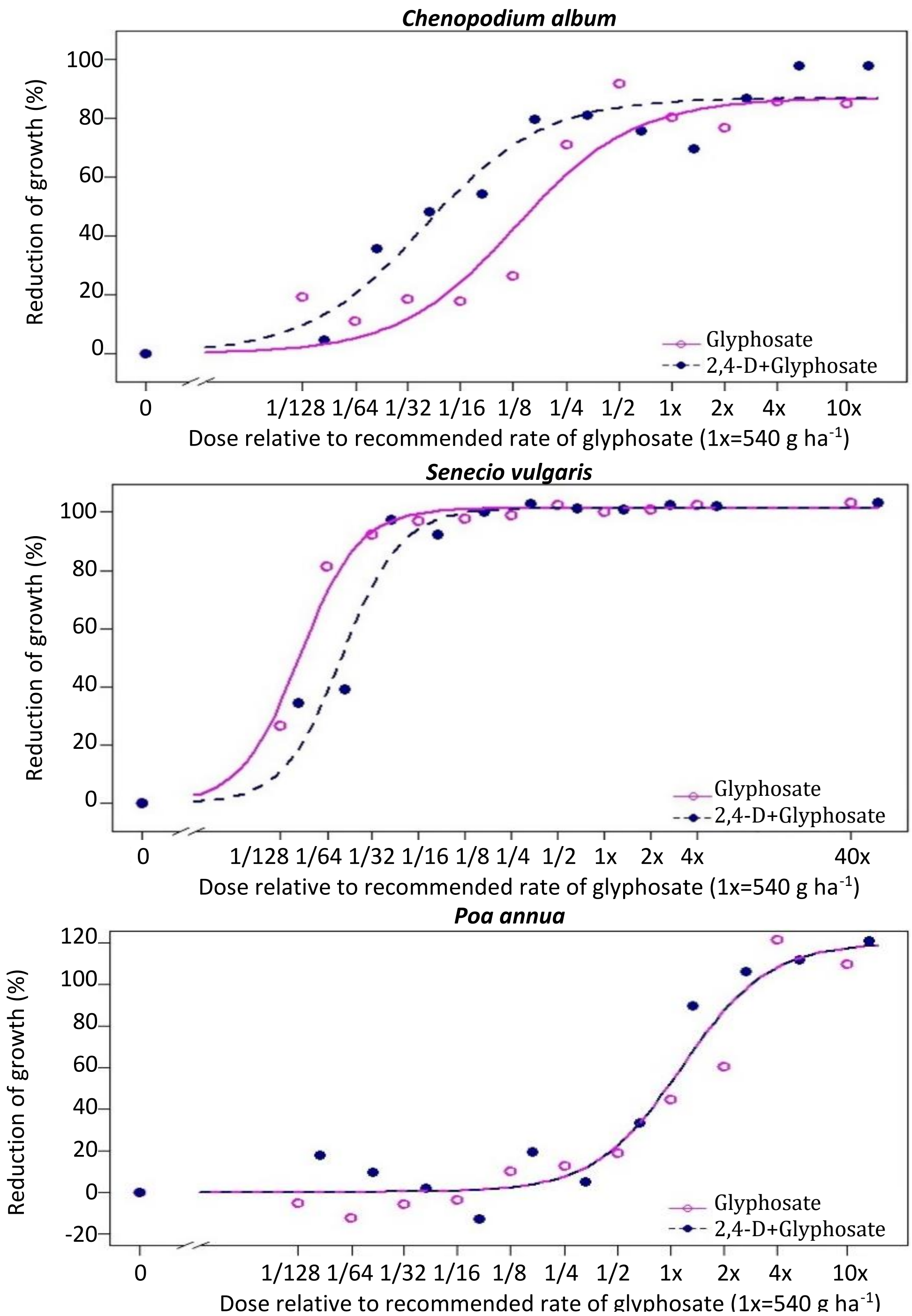
Chenopodium album



Poa annua



Supplementary figure 2.3. Dose-response curves of the *C. album* and *P. annua* separate lines model presented in Figure 2.5.



Supplementary figure 2.4. Dose-response curves of the reduction of growth (%) data when plotted against the doses relative to the recommended rate of glyphosate (540 g ha^{-1}) for the *C. album*, *S. vulagris* and *P. annua* trials. Dose-response models of *C. album* and *S. vulagris* have common slopes and upper limits and were not significantly different from the separate lines model (P=0.62 and P=0.08 respectively). A common line was fitted to plot the glyphosate and 2,4-D + glyphosate dose-response curves of *P. annua* which was not significantly different from the separate lines model (P=0.07). Lower limit of the curves was fixed at 0 (three-parameter model).

Chapter 3
Leaf-specific weed control in cabbage and leek fields

3.1 Abstract

A major challenge for vegetable growers in the UK is achieving effective weed control while complying with regulations regarding pesticide use. This difficulty has been exacerbated by losses of approval for herbicide actives and the increasing difficulty of gaining approvals for new compounds. To address these problems, the concept of leaf-specific weed control was tested in field experiments. Droplets of glyphosate and glufosinate-ammonium were applied manually to leaves of the natural weed infestation in transplanted cabbages (2016 & 2017) and leeks (2017 & 2018). Droplet applications of glyphosate were made on three occasions in the growing season of cabbages and controlled 92% of the weeds. There was a consistent lower trend of lower cabbage yields which was not significant than the weed-free controls (2016: 50.5 t ha^{-1} & 2017: 93.5 t ha^{-1}) while herbicide inputs were at least 91% lower compared to conventional pre and post-emergence spraying. In leeks, weekly applications of glyphosate and glufosinate-ammonium droplets were needed to control weeds without a significant yield penalty. Reductions in herbicide use in leeks ranged from 71% to 82%. Economic analysis demonstrated that droplet applications could increase profits by over £11,000 and £1,500 ha^{-1} per year for leeks and cabbages, respectively.

3.2 Introduction

Herbicide-based weed control for most vegetable growers in the UK is of the essence, with herbicides accounting for 40% of the total amount of pesticides applied while less fungicides and insecticides being used (31% and 24% respectively) (Garthwaite *et al.*, 2017). Weed control, apart from being important to maintain vegetable yield and quality, is becoming increasingly challenging because of UK and EU pesticide reviews and also because pesticide manufacturers are hesitant to seek to register herbicides for a market which is relatively small (Hillocks, 2012). Additionally, vegetable growers rely in a limited and old range of herbicides (first released in 60's and 70's) which require a lot of funding and effort in order to keep them in the market (Fennimore *et al.*, 2014). Legislation like the Regulation EC no. 1107/2009, the EU Water Framework Directive and the Sustainable Use of Pesticides (SUP) Directive along with UK's National Action Plan for SUP have resulted in actual losses of approval for some herbicide actives and have decreased the likelihood of new compounds gaining approval (Baker & Knight, 2017). In order to compensate for the lack of available herbicides, there has been an increase in physical and mechanical weed control methods which are often more expensive than spraying herbicides (Garthwaite *et al.*, 2017). These pressures act as a driver towards a paradigm shift in approaches to weed control which will balance the need to meet demand for sustainable vegetable production while maintaining and increasing productivity.

Although some support banning pesticides completely, that ignores the fact that food security is linked to the availability of adequate supplies of good quality food at affordable prices. Ignoring price, it is well-established that organically grown crops consistently yield less than conventionally-grown ones (Seufert *et al.*, 2012; Ponisio *et al.*, 2015). While the use of genetically-modified, herbicide-tolerant crops (GM-HT) is a paradigm shift in weed control, it would still require spraying herbicides on a whole-field basis, using broadcast application methods. Furthermore, no great differences were found in environmental impacts in the UK farm-scale between GM-HT and conventional crops (Hawes *et al.*, 2003). Because it is unlikely that weed control problems will be solved with new herbicides registrations or with the use of GM-HT crops, automated technologies which use precision weed management systems is the way forward (Fennimore *et al.*, 2014). The approach of leaf-specific weed control, in which herbicide is not applied to the crop and not directly to the soil, holds great promise in reducing herbicide inputs while ticking all the policy boxes mentioned here. This study explores an engineering solution to the problem of weed control in field vegetables as an alternative to selective chemistry or biotechnology by using droplets of broad-spectrum herbicides which are applied leaf-specifically.

Detecting single weed leaves and treating them using micro-rates of foliar-applied, translocated herbicides is the ultimate in precision weed management. A study on four arable crops measured losses of pesticides up to 99% (sugar beet) to the soil surface when they are applied using broadcast spraying methods (Jensen & Spliid, 2003). Assuming that a weed seedling covers a soil surface of 1 cm^2 with numbers ranging between 100 and 400 weeds m^{-2}, this corresponds to 1%-4% weed ground cover. To put it more simply, if a foliar-acting herbicide is applied by broadcast spraying, 96% will be lost in the soil surface or the crop, having potential environmental impact. Keeping this figure in mind, identifying and treating single weed plants has the potential of huge reductions in herbicide use (Zijlstra *et al.*, 2011). Blackmore (2014) predicted that with the use of a microdot system which sprays chemicals only on the leaf-area of the weed, should result in 99% reduction in herbicide inputs. The robotic weeder being developed by University of Reading and Concurrent Solutions llc. in the USA, uses an alternative method of herbicide application from spot spraying or microdot technology. Actively targeted droplets of broad-spectrum herbicides are applied to individual weed leaves and dosage rates are controlled to ensure the most effective control based on the weed's growth stage.

Lee *et al*. (1999) developed one of the first systems for real-time, intra-row weed control in tomato fields. Although the objective of the system was that no herbicides were applied to the crop but only to the weeds, it only recognized 76% of the tomato plants correctly and sprayed only 48% of the weeds. Lamm *et al*. (2002) improved on that precision system and although it sprayed correctly 89% of the weeds, it also treated with the blue dye mixture, 21% of the cotton plants. Giles *et al*. (2004) tested the biological performance of a micro-dosing system which was based on the ones developed by Lee *et al*. (1999) and Lamm *et al*. (2002). Although high levels of weed control were achieved, the equivalent application rate of the mixture (glyphosate & adjuvant) emitted from the micro-dosing system was relatively high

(4700 L ha^{-1} or 46 µL cm^{-2}). The micro-dosing system developed by Søgaard and Lund (2007) was able to deliver doses of 2.5 µl (containing 1 µg of glyphosate) as a spray to cells of 25 mm^2 by firing four shots, which was sufficient when treating individual weed seedlings. When it was tested in field conditions with *Brassica napus* L. (oilseed rape) as a test weed, the plant surface area played a major role in the system's performance. Targetting was acceptable when leaf area was greater than 100 mm^2 (86% of the weeds were treated). The average dose applied per plant was 22.6 µg of glyphosate (Søgaard *et al*., 2006). Assuming a weed density of 100 plants m^{-2}, weed control can be achieved with as little as 22.6 g ha^{-1} which corresponds to 96% reduction of the label recommendation for glyphosate (540 g ha^{-1}). Nieuwenhuizen *et al*. (2010) described a microsprayer system for volunteer potato control in sugar beet fields. The system emitted single droplets (20 ± 5 µl) using 5% glyphosate solution (Roundup Max, 450 g L^{-1}) and achieved 83% volunteer potato control while spraying approximately 1% of the sugar beet plants. A real-time micro-sprayer, which used an inkjet printer head as a spray system was developed by Midtiby *et al*. (2011) and was tested under indoor conditions. Droplets of 0.2 µl which contained 1 µg of glyphosate were used to control two weed species at speeds of 0.5 m s^{-1}. Although the system provided a 94% control of oilseed rape which had the largest leaf area, against the smaller *Tripleurospermum inodorum* L. (scentless mayweed) it only managed to control 37% of the weed. More recently the Australian Centre for Field Robotics at the University of Sydney developed an autonomous robotic platform (Ladybird) for real-time detection and precision spraying of fertilizers and herbicides for vegetable crops (Underwood *et al.*, 2015). A smaller version of the Ladybird robot has also been developed for Intelligent Perception and Precision Application (RIPPA) which, like its predecessor, is used for precision application of agrochemicals (Bogue, 2016). Lee *et al.* (2014) proposed an algorithm to improve the efficacy of spray applications of the Ladybird and RIPPA robots. Although some research has focused on using targeted applications of herbicides, little is known about the exact dose rates needed to control individual weed seedlings (Young & Giles, 2013). Likewise, little is known about the effect on crops when they are exposed to micro-doses of herbicides because of drift. Giles *et al.* (2004) observed phytotoxic effects to tomato plants which resulted in reduced crop yield by glyphosate "micro-drift" from a micro-dosing system.

Closer to the approach adopted here, Christensen *et al*. (2009) mentioned the use of Drop-on-Demand (DoD) technologies which apply low volume rates (approximately 1 μL) of glyphosate as a single droplet to weed leaves and have the potential of achieving herbicide savings higher than 95% when compared with broadcast application methods. The kind of DoD applicators that have been developed, utilize glyphosate and their efficacy has been studied in conjunction with surfactants and adjuvants (Basi *et al*., 2013; Urdal *et al*., 2014; Mathiassen *et al*., 2016). The robotic DoD system described by Utstumo *et al.* (2018) demostrated intra-row weed control in carrots using droplets of glyphosate (2.1 μl). However, the robotic applicator was treating with droplets all plants (weeds and carrots), no other herbicides than glyphosate were applied (either as droplets or overall spray) and no other vegetable crops were tested. Also, no yield data were recorded and it is envisaged that the robotic system would be used in conjunction with mechanical inter-row weed control.

In order to validate the concept of weed control by leaf-specific herbicide application, field trials were carried out, using manual droplet application of glyphosate and glufosinate-ammonium to the natural weed infestation in fields with cabbages and leeks. Efficacy of weed control and crop yield were estimated for herbicide droplet applications and were compared with weed-free, weedy and current herbicide spray applications. The hypotheses tested in this study were:

i. Efficacy of weed control by leaf-specific droplet application and crop yield are not significantly different than hand-weeding or current spraying methods of herbicides.
ii. Herbicide inputs will be significantly lower when droplet applications of herbicides are compared with spray applications of commercial herbicides.
iii. Multiple treatments will be required so that late-emerging seedlings are controlled and any failure of weed control is addressed during the critical weed-free period of the crop (Nieto *et al*., 1968).
iv. Fewer droplet applications will be needed in cabbages than in leeks. Leeks are known to be weak competitors against weeds (Baumann *et al.*, 2001) however, for cabbages, there is not a critical period for weed control and only a single weed control treatment is needed (Roberts *et al.* 1976; Weaver, 1984). A further hypothesis is, however, that more than one application of droplet treatments will be needed for cabbages.

3.3 Materials and Methods

3.3.1 Field experiments

Four field experiments were carried out at two fields in Sonning Farm, near Reading, two with cabbages (*Brassica oleracea* var. *capitata*) during summer 2016 and 2017 and two with leeks (*Allium porrum* L.) during summer 2017 and 2018 (Figure 3.1 (A)) (Table 3.1). The soil of the site for the 2016 trial was sand with 91.5% sand, 5.7% clay, 2.9% silt and 0.9% stone content and soil of the site for the 2017 and 2018 trial was loamy sand with 87.1% sand, 6.4% clay, 5.3% silt and 12.9% stone content. For the 2016 trial, savoy cabbage seeds, were provided from Elsoms Seeds (Elsoms Seeds Ltd, Lincolnshire, UK) and were sown under glasshouse conditions on Seed & Modular compost (Clover Peat, Dungannon, N. Ireland). Six weeks after sowing, cabbage seedlings were transplanted at the 3 to 4 leaf stage. For the 2017 trial white cabbage and leek seedlings were provided from the Westhorpe nursery (Westhorpe Plants Ltd, Boston, UK) and were transplanted in the field at the 3 to 4 leaf stage. For the 2018 trial leek seedlings were purchased from Farringtons (Farringtons Ltd, Preston, UK) and were transplanted at the 2 to 3 leaf stage.

For all trials, fertilizer was applied on the day of transplanting using sulfur (SO_3) and nitrogen (N) at the rates of 50 kg ha^{-1} and 100 kg ha^{-1} respectively. Plants were watered individually twice per day for 30 min, using an automated drip irrigation system. Weed control by manual droplet application took place in a central area of plots (treated area) between the two middle crop rows (Figure 3.1 (B)). An area between the plots remained unplanted which was 80 cm long for the leeks and 60 cm for the cabbages.

3.3.2 Herbicide treatments

The experimental design was a randomized complete block for all trials. Four replications of eight treatments were applied for 2016 and ten treatments for the 2017 trial with cabbages (Table 3.2). For the leeks nine and ten treatments were applied for the 2017 and 2018 trials, respectively, which were replicated three times for both years (Table 3.3). For all of the trials, control treatments consisted of weedy and weed-free plots which remained untreated and hand-weeded respectively. The commercial products Roundup® Biactive GL (360 g a.i. L^{-1}, SL, Monsanto (UK) Ltd.) and Harvest® (150 g a.i. L^{-1}, SL, Bayer CropScience Ltd) were used for the glyphosate (gly) and glufosinate-ammonium (glu-amm) applications respectively. For the inter-row spray of glyphosate for the 2016 trial, a spray shield (38 cm) was used to ensure that no herbicide was applied to the crop. For the pre-emergence spray pendimethalin (Stomp Aqua®, 455 g a.i. L^{-1}, CS, BASF plc) was applied whereas, for the post-emergence applications metazachlor (Sultan® 50 SC, 500 g a.i. L^{-1}, SC, Adama Agricultural Solutions UK Ltd) was used for the cabbages and bromoxynil (Buctril®, 225 g a.i. L^{-1}, EC, Bayer CropScience Ltd) for the leeks (Table 3.2, Table 3.3). Herbicide spray applications were carried out using an electric knapsack sprayer (CP 15 Electric, Cooper-Pegler, Villefranche-sur-Saone, France), equipped with a deflector nozzle (green colour 372021, Cooper-Pegler, Villefranche-sur-Saone, France) which was calibrated to deliver 1.310 L min^{-1} using a spray volume of 200 L ha^{-1}.

All droplets had a volume of 2 μl and were applied manually, using a pipette with a volume range from 0.1 to 2.5 μl (ErgoOne® Single-Channel, Starlab Ltd, Milton Keynes, UK) (Figure 3.1 (B)). When a single herbicide droplet per weed was applied, this involved the use of 36 μg of glyphosate and 60 μg of glufosinate-ammonium. The latter was applied for the 2017 trial only (Table 3.2, Table 3.3). Weeds growing in an area larger than 1 cm from the edge of the crop were treated. This was done to reduce the risk of accidental crop damage and also because a commercial system would have a 1 cm no-treated zone to avoid a direct hit on the crop.

Multiple droplet application was carried out according to the size of the weed or the size of individual leaves (Drop gly or glu-amm (adj)). For the 2016 trial droplets containing 9 μg of glyphosate were applied to a single leaf if the leaf area of the seedling was less than 1 cm^2 and if higher two droplets were applied to different leaves (Drop x3 gly (adj)) (Table 3.2). However, for 2017 and 2018 trials, 9 μg of glyphosate were applied to every visible leaf with area bigger than 1 cm^2. When dose of glufosinate-ammonium was adjusted, 7.5 μg were applied to leaves with area bigger than 1 cm^2 (Table 3.2, Table 3.3).

For the application of 36 μg and 9 μg of glyphosate 5% and 1.25% solutions of Roundup® Biactive GL were prepared, respectively. When droplets containing 60 μg and 7.5 μg of glufosinate-ammonium were applied the solutions of Harvest® used were 20% and 2.5% respectively. All droplet treatments contained 1% of the adjuvant AS 500 SL. Doses applied are based on ED_{50} and ED_{90} values which were needed to reduce the fresh weights of the *C. album* seedlings in dose-response studies (Chapter 2, Table 2.3). Numbers of droplets applied per plot was counted allowing the equivalent application rate per hectare to be calculated. For the 2017 trial with cabbages the last droplet application was carried out at six weeks after planting instead of seven. This is because of the white cabbage canopy closure was not allowing enough space for weed control.

3.3.3 Crop and weed measurements

Crops and weeds were harvested from the area of the plots treated with droplets. After cutting and weighing the above ground biomass, cabbages and leeks were trimmed and weighed as for commercial sale and dry biomass of the weeds was estimated after oven-drying fresh seedlings for 48h at 80 °C. Cabbages were harvested once when the majority had reached maturity (first cabbage head splitting) and ten to twelve outer leaves were removed in order to be left with a trimmed head. Marketable yield of savoy and white cabbage is based on number of trimmed heads weighing more than 500 g and 1000 g respectively (A. Blair harvesting manager TH Clements, pers. comm., 5 March 2018) although some supermarkets sell savoy cabbages weighing 400 g per head (e.g. Aldi, UK). For the savoy cabbages the cut-off weight was set at 300 g per head, because of heads were heavily trimmed.

Leeks were trimmed at 34 cm and the diameter of the stalk was measured at 10 cm from the root of the crop using digital callipers. Leek trimmed yield was classified into three marketable categories according to the diameter of the stalk (<25 mm, 25-35 mm and >35 mm). Leeks measuring less than 25 mm are likely to be sold for processing, whereas for the 25-35 mm and more than 35 mm categories are referred to as class 1 product yields and they are sold as pre-packed and loose produce respectively (T. Casey chairman of the Leek Growers Association, pers comm., 22 August 2018).

Figure 3.1. (A) Cabbage and leek experiments at Sonning Farm in 2017. The pipe in the foreground fed the irrigation system. (B) Manual application of droplets containing glufosinate-ammonium with a pipette in field with cabbages, at five weeks after planting the crop, for the 2017 trial. Blue hoops were placed so that the area could be cover with nets for bird protection.

Table 3.1. Field information and activities for the trials with cabbages (2016 & 2017) and leeks (2017 & 2018). See Tables 3.2 and 3.3 for herbicide treatments.

Activity	**2016**	**2017**	**2017**	**2018**
Field geo-reference	51°28'55"N, 0°53'51"W		51°28'24"N, 0°54'07"W	
Crop	Cabbage	Cabbage	Leek	Leek
Previous crop	Grass	Wheat	Wheat	Cabbage
Crop variety	Savoy, Famosa F1	White, Surprise F1	Krypton F1	Duraton F1
Crop planted	3 June	27 April	27 April	19 April
Crop harvested	3 October	24 July	21 August	09 August
Plants harvested per plot (number)	8	6	8	10
Plants planted per plot (number)	28		32	
Inter-row spacing (cm)	50		40	
Intra-row spacing (cm)	30		20	
Plot size (m^2)	5.25		3.2	
Plot arrangement	4 single rows			
Harvest/Droplet treated area (m^2)	1.2	0.9	0.64	0.80
Treatments applied (number)	8	10	9	10
Blocks (number)	4		3	
Insecticide	10 August: 1 kg ha^{-1} DiPel® DF	13 June: 1 kg ha^{-1} DiPel® DF	13 June: 1 kg ha^{-1} DiPel® DF	8 & 20 June: 0.8 L ha^{1} Conserve®
Fungicide	N.A.	7 July: 1 L ha^{-1} Amistar®	N.A.	N.A.
Weed species (at harvest)	SENVU, CHEAL, SPRAR, MATRE, and CAPBP	SENVU, MATRE, CHEAL, POAAN, POLPE, CAPBP, TRZAX, ACHMI, GERMO, POLAR, TAROF and TRFDU.		SENVU, MATRE, CHEAL, POAAN, CAPBP, SOLNI, TAROF and ACHMI.

SENVU: *Senecio vulgaris*, CHEAL: *Chenopodium album*, SPRAR: *Spergula arvensis*, MATRE: *Matricaria recutita*, POAAN: *Poa annua*, CAPBP: *Capsella bursa-pastoris*, POLPE: *Polygonum persicaria*, SOLNI: *Solanum nigrum*, TRZAX: *Triticum aestivum*, ACHMI: *Achillea millefolium*, GERMO: *Geranium molle*, TAROF: *Taraxacum officinale*, POLAR: *Polygonum arenastrum*, TRFDU: *Trifolium dubium*

Table 3.2. Treatments applied with their respective doses and timing of application for the 2016 and 2017 trials with cabbages. All droplet treatments contained 1% of the adjuvant AS 500 SL.

Treatments	Dose	Weeks After Planting
	2016	
Drop x1 gly	36 μg of glyphosate/seedling	3
Drop x3 gly	36 μg of glyphosate/seedling	3, 5 and 7
Drop x3 gly (adj)	9 or 18 μg of glyphosate/seedling	3, 5 and 7
Inter-row spray	1.5 L ha^{-1} Roundup® Biactive GL	3
Inter-row spray + Drop x1 gly	1.5 L ha^{-1} Roundup® Biactive GL + 36 μg of glyphosate/seedling	3 (Inter-row spray) and 5 (Droplet x1 gly)
Pre-emergence	2.9 L ha^{-1} Stomp Aqua®	One week before planting
	2017	
Drop x1 gly	36 μg of glyphosate/seedling	3
Drop x2 gly	36 μg of glyphosate/seedling	3 and 5
Drop x3 gly	36 μg of glyphosate/seedling	3, 5 and 6
Drop x3 gly (adj)	9 μg of glyphosate/leaf	3, 5 and 6
Drop x3 glu-amm	60 μg of glufosinate -ammonium/seedling	3, 5 and 6
Drop x3 glu-amm (adj)	7.5 μg of glufosinate-ammonium/leaf	3, 5 and 6
Post-emergence	1.5 L ha^{-1} Sultan® 50 SC	4
Pre-emergence	2.9 L ha^{-1}, Stomp Aqua®	Five days before planting

Table 3.3. Treatments applied with their respective doses and timing of application for the 2016 and 2017 trials with leeks. All droplet treatments contained 1% of the adjuvant AS 500 SL.

Treatments	Dose	Weeks After Planting
	2017	
Drop x5 gly	36 µg of glyphosate/seedling	2, 6, 8, 10 and 12
Drop x10 gly	36 µg of glyphosate/seedling	2, 4, 5, 6, 7, 8, 9, 10, 11 and 12
Drop x10 gly (adj)	9 µg of glyphosate/leaf	2, 4, 5, 6, 7, 8, 9, 10, 11 and 12
Drop x10 glu-amm	60 µg of glufosinate - ammonium/seedling	2, 4, 5, 6, 7, 8, 9, 10, 11 and 12
Drop x10 glu-amm (adj)	7.5 µg of glufosinate-ammonium/leaf	2, 4, 5, 6, 7, 8, 9, 10, 11 and 12
Post-emergence	1.5 L ha^{-1}, Buctril®	4 and 7
Pre-emergence	2.9 L ha^{-1}, Stomp Aqua®	Five days before planting
	2018	
Drop x5 gly	36 µg of glyphosate/seedling	3, 5, 7, 9 and 11
Drop x5 gly (adj)	9 µg of glyphosate/leaf	3, 5, 7, 9 and 11
Drop x10 gly	36 µg of glyphosate/seedling	3, 4, 5, 6, 7, 8, 9, 10, 11 and 12
Drop x10 gly (adj)	9 µg of glyphosate/leaf	3, 4, 5, 6, 7, 8, 9, 10, 11 and 12
Drop x10 glu-amm (adj)	7.5 µg of glufosinate-ammonium/leaf	3, 4, 5, 6, 7, 8, 9, 10, 11 and 12
Post-emergence	1.5 L ha^{-1}, Buctril®	4
Pre-emergence	2.9 L ha^{-1}, Stomp Aqua®	Two days before planting
Pre + Post-emergence	2.9 L ha^{-1} Stomp Aqua® + 1.5 L ha^{-1} Buctril®	Two days before planting (Pre) + 4 (Post-emergence)

3.3.4 Economic analysis

Preliminary economic analysis was carried out in order to determine the profit or loss likely to be associated with use of an automated platform when compared with pre and post-emergence sprays or with hand-weeding. The yield and the economic value of the weed-free plots is assumed to be the same with commercial hand-weeding cabbages and leeks. The gross margins of droplet treatments (GM_d), conventional spraying (GM_s) and hand-weeding (GM_h) were calculated as follows:

$GM_d = V_d - H_d - M_d$

$GM_s = V_s - H_s - A_s$ and

$GM_h = V_h - L_h$

where V is the crop value (£ ha^{-1}), H is the herbicide cost (£ ha^{-1}), M_d is the machine cost (£ ha^{-1}), A_s is the contractor charge (£ ha^{-1}) and L_h is the labour charge (£ ha^{-1}). The net benefit is given by the difference in the gross margins. The economic assumptions about the value of the crop and the cost for hand-weeding are presented in Table 3.4. A single hand-weeding is assumed for cabbages as the crop does not have a critical period for weed control and a single weed control treatment is needed to avoid yield loss (Weaver, 1984). Three hand-weeding treatments were assumed for leeks (after planting, in the middle of the critical period and close to harvest) because of the crop's long critical period of weed control (1 to 12 weeks after planting) and its low competitive ability against weeds (Melander & Rasmussen, 2001; Tursun *et al.*, 2007). Assumptions about the material cost of herbicides are presented in Table 3.5. Other costings related to growing and harvesting the crops were not taken into account as they would be similar in both cases of weed control (automated platform or conventional spraying) although, higher yielding crop would cost more to harvest. Further assumptions regarding the automated platform were:

i. The platform would treat an area of 4 ha per day (8 h) and would retreat the same area every 14 days for both cabbages and leeks or every week for leeks only,
ii. To allow for adverse weather and machine downtime, various costs have been assessed, assuming that the platform could operate 1, 3.5, 5 and 7 days per week, which means it could treat 4, 14, 20 and 28 ha per week, respectively
iii. Based on the actual treatments applied in the field experiment and in the case of leeks, the known critical period for weed control (as described above), crops

would need to be treated:

- ➢ three times at 14-day intervals for cabbages
- ➢ five times at 14-day intervals for the leeks in the case of fortnightly treatment or ten times in the case of weekly application,

iv. The cost of the platform including a maintenance contract is assumed to be not less than £25,000 and not more than £100,000. It is further assumed that this cost is depreciated over a period of five years giving an annual cost of £5,000, 10,000, 15,000 and 20,000 per year assuming platform costs of £25,000, £50,000, £75,000 and £100,000, respectively. The platform costs per hectare per year are presented in Table 3.6.

Each platform could be used for a maximum of 140 ha of cabbages or for 56 or 28 ha when applying droplets fortnightly or weekly to leeks, respectively in the unlikely event that it was operating seven days per week. To examine how the variability in the platform's cost and the operating days affect the gross margin, a sensitivity analysis was carried out using assumptions (ii) and (iv) above.

Table 3.4. Economic assumptions for hand-weeding and crop value for cabbages (2016 & 2017) and leeks (2017 & 2018). Time for each hand-weeding derived from the actual time spent over the growing season (leeks: 75 sec m^{-2} and cabbages: 46 sec m^{-2}).

		Leeks			Cabbages	
Manual labour cost (£ h^{-1}) *		10.16			10.16	
No. of hand-weeding		3			1	
Time per hand-weeding (h ha^{-1})		208 h ha^{-1}			126 h ha^{-1}	
Total cost of hand-weeding (£ ha^{-1})		6,340			1,280	
	Stalk diameter	2017	2018		2016 (savoy)	2017 (white)
Crop value† (£ kg^{-1})	<25mm	0.50	0.60			
	=25-35mm	1.00	1.22	£ $head^{-1}$	0.42	—
	>35mm	0.82	1.00	£ kg^{-1}	0.84	0.34

*Minimum manual labour cost (Redman, 2017); † Leeks (T. Casey. pers comm., 22 August 2018), Value for cabbages is the average wholesale market prices for 2016 and 2017 (Brigham, 2017).

Table 3.5. Material costs of the chemicals (herbicides and adjuvant) used for the 2017 and 2018 trials with leeks. Costings were obtained from personal communication in November 2018. Spraying application cost was 12.50 £ ha^{-1} (Redman, 2017). The material cost for spraying glyphosate based on the label recommendation ranges from 8 to 32 £ ha^{-1} and for glufosinate-ammonium costs between 6 and 10 £ ha^{-1}. The material cost of spraying pendimethalin is £20.3 ha^{-1} and for bromoxynil is £28 ha^{-1}.

Chemicals	Cost (£ L^{-1})	Reference
Roundup® Biactive GL	5.30	R. Casebow (Manager of Crops Research Unit, Sonning Farm)
Harvest®	2.00	I. Ford (Business Development Manager, BASF Agricultural Solutions)
AS 500 SL (adjuvant)	2.59	Prof. Zenon Woznica (Poznan University of Life Sciences)
Stomp Aqua®	7.00	P. Liley (Crop Production Director, Hammond Produce Ltd.)
Buctril®	19.0	Gordon Anderson-Taylor (Dr.) (Development Manager, Bayer CropScience Ltd.)

Table 3.6. Annual platform costs (£ ha^{-1} $year^{-1}$) when operating for 1 to 7 days per week and covering areas from 4 to 28 ha per week. When it is used on a fortnightly basis the platform could cover areas ranging from 8, to 56 ha. Platform costs assume the total cost is spread over five years.

			Annual platform cost, £ ha^{-1} $year^{-1}$			
			Operating days/week			
Crop	**Treatments**	**Platform cost, £/year**	**1**	**3.5**	**5**	**7**
Cabbages	Droplet x3 (Fortnightly)	£5,000	£250	£71	£50	£36
		£10,000	£500	£143	£100	£71
		£15,000	£750	£214	£150	£107
		£20,000	£1,000	£286	£200	£143
Leeks	Droplet x10 (Weekly)	£5,000	£1,250	£357	£250	£179
		£10,000	£2,500	£714	£500	£357
		£15,000	£3,750	£1,071	£750	£536
		£20,000	£5,000	£1,429	£1,000	£714
	Droplet x5 (Fortnightly)	£5,000	£625	£179	£125	£89
		£10,000	£1,250	£357	£250	£179
		£15,000	£1,875	£536	£375	£268
		£20,000	£2,500	£714	£500	£357

3.3.5 Statistical analysis

Crop and weed biomass data, harvest index, economic value and amounts of herbicide applied were analysed with one-way analysis of variance as a randomized complete blocks design. All analyses were performed using Genstat 16th Edition (Payne *et al.*, 2002). Weed biomass data were expressed as per cent biomass reduction using Eqn 1:

$$(\%) \text{ Reduction of biomass} = (1 - T_i / T_0) \times 100 \quad (1)$$

where, T_i is the sum of the weeds' dry weights of each treatment and T_0 is the total weeds' dry weights of the weedy control treatment. Yield and biomass data of the crops were expressed as a per cent relative to the weed-free control. Harvest index (HI) is the ratio of the trimmed marketable yield divided by the untrimmed biomass.

3.4 Results

The efficacy of weed control in cabbages varied between weed control treatments in both 2016 and 2017 ($P<0.001$, Figure 3.2). When single droplets of glyphosate were applied per weed, on three occasions (Drop x3 gly) weed control efficacies were 92% for 2016 and 93% for 2017 (Figure 3.2). Furthermore, weeds were satisfactorily controlled when droplet application of glyphosate was adjusted according to the size of weed seedlings for the 2016 trial (89% control) and according to the area of individual leaves of the weeds for the 2017 trial (91% control) (Drop x3 gly (adj)). However, treating the crop on one occasion only (Drop x1 gly) gave the worst weed control in both years, but when droplets of glyphosate were applied on two occasions (Drop x2 gly) for the 2017 trial, weed control was much improved (72% control) (Figure 3.2). The pre-emergence spray treatment with pendimethalin was unsatisfactory in 2016 (62% control) whereas in 2017, it controlled 88% of the weeds. A single post-emergence spray of metazachlor in 2017 only controlled 68% of the weeds (Figure 3.2).

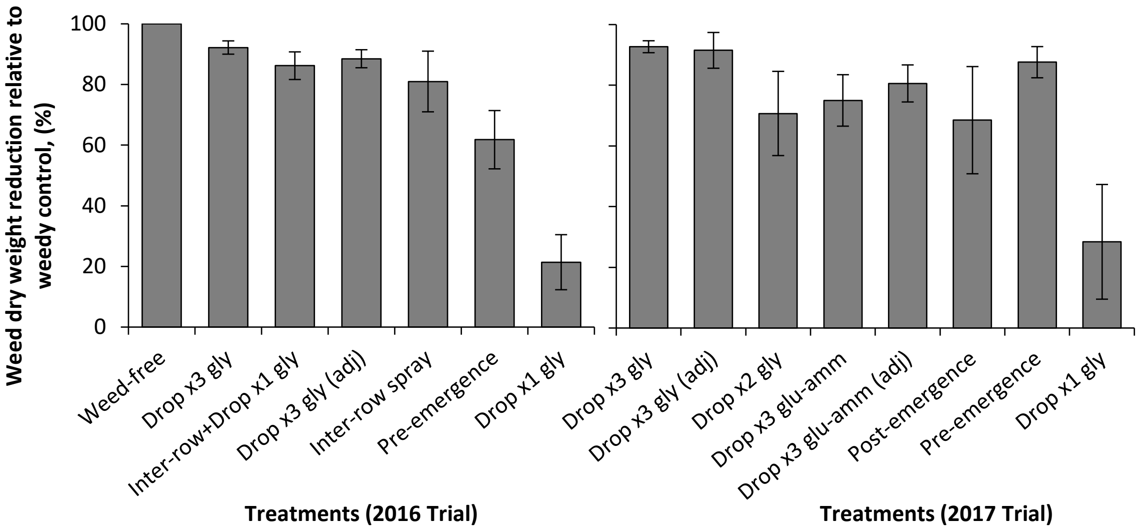


Figure 3.2. Efficacy of weed control expressed as percent dry weight reduction relative to weedy control (2016: 244 g/m^2 and 2017: 393 g/m^2) for the 2016 (SED: 11.6, d.f.: 21) and 2017 (SED: 15.9, d.f.: 27) trials. Adjusted treatment for 2016 received 9 or 18 µg if the leaf area of the weed was less or more than 1 cm^2 respectively. For the 2017 trial adjusted treatments received 9 µg of glyphosate (gly (adj)) or 7.5 µg of glufosinate-ammonium (glu-amm (adj)) to each leaf with an area bigger than 1 cm^2 (Table 3.2). Analysis of variance is shown in Supplementary table 3.1.

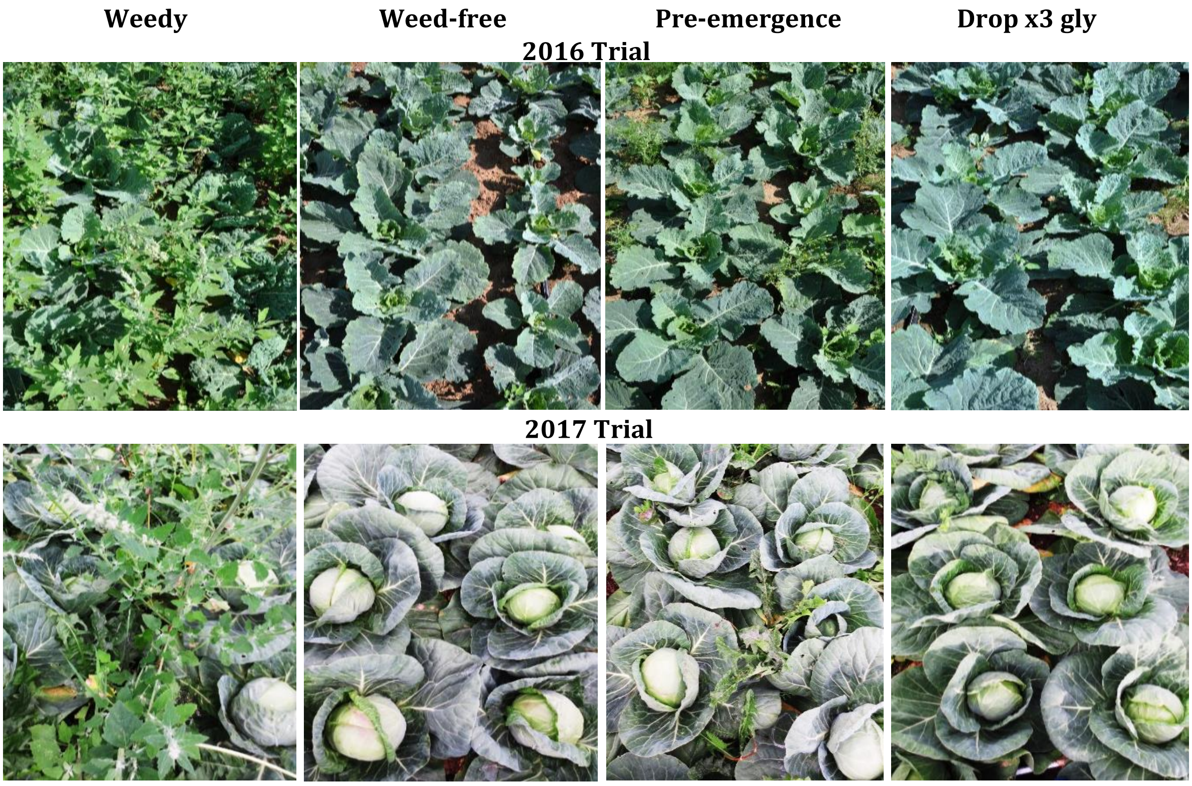


Figure 3.3. Pictures of the plots' area where cabbages and weeds were harvested, for the Weedy, Weed-free, Pre-emergence and Drop x3 gly treatments, at seven weeks after planting the savoy cabbages and nine weeks after planting the white cabbages for the 2016 and 2017 trials, respectively. The same area was treated with droplets for the Drop x3 gly treatment.

Significant differences were observed among the treatments applied at the 2016 trial for the trimmed (Figure 3.4) and marketable yield and harvest index of the savoy cabbages ($P \leq 0.01$) (Supplementary table 3.1). The trimmed and marketable yields from the single and multiple droplet treatments and the combined inter-row spray plus single droplet treatment (Drop x3 gly; Drop x3 gly (adj); Inter-row spray + Drop x1 gly) for the 2016 trial, were lower than the 50.5 t ha^{-1} achieved in the hand-weeded plots however, this trend was not significant (Figure 3.5). The trimmed yield of cabbages produced from the single droplet application of glyphosate (Drop x1 gly), for the 2016 trial was only half that of that of the weed-free control. Overall 36% of the harvested savoy heads were characterised as unmarketable (head weight < 300 g) whereas for the 2017 trial the overall rejection rate of unmarketable white cabbages was 11% (Supplementary figure 3.1). Marketable yields from the pre-emergence spray of pendimethalin and inter-row spray of glyphosate for the 2016 trial were not significantly different than the hand-weeded control (Figure 3.5). No significant differences occurred for trimmed yield (P=0.26), marketable yield (P=0.24) and harvest index (P=0.08) for the 2017 trial (Supplementary table 3.1). However, the lowest yield measurements and the highest rejection rate of unmarketable produce were observed when droplets of glyphosate were applied on one occasion (Figure 3.5) (Supplementary figure 3.1).

When dose was adjusted according to the size of the leaves for the 2017 trial more glufosinate-ammonium droplets were applied than glyphosate at 5 and 6 weeks after planting. Furthermore, 100 more droplets were applied at the starting point of droplet application for the 2016 single droplet treatments (Figure 3.6). The optimal glyphosate droplet treatments (Drop x3 gly and Drop x3 gly (adj)) which achieved high levels of weed control and no significant yield penalties, reduced the use of herbicide actives relative to pendimethalin spray by at least 91% and 96% for the 2016 and 2017 trials respectively (Table 3.7). When the dose of glufosinate-ammonium was adjusted for the 2017 trial it reduced herbicide use by 95% and 97% relative to the post-emergence spray of metazachlor and the pre-emergence spray respectively.

Figure 3.4. Trimmed savoy and white cabbage heads at harvest of the 2016 and 2017 trial, respectively for the treatments used each year. Head of weed-free for the 2016 trial is not a representative size of the heads harvested from this treatment.

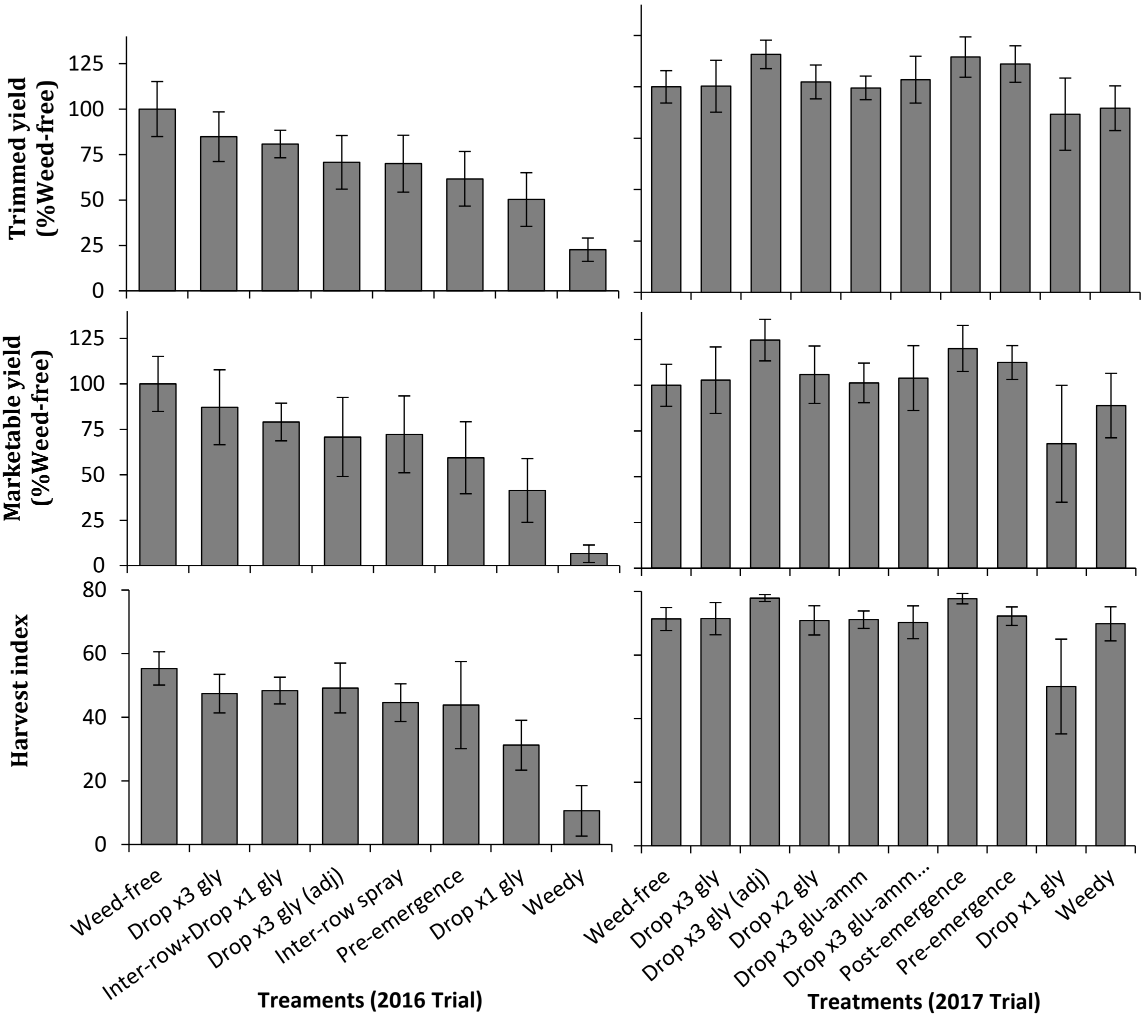


Figure 3.5. Yield of trimmed cabbages expressed as per cent relative to the yield of weed-free plots (mean ± SEM) for the 2016 (Weed-free: 50.5 t ha^{-1}, SED: 16.7, d.f.: 21 and 2017(Weed-free: 93.5 t ha^{-1}, SED: 11.8. d.f.: 27) trials. Marketable yield was determined on number of trimmed heads weighing more than 300 g and 1000 g for the 2016 (Weed-free: 50.5 t ha^{-1}, SED: 21.6, d.f.: 21) and 2017 (Weed-free: 88.5 t ha^{-1}, SED: 19.1, d.f.: 27) trials respectively. Rejection rate of unmarketable cabbage heads is presented in Supplementary figure 3.2. Harvest index is the ratio of the trimmed marketable yield divided by the untrimmed biomass (2016: SED: 10.5, d.f.: 21), 2017: SED: 7.7, d.f.: 27)).

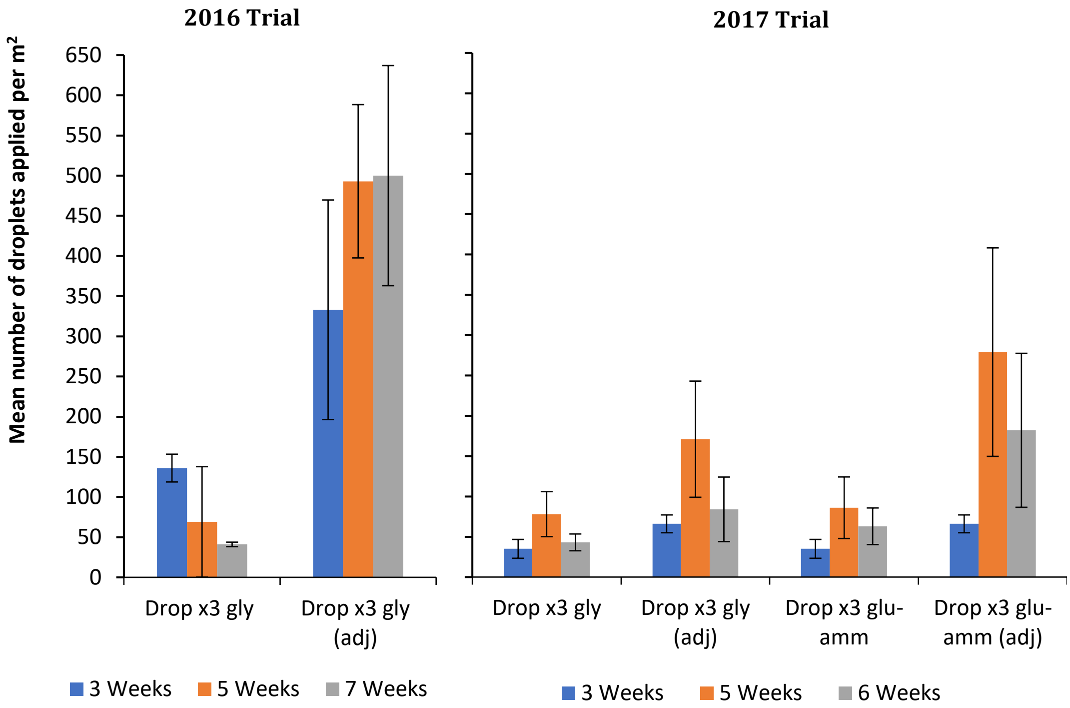


Figure 3.6. Mean number of droplets (±SEM) applied per m^2 for the 2016 and 2017 trials with cabbages. For the 2017 trial, the number of droplets for the single droplet application treatments at 3 weeks after planting is the mean number of the Drop x1 gly, Drop x2 gly, Drop x3 gly and the Drop x3 glu-amm. For the droplets applied at 5 weeks the number represents the mean from the Drop x2 gly and Drop x3 gly treatments. For the 2017 adjusted treatments the number of droplets applied at 3 weeks is the mean number of the Drop x3 gly (adj) and Drop x3 glu-amm (adj). For the single droplet treatment of the 2016 trial, the droplets applied at 3 weeks is the mean number of the Drop x1 gly and Drop x3 gly. Final droplet application was carried out at 7 and 6 weeks after planting for the 2016 and 2017 trials respectively.

Table 3.7. Average of total amounts (±SEM) of herbicide applied (g of a.i. ha^{-1}) for the droplet treatments and reductions relative to the pre-emergence and post-emergence spray treatments. Label recommendation for pre-emergence spray of pendimethalin is 1320 g ha^{-1}. For spraying glyphosate, the label recommendations range from 540 to 1800 g ha^{-1} and for glufosinate-ammonium range from 450 to 750 g ha^{-1} with a maximum of 1500 g ha^{-1} per year if two treatments are applied.

		% Reduction relative to	
Treatments	**Total amount of herbicide (g of a.i. ha^{-1})**	**Pre-emergence**	**Post-emergence**
	2016		**Inter-row spray (glyphosate, 540 g ha^{-1})**
Drop x1 gly	53.9 (7.4)	95.9 (0.6)	90.0 (1.4)
Drop x3 gly	83.3 (11.7)	93.7 (0.9)	84.6 (2.2)
Drop x3 gly (adj)	119.3 (29.3)	91.0 (2.2)	77.9 (5.4)
Inter-row spray + Drop x1 gly	562.1 (3.2)	57.4 (0.2)	- 4.1 (0.6)
SED (d.f.)	17.7 (9)	1.3 (9)	3.3 (9)
	2017		**Broadcast spray (metazachlor, 750 g ha^{-1})**
Drop x1 gly	16.4 (5.3)	98.8 (0.4)	97.8 (0.7)
Drop x2 gly	41.0 (13.4)	96.9 (1.1)	94.5 (1.8)
Drop x3 gly	55.2 (17.9)	95.8 (1.4)	92.6 (2.4)
Drop x3 gly (adj)	28.1 (9.9)	97.9 (0.7)	96.3 (1.3)
Drop x3 glu-amm	104.6 (42.3)	92.1 (3.2)	86.1 (5.6)
Drop x3 glu-amm (adj)	40.2 (17.6)	97.0 (1.3)	94.6 (2.4)
SED (d.f.)	24.2 (15)	1.8 (15)	3.2 (15)

Weed control of 97% and higher was achieved when glyphosate was applied as single droplet (9 µg) to every leaf with area bigger than 1 cm^2 (gly (adj)) and as a single droplet per weed, on a weekly basis (10 times over 12 weeks) in fields with leeks for the 2017 and 2018 trials (Figure 3.7). Same levels of weed control were achieved when the adjusted glyphosate treatment was applied fortnightly (5 times) in 2018 (Figure 3.8). When the single droplet per weed treatment was applied 5 times (2, 6, 8, 10 and 12 weeks after planting) it controlled 85% of weeds for the 2017 trial. When dose of glufosinate-ammonium (7.5 µg) was adjusted according to the size of individual leaves (glu-amm (adj)) it reduced the weed dry biomass by 91% and 99% for the 2017 and

2018 trials respectively (Figure 3.8). Efficacy of weed control from the spray applications of pre and post-emergence herbicides was significantly lower (P<0.001) than then hand-weeded controls for both years (Supplementary table 3.2).

Yields of trimmed leeks produced from the weekly glyphosate droplet-to-every-leaf treatment, were the highest among the plots treated with herbicides (either as droplets or overall spray). Although there was a consistent trend of lower yields compared to hand-weeded controls of the 2017 (42 t ha^{-1}) and 2018 (39 t ha^{-1}) trials, this was not significant. Similarly, the yield for the weekly application of the adjusted glufosinate-ammonium droplets (glu-amm (adj)) was 87% of the yield from the weed-free control for the 2018 trial (Figure 3.9). However, yield and efficacy of weed control achieved from the single droplet of glufosinate-ammonium per weed treatment were significantly lower than the weed-free controls and therefore it was not repeated in the 2018 trial (Figure 3.8, Figure 3.9). Yields achieved from all the other herbicide treatments applied as droplets or overall spray were significantly lower than the weed-free controls for both years (P<0.001, Supplementary table 3.2). Optimal treatments which achieved high levels of weed control (> 97% efficacy) and high yields produced more leeks with stalks measuring more than 25 mm (class 1 marketable yield). The mean harvest indices were 65% and 72% in 2017 and 2018, respectively and no significant differences occurred between treatments in either year (2017: P=0.9, 2018: P=0.7))

Droplet application was initiated at two and three weeks after planting the leeks for the 2017 and 2018 trials, respectively. Overall more herbicide droplets were applied for every droplet treatment for 2017 compared to the 2018 trial . Within the year, for the weekly droplet application of the adjusted treatments, more glufosinate-ammonium droplets were applied compared to glyphosate for both years. Droplet application of the weekly treatments for both herbicides started to reduce at seven weeks after planting during the 2018 trial (Figure 3.10). Regarding the amounts of herbicide applied, the weekly droplet treatment of glyphosate received the equivalent of 340 and 299 g of glyphosate ha^{-1} which was 74% and 77% lower than spraying the recommended rate of pendimethalin (1320 g ha^{-1}) for the 2017 and 2018 trials respectively and 82% lower than spraying both pre and post-emergence herbicides. The fortnightly application of glyphosate droplets, in 2018 reduced the herbicide inputs by 71% and 77% relative to pre-emergence and the pre+post-emergence sprays, respectively. Weekly application of glufosinate-ammonium droplets reduced herbicide a.i. amounts by 51% and 74% in 2017 and 2018 trials, respectively (Table 3.8). When glufosinate-ammonium was applied as a single droplet per weed for the 2017 trial the maximum label recommendation of 1500 g ha^{-1} was exceeded (2120 g ha^{-1}). With this exception, the amounts of a.i. applied per unit land area never exceeded label recommendations for conventional spraying.

Weedy **Weed-free** **Pre-emergence** **Drop x10 gly**

Figure 3.7. Pictures of the plots' area where weeds and leeks were harvested and weeds were treated with droplets, for the Weedy, Weed-free, Pre-emergence and Drop x10 gly treatments, at eleven weeks after planting the crop for the 2017 trial.

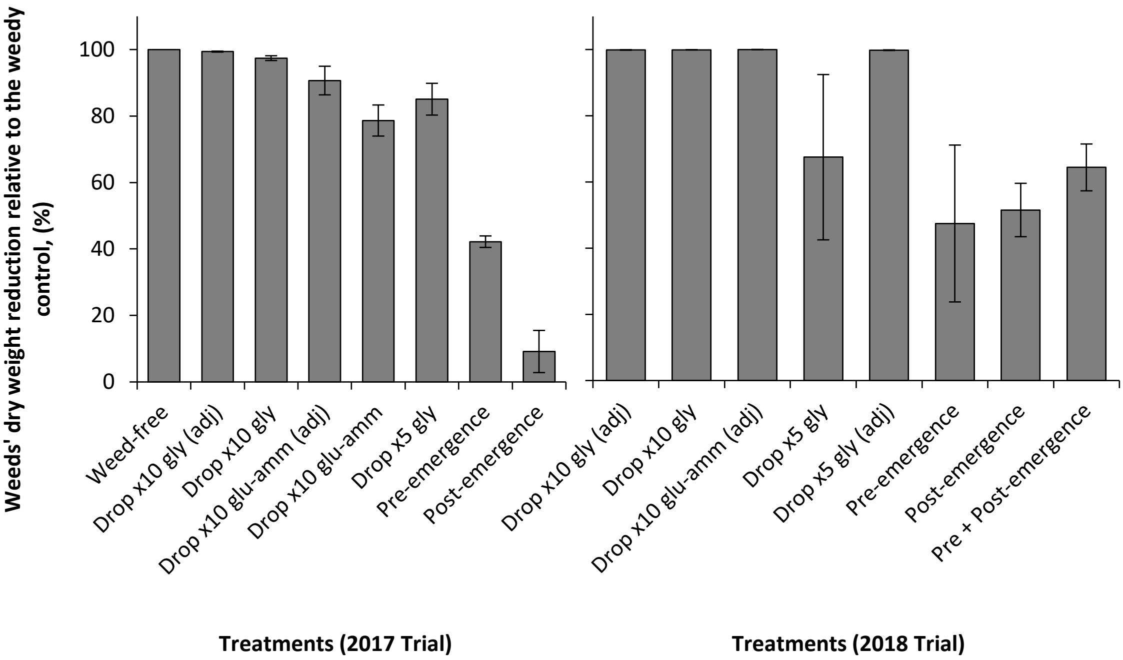


Figure 3.8. Efficacy of weed control expressed as percent dry weight reduction relative to weedy control for the 2017 (SED: 8.69, d.f.:16) and 2018 (SED: 15.5, d.f.: 18) trials. Dry weights of weeds in the weedy controls were 537 g/m^2, and 547 g/m^2 in 2017 and 2018, respectively. Adjusted treatments received 9 μg of glyphosate (gly (adj)) or 7.5 μg of glufosinate-ammonium (glu-amm (adj)) to each leaf with area bigger than 1 cm^2 (Table 3.3).

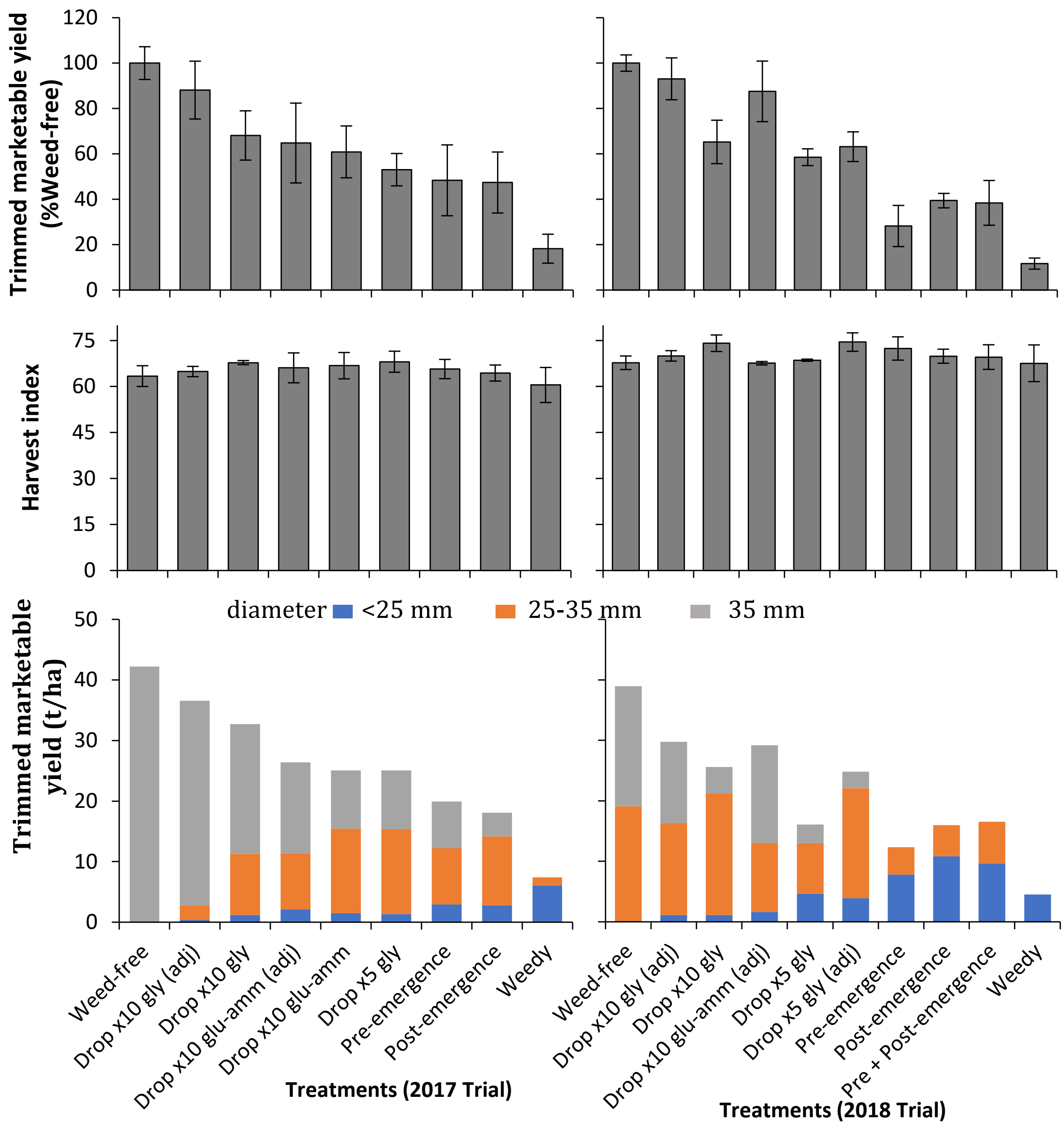


Figure 3.9. Trimmed marketable yield of leeks expressed as % relative to the yield of the weed-free (mean ±SEM) for the 2017 (Weed-free yield: 42.2 t ha^{-1}, SED: 11, d.f.:16) and 2018 (Weed-free yield: 39 t ha^{-1}, SED: 10.5, d.f.:18) trials. Marketable yield (t ha^{-1}) was classified into three categories according to stalk diameter (<25mm, 25-35mm and >35 mm). Harvest index is the ratio of the trimmed marketable yield divided by the untrimmed biomass (2017: (SED: 4.88, d.f.:16), 2018: (SED 4.53, d.f.:18)).

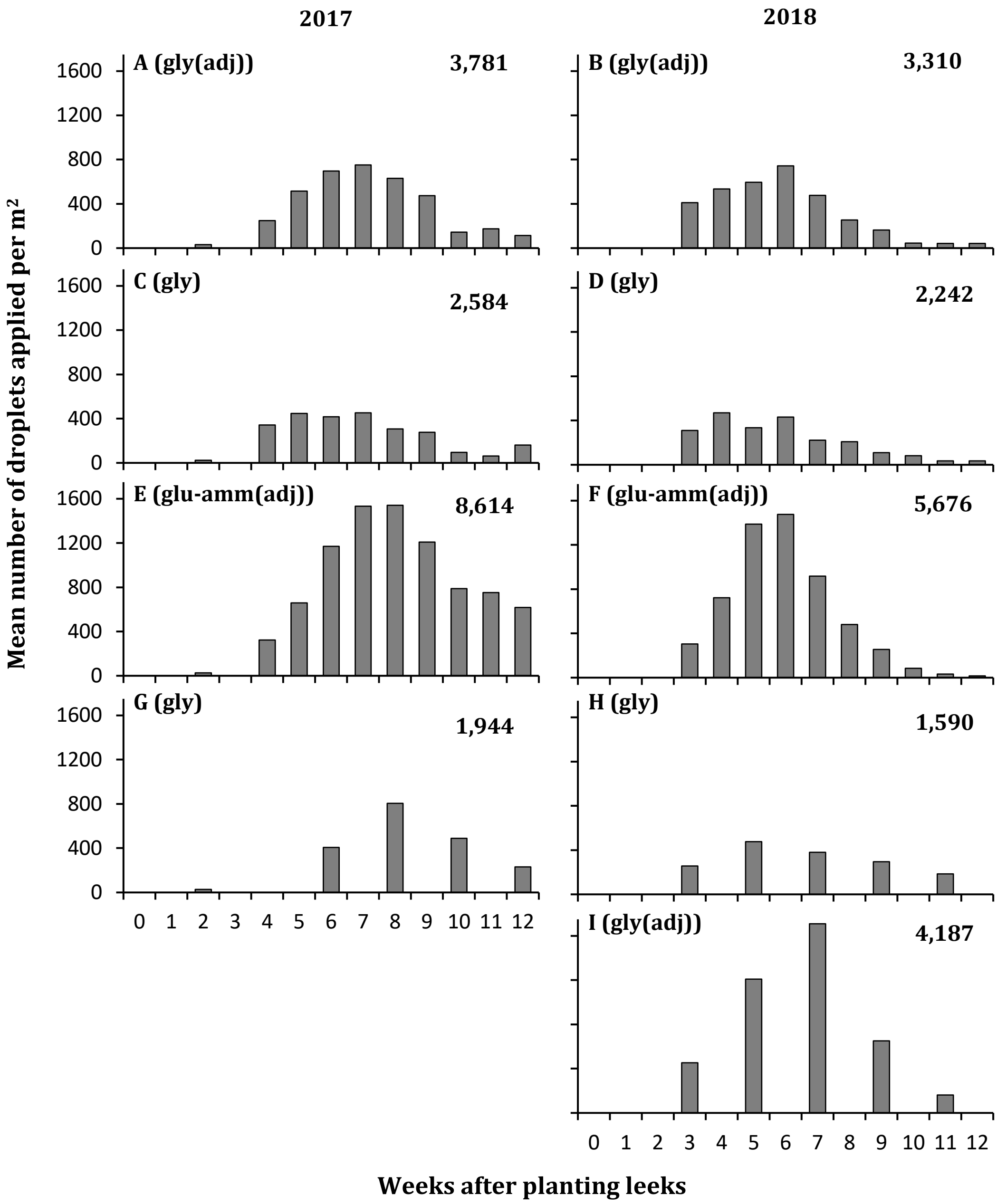


Figure 3.10. Mean number of droplets applied per m^2 for the droplet treatments: Drop x 10 gly (adj) (A &B), Drop x10 gly (C & D), Drop x10 glu-amm (adj) (E & F), Drop x5 gly (G & H) and Drop x5 gly (adj) (I) from the day of planting up to 12 weeks after planting the leeks for the 2017 and 2018 trials. Number on the right corner of each graph represents the total number of droplets applied per m^2 for each treatment. Description of droplet treatment for the leek trials is presented in Table 3.3.

Table 3.8. Total amounts of herbicide applied (g a.i. ha^{-1}) for the droplet treatments and reductions relative to the pre-emergence (1319.5 g ha^{-1} pendimethalin) and post-emergence (337.5 g ha^{-1} bromoxynil) spray and the combined treatment of the two herbicides. For the 2017, trial no combined treatment was applied and two spray applications of the post-emergence treatment were carried out (675 g ha^{-1} bromoxynil). For spraying glyphosate, the label recommendations range from 540 to 1800 g ha^{-1} and for glufosinate-ammonium range from 450 to 750 g ha^{-1} with a maximum of 1500 g ha^{-1} per year if two treatments are applied. Figures are means (±SEM).

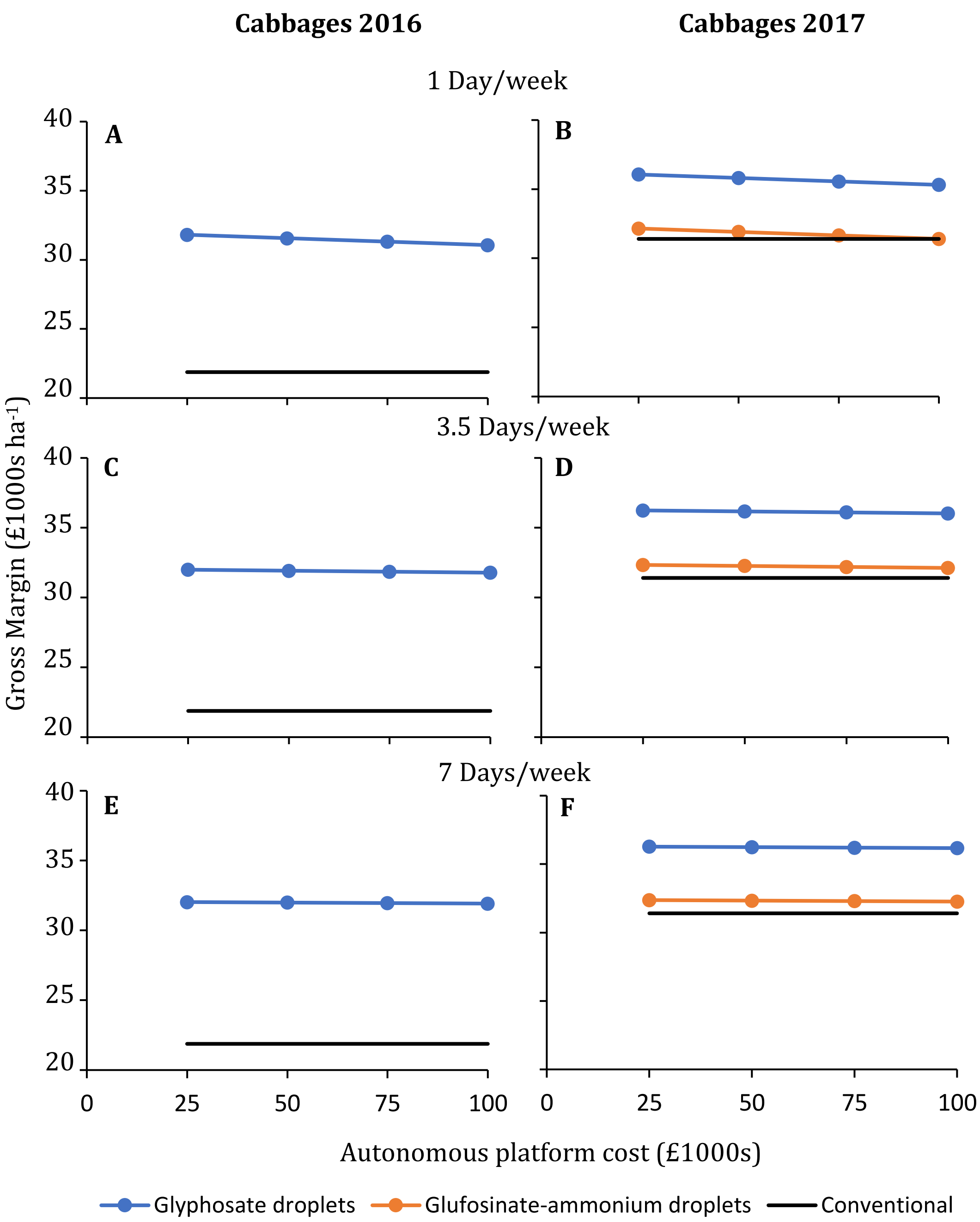
		%Reduction relative to		
Treatments	**Total amount of herbicide (g of a.i. ha^{-1})**	**Pre-emergence**	**Post-emergence**	**Pre + Post-emergence**
		2017		
Drop x5 gly	699.9 (139.3)	47.0 (10.6)	-3.69 (20.6)	N.A.
Drop x10 gly	930.1 (34.8)	29.5 (2.64)	-37.8 (5.16)	N.A.
Drop x10 gly (adj)	340.3 (26.2)	74.2 (1.99)	49.6 (3.9)	N.A.
Drop x10 glu-amm	2120.5 (139.9)	-60.7 (10.6)	-214 (20.7)	N.A.
Drop x10 glu-amm (adj)	646.1 (122.6)	51.0 (9.29)	4.29 (18.2)	N.A.
SED (d.f.)	125.6 (8)	9.52 (8)	18.6 (8)	N.A.
		2018		
Drop x5 gly	572.5 (26.4)	56.6 (2)	-69.6 (7.83)	65.5 (1.59)
Drop x5 gly (adj)	376.8 (24)	71.4 (1.82)	-11.6 (7.12)	77.3 (1.45)
Drop x10 gly	807 (11.6)	38.8 (8.46)	-139.1 (33.1)	51.3 (6.74)
Drop x10 gly (adj)	299.2 (47.4)	77.3 (3.56)	11.3 (14.1)	81.9 (2.86)
Drop x10 glu-amm (adj)	425.7 (18.8)	67.7 (1.42)	-26.1 (5.56)	74.3 (1.13)
SED (d.f.)	66.4 (8)	5.03 (8)	19.7 (8)	4.01 (8)

N.A.: Not Applicable

The £32,166 ha^{-1} value achieved from the treatments where glyphosate droplets were applied on three occasions (Drop x3 gly) and from the combined treatments of inter-row spray and droplet application was not significantly different to that of the weed-free (£42,441 ha^{-1}) for the savoy cabbages. The values of all other treatments in 2016 were, however, significantly lower (Figure 3.12). In 2017, no significant differences were observed for the economic value among the treatments applied for the 2017 trial with white cabbages (P=0.13). Although higher leek yields were produced for the 2017 trial, because of the differences in pricing between years the value of the crop was higher for the 2018 trial. The value of the crop when droplets of glyphosate were applied either as a single droplet or multiple droplets per weed, on a weekly basis did not differ significantly than that of the weed-free for both trials (2017: £34,602 ha^{-1} and 2018: £43,187 ha^{-1}) (Figure 3.9). Because of the high value of the crops and low cost of weed control the gross margins over weed control costs differed little from the economic values (Supplementary figure 3.2).

With one exception, reductions in gross margins when using glyphosate and glufosinate-ammonium droplets, associated with an increase in the cost of the platform from £25,000 to £100,000 and a decrease in the operating days from seven to one day per week, were less than 0.7% and 4.8% for cabbages and leeks, respectively (Figure 3.11, Supplementary table 3.3). The exception was when the platform was operating one day per week, a larger decrease in the gross margins (2.3% and 18% for cabbages and leeks) was observed with increased cost of the platform (Figure 3.11, (A), (B), (F) and (G), Supplementary table 3.3).

Preliminary economic analysis suggested that if the platform's cost is £25,000 and droplet treatments are carried out 5 days per week, the system would have been less profitable than hand-weeding the leek crop. On this basis, only when droplets of glyphosate were applied on ten occasions for the 2017 trial was the droplet treatment more profitable than hand-weeding. Gross margins of weekly applications of glyphosate droplets greatly exceeded those of conventional pre-emergence spraying, by £12,980 ha^{-1} and £22,255 ha^{-1} in 2017 and 2018, respectively (Table 3.9). Similarly, high values of additional profit were observed when applying droplets of glufosinate-ammonium compared with pre-emergence spray (up to £20,617 ha^{-1}). For cabbages just one droplet treatment (Drop x3 gly (adj)) was more profitable when compared with spray applications of pendimethalin in both years (2016: £4,521 ha^{-1} and 2017: £1,489 ha^{-1}) (Table 3.9). Droplet treatments appeared to be more profitable only for 2017 trial when compared to hand-weeding the cabbages. Based on the material cost of the glyphosate when using droplet applications, savings of up to £13 ha^{-1} and £19 ha^{-1} in herbicide costs were achieved when compared with spraying the pre-emergence pendimethalin for leeks and cabbages respectively (Table 3.9).



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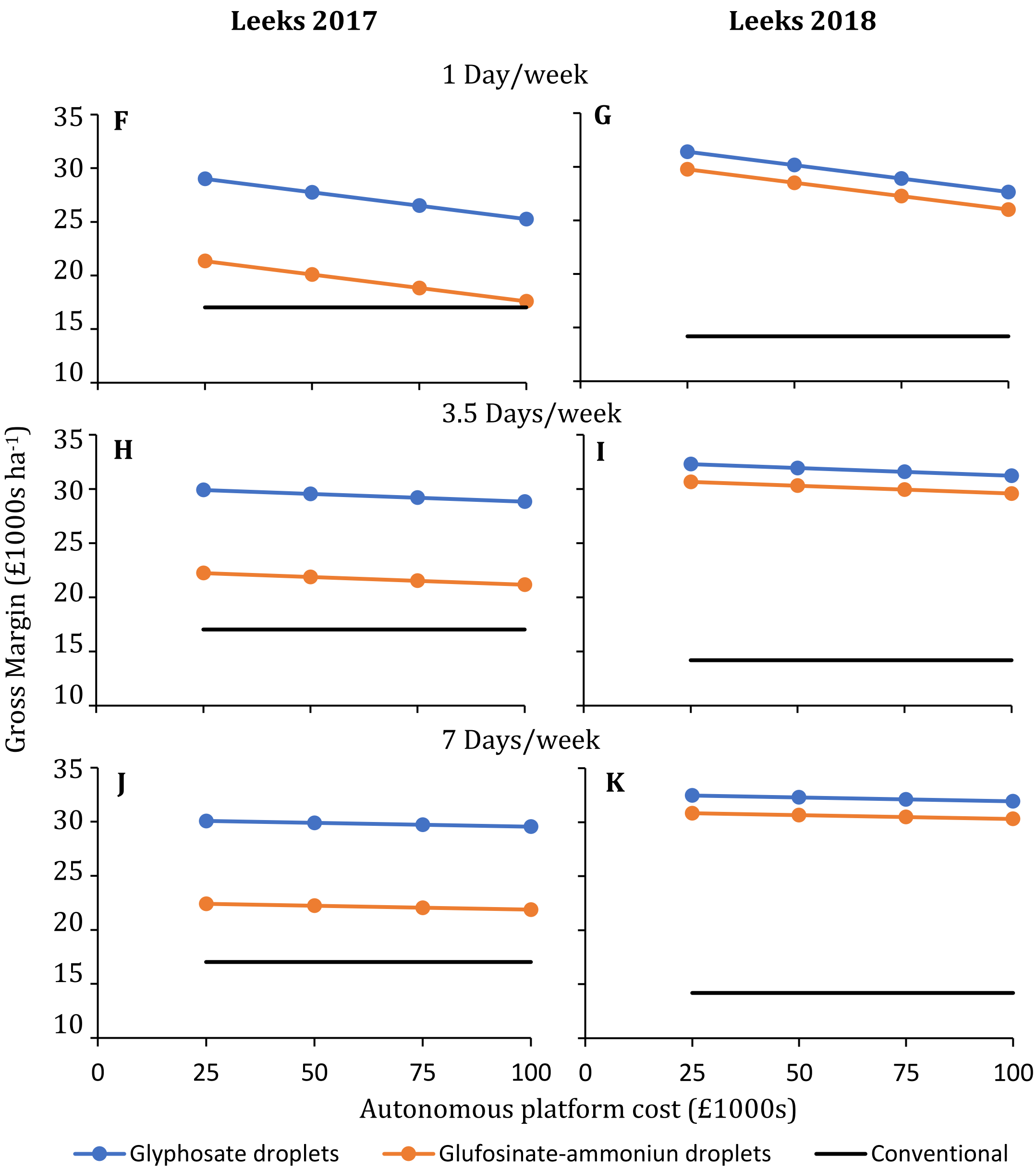


Figure 3.11. Mean gross margin (1000s ha^{-1}) plotted against different prices of an autonomous platform performing leaf-specific weed control when operating at 1, 3.5 and 7 days per week. For cabbages glyphosate and glufosinate-ammonium droplets were applied in three occasions every two weeks, whereas in leeks droplets of both herbicides were applied every week in ten occasions. Conventional treatment was a pre-emergence spray of pendimethalin and only for leeks 2018 a pre-emergence and a post-emergence spray of bromoxynil were applied. Gross margin values are presented in Supplementary table 3.3.

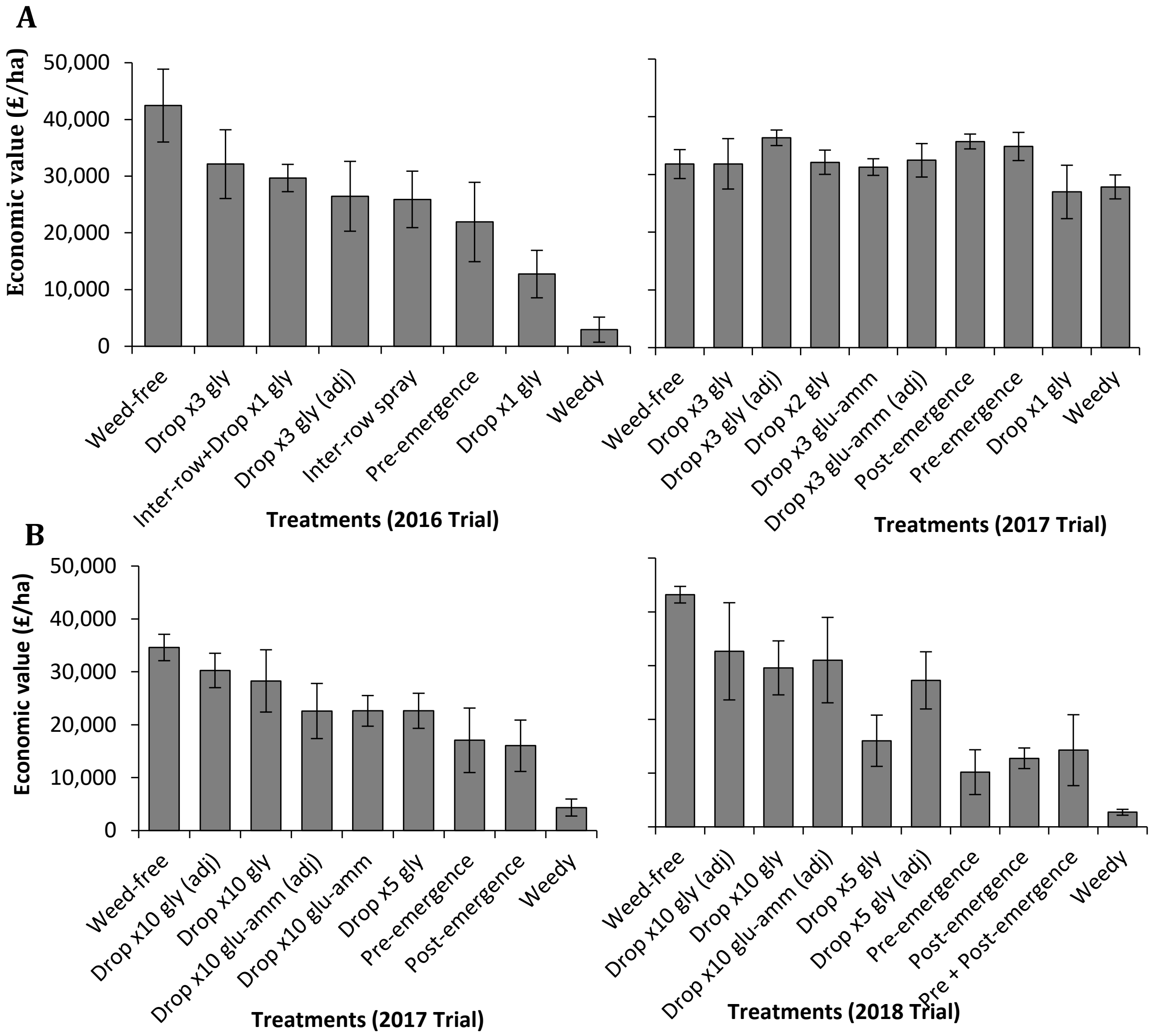


Figure 3.12. Economic values of cabbages (A) and leeks (B) for the 2016, 2017 and 2018 trials. Marketability of cabbages was calculated based on minimum marketable weights of 300 and 1000 g/head for savoy and white cabbages, respectively. Value of leeks was estimated according to the crop's stalk diameter and a different value was given for the three categories each year. Economic values for cabbages and leeks are presented in Table 3.4. SED (d.f.) were 6,825 (21) and 3,284 (27) for cabbages in 2016 and 2017 and 4,269 (16) and 7,854 (18) for leeks in 2017 and 2018, respectively.

Table 3.9. Gross margin and material cost of the herbicides for the droplet treatments applied in the trials with cabbages (2016 & 2017) and leeks (2017 & 2018) and net profit or loss (in red) relative to pre-emergence spray, pre+post-emergence spray and hand-weeding (weed-free) treatments. Gross margins were calculated on the basis that the applicator would cost £25,000 and could operate five days per week. Pre + Post-emergence spray was not applied to cabbages. Material costs of herbicides are based on the assumptions presented in Table 3.5. Gross margins are presented in Supplementary figure 3.2.

Treatments	Gross Margin (£ ha^{-1})		Material cost (£ ha^{-1})		Difference in gross margin (£ ha^{-1}) of droplet treatments compared to				
					Pre-emergence		Hand-weeding		Pre+Post-emergence
(A) Leeks	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2018**
Drop x5 gly	22,628	15,885	11	9.2	5,478	5,744	-5,759	-20,961	1,572
Drop x5 gly (adj)	N.A.	27,116	N.A.	7.7	N.A.	16,974	N.A.	-9,731	12,802
Drop x10 gly	28,273	29,308	15	13	10,998	19,166	-240	-7,539	14,869
Drop x10 gly (adj)	30,255	32,396	7	6.1	12,980	22,255	1,743	-4,451	17957
Drop x10 glu-amm	22,602	N.A.	25	N.A.	5,327	N.A.	-5,911	N.A.	N.A.
Drop x10 glu-amm (adj)	22,586	30,758	13	8.6	5,311	20,617	-5,926	-6,089	16,319
SED (d.f.)	4,270 (16)	7,854 (18)	3.6 (8)	1.1 (8)	3,394 (8)	9,628 (8)	3,394 (8)	9,628 (8)	9,628 (8)
(B) Cabbages	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	
Drop x1 gly	12,680	26,975	0.9	0.3	-9,197	-7,987	-28,481	-3,599	
Drop x2 gly	N.A.	32,074	N.A.	0.7	N.A.	-2,748	N.A	1,550	
Drop x3 gly	32,065	31,795	1.4	0.9	10,188	-3,027	-9,097	1,271	
Drop x3 gly (adj)	26,398	36,311	2.1	0.6	4,521	1,489	-14,764	5,787	
Drop x3 glu-amm	N.A.	31,235	N.A.	1.5	N.A.	-3,586	N.A.	712	
Drop x3 glu-amm (adj)	N.A.	32,407	N.A.	0.8	N.A.	-2,415	N.A.	1,883	
SED (d.f.)	6,825 (21)	3,430 (24)	0.3 (6)	0.4 (15)	7,444 (6)	3,387 (15)	7,444 (6)	3,387 (15)	

N.A. Not Applicable

3.5 Discussion

Results presented in this paper validate the concept of weed control by applying micro-doses of herbicides using droplets applied leaf-specifically in fields with cabbages and leeks. The primary hypothesis was accepted for both crops since the efficacy of weed control and the yield produced from the droplet treatments were not statistically significantly lower than the weed-free controls and were higher than current spraying methods.

For cabbages when micro-doses of glyphosate were applied either as a single or multiple droplets per weed on three occasions, they achieved over 90% weed control without any significant yield penalty. The hypothesis that multiple treatments would be needed was accepted since a single treatment using glyphosate droplets gave poorer levels of weed control and yielded even lower than the weedy controls for both years. However, according to Weaver (1984) a single weed control treatment between three and five weeks after planting was enough to avoid yield loss, and Roberts *et al.* (1976) similarly found that, for summer seeded cabbage in the UK, a single weeding three weeks after crop emergence was sufficient. Both authors agreed that there is not a critical period of weed control for the cabbage crop. In a more recent Danish study, which tested two types of mechanical intra-row weeding in transplanted white cabbage, a single weeding method (either as a post-emergence spray or mechanical weeding) at two weeks (in 2012) and three weeks (in 2013) after planting produced yields which were not significantly different from the manual weeding controls (Melander *et al.* 2015). It is also worth mentioning that the yield of white cabbage from the hand-weeded control in this study for 2013 was the same as with the weed-free trimmed yield produced here (93.5 t ha^{-1}).

Why then, may more than one droplet treatment be necessary? All the above studies utilize physical, mechanical or chemical weed control methods and total weed control is achieved when they are applied. However, a single leaf-specific application of herbicides as tested in this study may not achieve 100% efficacy for two reasons. First, the aim of applying droplets to weed leaves is that no herbicide will end up on the crop either directly or by run-off after rain. Weeds growing in an area less than 1 cm from the edge of the crop were therefore deemed unsafe to treat due to a risk of collateral damage. Secondly, to avoid accidental direct applications to soil, very small seedlings (leaf area <1 cm^2) were also left untreated on the basis that they might not be targeted accurately enough by an automated system. Therefore, multiple treatments were required to control weeds that were omitted on the first visit. A third reason was to ensure effective weed control if weeds were poorly controlled or emerged after previous applications. So even though cabbages do not have a critical period for weed control, it is recommended that herbicide droplets should be applied on three occasions between 3 and 7 weeks after planting as demonstrated here.

Unlike cabbages, leeks have a clear critical weed-free period and in order to avoid yield losses of over 5% in leeks, the crop needs to remain weed-free during the critical weed-free period from 1 to 12 weeks after planting (Tursun *et al.* 2007). Not surprisingly therefore, multiple treatments using droplets of glyphosate and glufosinate-ammonium were found necessary to keep the crop largely weed-free. For both years, weekly applications of glyphosate droplets applied to every leaf starting from 3 up to 12 weeks after planting, achieved at least 91% weed control efficacy without significantly lower yields. Also, a fortnightly application of the same treatment for 2018 controlled 100% of the weeds however, it yielded significantly lower than the hand-weeded controls. A possible reason for that could be an infestation with leek miner which resulted in overall lower leek yields for the 2018 trial. Efficacy of weed control also appeared satisfactory by applying a single glyphosate droplet per weed (36 µg) on a weekly basis however yields for both years were significantly lower.

The amounts of herbicide a.i. applied in the optimal droplet treatments for cabbages (Drop x3 gly and Drop x3 gly (adj)) were 91% to 98% lower than for the conventional pre-emergence spray of pendimethalin. For leeks, applications of droplets containing glyphosate or glufosinate-ammonium demonstrated that herbicide inputs can be reduced by up to 82% and 74% respectively. So, the hypothesis that targetted droplet applications would significantly reduce herbicide inputs was accepted. From a regulatory perspective, the amounts of glyphosate applied in cabbages over the growing season (28.1 g ha^{-1} and 83.3 g ha^{1}) were from 85% to 98% lower than the minimum label recommendation for spraying the herbicide (540 g ha^{-1}). When a DoD system (Asterix robot) was used for intra-row weed control in fields with carrots applying 2.1 µl droplets of glyphosate , the equivalent of 191 g glyphosate ha^{-1} was applied, which is 73 to 91% lower than the minimum and the maximum label recommendations (540 g ha^{-1} to 2,160 g ha^{-1}) (Utstumo *et al.*, 2018). In this study, for glufosinate-ammonium the amount applied (40.2 g a.i. ha^{-1}) was also much lower than the recommended rates of 450 to 750 g ha^{-1}. Although more droplets were applied to the fields with leeks, the amounts of herbicides remained within the range of recommended doses, if not lower. Only in the case of the single droplet per weed of glufosinate-ammonium for the 2017 trial with leeks the amounts applied (2120.5 g ha^{-1}) exceeded the maximum recommendation of 1500 g ha^{-1} (two applications of 750 g ha^{-1}).

The doses of glyphosate applied here (36 µg per weed or 9 µg per leaf) are similar to those applied by a robotic application system described by Søgaard *et al*. (2006). In field conditions, the test weed *Brassica napus* on average received 22.6 µg of glyphosate per plant, which was applied as droplets of 2.5 µl containing 5 µg of glyphosate to leaves with area of approximately 1 cm^2. Also, the Asterix robot achieved total weed control using droplets of 2.1 µl volume which contained 5.3 µg of glyphosate (Utstumo *et al*., 2018).

Franco *et al.* (2017) predicted that the potential savings in herbicides using a micro-spraying system would be 11-22 £ ha^{-1}. This study supports this prediction: compared to spraying the full dose of a commercial herbicide, weed control by droplet application demonstrated savings in herbicide costs of up to £19 ha^{-1} for cabbages and £13 ha^{-1} for leeks. To answer the question if the reductions in costs and amount of herbicides applied would justify the investment in an automated platform, the preliminary economic analysis presented here predicted a very high increase in crop profitability associated with droplet applications of both glyphosate (£22,255 ha^{-1}) and glufosinate-ammonium (£20,617 ha^{-1}) compared with spraying commercial herbicides (pre-emergence). These differences are due to the high value of leeks and the lower yields with pre-emergence herbicides. For cabbages however, only the adjusted glyphosate treatment appeared to be profitable for both years when compared with pre-emergence spraying. When compared with hand-weeding, controlling weeds by leaf-specific droplet application was, however, less profitable for leeks and savoy cabbages, assuming it would take 208 h ha^{-1} and cost £6,340 ha^{-1} to hand-weed a leek field three times. For the white cabbages (2017 trial) the optimum droplet treatments appeared to be more profitable than hand-weeding and that was because these treatments achieved higher yields than the weed-free control. These comparisons are, however, based on the assumption that yield of a commercial hand-weeded crop can be equated with the hand-weeded, weed-free controls in these experiments. The latter were hand-weeded on more than three occasions.

The sensitivity analyses of the foregoing economic analysis was particularly encouraging. The gross margins were relatively insensitive to changes in platform costs to values much higher than anticipated. For example, Miller et al.'s (2011, 2013) spot spraying system costs approximately £45,000 and the applicator envisaged here is likely to be priced similarly. Concerns about the number of operational days were also addressed and provided the machine could operate for more than one day per week, profitability is not likely to be seriously compromised.

In conclusion, manually applied droplets of glyphosate achieved satisfactory weed control without a significant yield penalty and reduced amounts of herbicide active ingredients applied to field grown cabbages by up to 94%. In leek crops, a sequence of applications of glyphosate or glufosinate-ammonium achieved high levels of weed control, no yield penalty and reduced herbicide applications by over 70% compared to conventional pre- and post-emergence sprays. Because of the high value of both crops, droplet applications could increase profits by over £11,000 ha^{-1} and £1,500 ha^{-1} per year for leeks and cabbages, respectively.

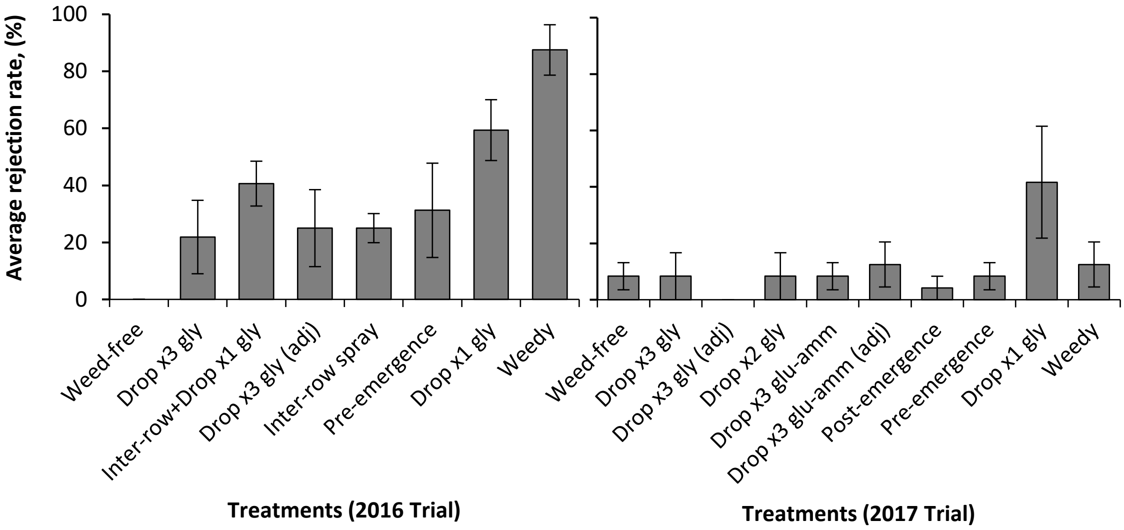
3.6 Supplementary material

Supplementary table 3.1. One-way ANOVA analysis of trimmed and marketable yield, harvest index, reduction of weed biomass and rejection rate of unmarketable savoy cabbage heads for the 2016 and white cabbage heads for the 2017 trials.

Year	Variate	Source of variation	d.f.	Sum of Squares	Mean Sum of Squares	*P*-value
2016	Trimmed yield (% Weed-free)	Blocks	3	5365	1788.3	
		Treatments	7	15565	2223.5	**0.006**
		Residual	21	11680	556.2	
	Marketable yield (% Weed free)	Blocks	3	9293.1	3097.7	
		Treatments	7	24016.1	3430.9	**0.01**
		Residual	21	19561	931.5	
	Economic value (£/ha)	Blocks	3	659,200,000	219,700,000	
		Treatments	7	4,090,000,000	584,300,000	**<0.001**
		Residual	21	1,956,000,000	93,160,000	
	Harvest index	Blocks	3	1000.9	333.6	
		Treatments	7	6428.9	918.4	**0.014**
		Residual	21	5671.4	270.1	
	Reduction of weeds' biomass relative to weedy control, (%)	Blocks	3	1097.5	365.8	
		Treatments	7	37386.8	5341.0	**<0.001**
		Residual	21	5616.0	267.4	
	Cabbage heads rejection rate, (%)	Blocks	3	483.4	161.1	
		Treatments	7	19917	2845.3	**<0.001**
		Residual	21	10336.9	492.2	
2017	Trimmed yield (% Weed-free)	Blocks	3	5767.2	1922.4	
		Treatments	9	3329.9	370.0	0.265
		Residual	27	7476.3	276.9	
	Marketable yield (% Weed free)	Blocks	3	14369.1	4789.7	
		Treatments	9	9164.3	1018.3	0.242
		Residual	27	19793.3	733.1	
	Economic value (£/ha)	Blocks	3	321,800,000	107,300,000	
		Treatments	9	336,500,000	37,390,000	0.13
		Residual	27	582,500,000	21,570,000	
	Harvest index	Blocks	3	1078.4	359.5	
		Treatments	9	2114.2	234.9	0.082

Supplementary table 3.1 continued

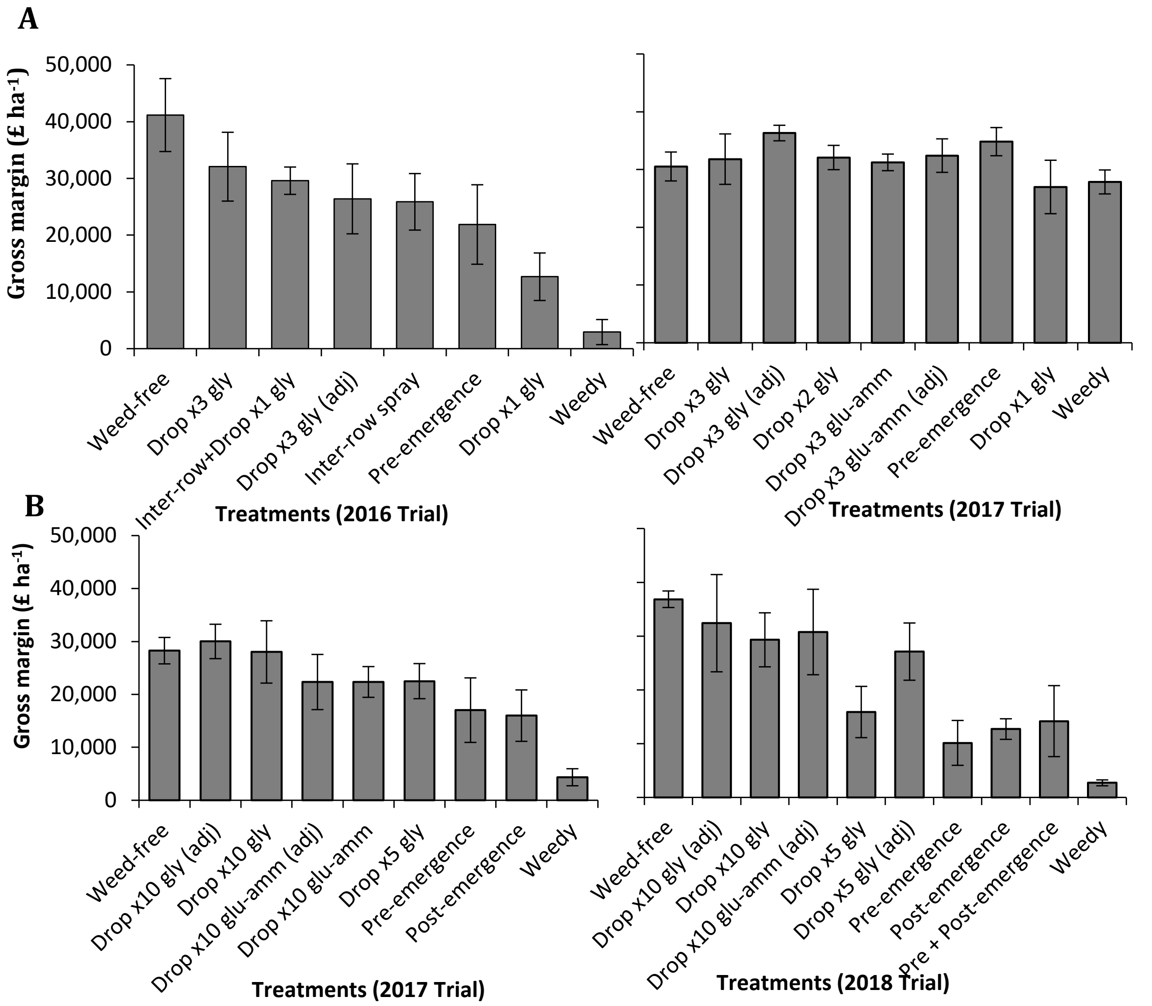
Year	Variate	Source of variation	d.f.	Sum of Squares	Mean Sum of Squares	*P*-value
		Residual	27	3204.9	118.7	
2017	**Reduction of weeds' biomass relative to weedy control, (%)**	Blocks	3	2444.7	814.9	
		Treatments	9	35820.3	3980.0	**<0.001**
		Residual	27	13632.7	504.9	
	Cabbage heads rejection rate, (%)	Blocks	3	1909.7	636.6	
		Treatments	9	4590.3	510	0.09
		Residual	27	7048.6	261.1	



Supplementary figure 3.1. (%) Average rejection rate (±SEM) of unmarketable savoy and white cabbage heads for the 2016 (SED:15.7, d.f.:21) and 2017 (SED: 23.4, d.f.:27) trials. The overall rejection rates were 36.3% and 11.3% of the savoy (2016 Trial) and white cabbages (2017 Trial) respectively.

Supplementary table 3.2. One-way ANOVA analysis of trimmed marketable yield (% Weed-free), harvest index, economic value and reduction of weed biomass for the 2017 and 2018 trials with leeks.

Year	Variate	Source of variation	d.f.	Sum of Squares	Mean Sum of Squares	*P*-value
2017	**Trimmed marketable yield (% Weed-free)**	Blocks	2	4828.4	2414.2	
		Treatments	8	13673.2	1709.1	**<0.001**
		Residual	16	2900.3	181.3	
	Harvest index	Blocks	2	137.09	68.55	
		Treatments	8	134.38	16.80	0.860
		Residual	16	572.34	35.77	
	Economic value (£/ha)	Blocks	2	530,900,000	265,400,000	
		Treatments	8	1,918,000,000	239,700,000	**<0.001**
		Residual	16	437,400,000	27,340,000	
	Reduction of weeds' biomass relative to weedy control, (%)	Blocks	2	178.8	89.4	
		Treatments	8	37602.7	4700.3	**<0.001**
		Residual	16	1813.9	113.4	
2018	**Trimmed marketable yield (% Weed-free)**	Blocks	2	752.6	376.3	
		Treatments	9	23126.1	2569.6	**<0.001**
		Residual	18	2966.3	164.8	
	Harvest index	Blocks	2	22.77	11.38	
		Treatments	9	184.44	20.49	0.73
		Residual	18	554.33	30.80	
	Economic value (£/ha)	Blocks	2	63,760,000	31,880,000	
		Treatments	9	4,264,000,000	473,800,000	**0.002**
		Residual	18	1,666,000,000	92,530,000	
	Reduction of weeds' biomass relative to weedy control, (%)	Blocks	2	1500.6	750.3	
		Treatments	9	30507.9	3389.8	**<0.001**
		Residual	18	6452.8	358.5	



Supplementary figure 3.2. Mean gross margins (±SD) (£ ha^{-1}) of cabbages (A) and leeks (B) for the 2016, 2017 and 2018 trials. SED (d.f.) in 2016 and 2017, respectively were 6,825 (21) and 3,430 (24) for cabbages and 4,269 (16) and 7,854 (18) for leeks in 2017 and 2018.

Supplementary table 3.3. Gross margins (£ ha^{-1}) of the glyphosate and glufosinate-ammonium droplet treatments used for the sensitivity analysis. Gross margins of conventional spraying treatments were £21,877 and £31,061 for the cabbages 2016 and 2017, respectively and £17,025 and £14,189 for the leeks 2017 and 2018, respectively. Glufosinate-ammonium was not applied in the 2016 trial with cabbages.

Crop	Platform cost, £/year	Operating days/week	Gross margin, £ ha^{-1} Treatments: Glyphosate droplets	Gross margin, £ ha^{-1} Treatments: Glufosinate-ammonium droplets
Cabbage 2016	5,000	1	31,815	N.A.
	10,000		31,565	
	15,000		31,315	
	20,000		31,065	
	5,000	3.5	31,994	
	10,000		31,922	
	15,000		31,851	
	20,000		31,779	
	5,000	5	32,015	
	10,000		31,965	
	15,000		31,915	
	20,000		31,865	
	5,000	7	32,029	
	10,000		31,994	
	15,000		31,958	
	20,000		31,922	
Cabbage 2017	5,000	1	36,061	32,156
	10,000		35,811	31,906
	15,000		35,561	31,656
	20,000		35,311	31,406
	5,000	3.5	36,240	32,335
	10,000		36,168	32,263
	15,000		36,097	32,192
	20,000		36,025	32,120
	5,000	5	36,261	32,356
	10,000		36,211	32,306
	15,000		36,161	32,256
	20,000		36,111	32,206
	5,000	7	36,275	32,370
	10,000		36,240	32,335
	15,000		36,204	32,299
	20,000		36,168	32,263

Crop	Platform cost, £/year	Operating days/week	Gross margin, £ ha^{-1} Treatments: Glyphosate droplets	Glufosinate-ammonium droplets
Leek 2017	5,000	1	29,005	21,336
	10,000		27,755	20,086
	15,000		26,505	18,836
	20,000		25,255	17,586
	5,000	3.5	29,898	22,229
	10,000		29,541	21,872
	15,000		29,184	21,515
	20,000		28,826	21,157
	5,000	5	30,005	22,336
	10,000		29,755	22,086
	15,000		29,505	21,836
	20,000		29,255	21,586
	5,000	7	30,076	22,407
	10,000		29,898	22,229
	15,000		29,719	22,050
	20,000		29,541	21,872
Leek 2018	5,000	1	31,396	29,758
	10,000		30,146	28,508
	15,000		28,896	27,258
	20,000		27,646	26,008
	5,000	3.5	32,289	30,651
	10,000		31,932	30,294
	15,000		31,575	29,937
	20,000		31,217	29,579
	5,000	5	32,396	30,758
	10,000		32,146	30,508
	15,000		31,896	30,258
	20,000		31,646	30,008
	5,000	7	32,467	30,829
	10,000		32,289	30,651
	15,000		32,110	30,472
	20,000		31,932	30,294

N.A. Not Applied

Chapter 4
Targeting accuracy of a DoD applicator for the leaf-specific weed control

4.1 Summary

Using broadcast spraying over an entire field as a method of herbicide application can have potential impacts on the environment and human health. Pressures from policy makers and consumers to lower pesticide use have resulted into researching new methods for precision weed management. One technology is Drop-on-Demand (DoD) systems where herbicide application is carried out using individual droplets. This method of weed control targets only the weeds and therefore, avoiding the soil and the crop. Although, several studies have researched the effect of a DoD applicator on droplet formation and the biological efficacy, there is limited work on the targeting accuracy and the displacement of droplets. The applicator which was developed by Concurrent Solutions LLC in the USA was tested under indoor conditions. A calibration test indicated that at 138 kPa pressure the applicator needed 4 ms to dispense a droplet of 1 µl. The effect of pressure, distance from the target, wind direction and motion of the applicator was tested on the targeting accuracy of the applicator. Droplet disintegration was observed when the applicator was operating at 207 and 276 kPa pressures. The highest droplet displacement (19 mm) was observed at 69 kPa pressure. Droplets were displaced from 1.9 up to 3.2 mm from a target set at 15 cm distance, for both motions of the applicator (moving & static) and when a 10 km h^{1} was coming from three directions (head, tail & cross). Recommendations for future field applications suggested that the applicator should operate at 138 kPa pressure and set at 15 cm height from weeds.

4.2 Introduction

The most common method of herbicide application is using nozzles mounted on a conventional boom sprayer delivering a uniform dose over an entire field. However, a patchy distribution of weeds can make this method inefficient and wasteful, particularly in the case of post-emergence herbicide applications (Jensen *et al.,* 2013). Furthermore, the use of herbicides is receiving a lot of negative attention because of potential adverse effects on human health (e.g. due to residues on fruit and vegetables) and the environment (e.g. ground water contamination) (Young, 2012). In order to address these and other concerns, strict regulations have resulted in losses of approval of herbicide actives and have decreased the likelihood of new products gaining approval (Baker & Knight, 2017). These pressures from consumers and regulations from policy makers signal the need for a paradigm shift from spraying whole fields as a method of weed control.

As a result, a lot of research has focused on precision spray applications of herbicides. One example is the use of Drop-on-Demand (DoD) weed control systems where herbicide application is carried out using low volume single droplets directly to the target and therefore, avoiding application to the crop and the soil. Furthermore when using these systems, the potential reductions in herbicide use could be over 95% (Christensen *et al.*, 2009). The systems that have already been described for DoD herbicide application, in order to form droplets, they use an arrangement of solenoid valves (Giles *et al.*, 2004; Urdal *et al.*, 2014), needles (Nieuwenhuizen *et al.*, 2010) and inkjet printer heads (Lund & Mathiassen, 2010; Midtiby *et al.*, 2011). Also, the use of pneumatic valve was described by Basi *et al*. (2012) for generating single droplets of 1 μl.

One of the first automated systems for precision DoD was developed by Lee *et al*. (1999) for weed control in a tomato crop. The system was comprised of a machine vision system for crop and weed recognition and a precision sprayer with eight solenoid valves. After the images have been divided into grid cells of 0.81 cm^2 (1.27 cm x 0.64 cm), it took 10 ms to spray each cell. When travelling at a speed of 0.8 km h^{-1} the displacement of the spray droplets from the target ranged from 1.7 mm to 11.5 mm with an average of 6.6 mm. However, the system was unsuccessful, as at this speed it recognized 73% of the crop and sprayed correctly only 48% of the weeds. Trying to improve on that precision, Lamm *et al*. (2002) used the prototype system described by Lee *et al*. (1999) to develop a machine for precision weeding in cotton. When it was tested in the field at constant travelling speed of 1.62 km h^{-1}, the system correctly recognized 89% of the weeds. However, it also sprayed 21% of the crop.

Søgaard *et al*. (2006) developed and tested an autnomous robotic micro-dosing system. In indoor conditions, it was demonstrated that areas of 110 mm^2 were treated with an average of four droplets, 2.5 µl (5 µg of glyphosate) each (10 ms shot at 40 kPa pressure). Although, one out of four droplets fired was off-target, the droplets that were hitting the target were assumed to be enough for weed control. When the same system was tested in the field trying to control *Brassica napus* (test weed), the targeting performance was acceptable, with 86% of plants with 100 mm^2 leaf area being treated. However, when treating plants with leaf areas of 75 mm^2 and lower, the targeting performance was reduced significantly.

The tractor-pulled, automated system developed by Nieuwenhuizen *et al*. (2010), was designed for control of volunteer potatoes in sugar-beet fields. The algorithm used was able to create 10 mm x 40 mm grids in which the crop was distinguished from the volunteer potato. The latter was then treated with 20 ± 5 µl droplets containing glyphosate. Tests in indoor conditions showed that the displacement of droplets in the direction of travel was 4 mm with a standard deviation of 12 mm and at an angle of 90° to the direction of travel the displacement was 5.4 with a standard deviation of 6 mm. The system's targeting accuracy was deemed acceptable as the minimum leaf area of a single plant was 1200 mm^2 and so the droplet displacement would not affect targeting. To achieve satisfactory weed control, Fennimore *et al*. (2016) suggested that for most vegetable crops, the system needs to target weeds with leaf area smaller 400 mm^2 (i.e. 10 x 40 mm).

The micro-sprayer system developed by Midtiby *et al*. (2011) was able to detect and control weeds larger than 11 mm x 11 mm, when travelling at a constant speed of 1.8 km h^{-1}. Therefore, from these studies it is becoming clear that neither of the systems described earlier will be appropriate for weed control early in the growing season when weeds leaf area can be smaller than 100 mm^2.

When using conventical sprayers as a method of pesticide application meteorological factors like wind speed and direction are important as they can influence the spray drift (Carlsen *et al*., 2006). Sprayer settings such as nozzle type and spray pressure affect the number of drops that are likely to be carried away by the wind (Southcombe *et al*., 1997; Van De Zande *et al*., 2008). The use of much larger single droplets of herbicides as a method of pesticide application is expected to minimize drift and therefore, deposition of the chemicals to off-target locations (Zijlstra *et al*., 2011). Although the studies on DoD application mentioned earlier are important in order to decide the growth stage at which a weed can be accurately detected and treated, experiments were carried out under ideal conditions and the effect of wind on the targeting accuracy of the system is not demonstrated. Slaughter *et al.* (2008) highlights the need for more robust and accurate methods of plant recognition and herbicide application which will suit real field conditions and therefore, meet the need for commercializing a robotic weed control system.

Similar to the applicator setup used in this study, Urdal *et al*. (2014) designed a DoD system for application of herbicide droplets 1 μl, which comprised of a solenoid valve and a nozzle. The tests were mainly carried out to study the effect of the applicator on droplet formation. The objectives of this paper are to estimate the volume of droplets emitted by a prototype DoD applicator as a function of hydraulic pressure and to study the effects of wind, pressure and distance from the target on the displacement of droplets. The hypotheses tested in this study were:

i. The solenoid valve open times of the prototype system in order to achieve droplet volumes appropriate for DoD application (1 to 5 μl) will not exceed 10 ms (based on Urdal *et al*., 2014).
ii. The higher the hydraulic pressure:
    a. the larger the volume of droplets and
    b. the smallest and displacement of droplets
iii. The greater the distance of the applicator from the target:
    a. the lower accuracy of droplet targeting and
    b. the greater the likelihood of split droplets.
iv. When the applicator is moving, a tail wind (coming in the direction of travel) will cause a greater displacement than a head wind (against the direction of travel).

4.3 Materials and Methods

A calibration test and a targeting accuracy test were carried out using a prototype Drop-on-Demand applicator system built by Concurrent Solutions llc, for bench-scale testing in indoor conditions (Figure 4.1). For both experiments distilled water was used which was coloured with blue food dye (McCormick® Assorted Food Colors & Egg Dye, McCormick & Company, Inc., Maryland, USA).

4.3.1 Drop on Demand system

The prototype consisted of two subsystems: a pneumatically-driven fluid application system and an electronic control system operated via a graphical user interface (GUI) on a personal computer. The subsystems were mounted on a miniature gantry, 1 m tall and 0.5 m wide, which included a motor and linear actuator which moved the applicator from right to left (Figure 4.1). The test rig allowed the operator to vary different pneumatic pressures, dispensing times as well as the lateral speed of the mini-gantry.

The volume and initial velocity of dispensed droplets were affected by three variables: fluid pressure, dispensing duration and the shape and size of the nozzle orifice. The nozzle was a constant for these experiments, meaning fluid pressure and dispensing duration determined droplet volume and initial velocity. The nozzle geometry is not described here, as this part of the system was built by Concurrent Solutions llc and is proprietary information. Fluid pressure was controlled manually by adjusting the air pressure within the system (Figure 4.1 pressure regulator (2)) and it could varied from 0 to 60 psi. Dispensing was regulated by the opening and closing of a solenoid valve mounted before the nozzle and the duration for which the valve was open was controlled electronically by the operator via the GUI. The solenoid valve could be opened for a minimum of 1 ms, enabling very fast cycling of the system. The solenoid used here had a smaller internal liquid capacity and could operate at speeds 100-times faster than those used for broadcast spraying. This performance would be critical to the accurate small dosing and timing needed for precise droplet application to leaves of small weed seedlings. As with the nozzle, the solenoid valve was purpose-built for the applicator and details are proprietary to Concurrent Solutions llc.

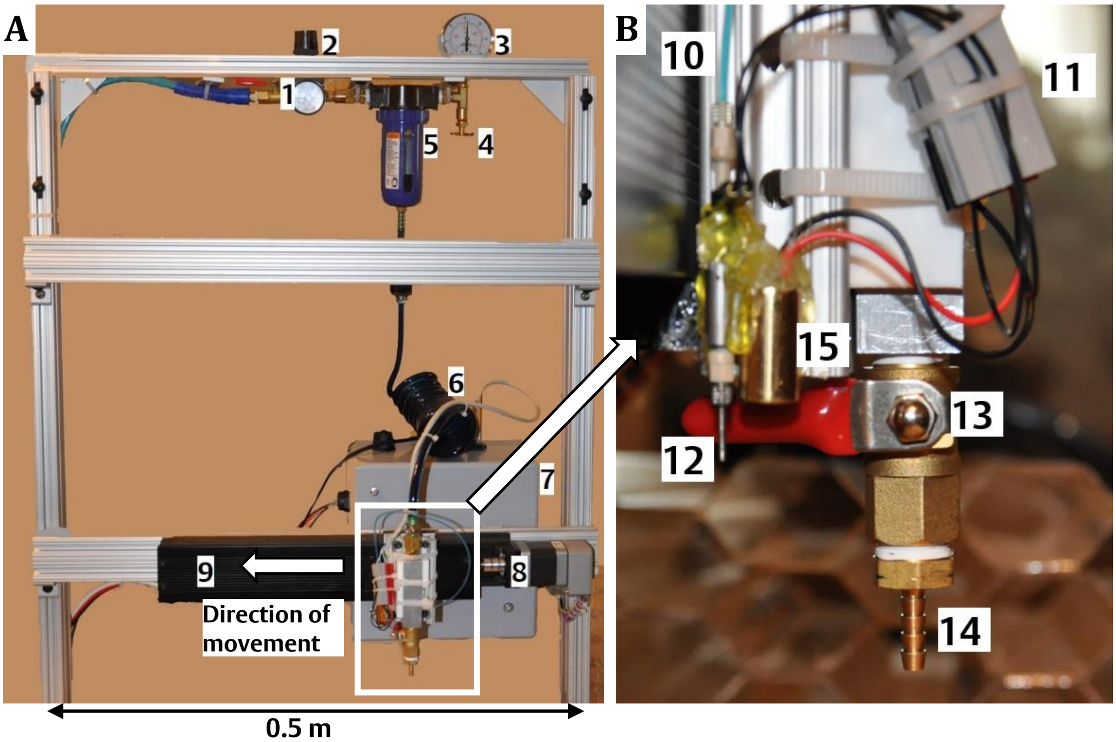


Figure 4.1. Gantry system mounted on an aluminium frame comprising an air pressure shut off valve (1), pressure regulator (2), pressure gauge (3), pressure release valve (4), liquid reservoir (5), flexible tubing (6), controller box (7), motor (8), linear actuator (9), ejector tubing (10), manifold (11), ejector nozzle (12), drain valve (13), liquid drain (14) and a laser pointer (15). Pressure was delivered using a Husky 8G 150 PSI Hotdog portable Air Compressor. The overall gantry is shown in (A) and the detail of the applicator in (B). Note that (B) is viewed along the direction of movement and is not the front view shown in (A).

4.3.2 Calibration test

In order to estimate the time needed for the applicator to dispense a single droplet of 1 µl under different pressures, a calibration test was carried out. The pressures tested were 69 kPa (10 psi), 138 kPa (20 psi), 207 kPa (30 psi) and 276 kPa (40 psi) and the time that the ejector nozzle/applicator operated ranged from 1 to 10 ms. Overall 40 treatments of pressure x time were tested, which were replicated four times. The average volume of each droplet was estimated by dispensing 1000 droplets of distilled water into an empty 1.5 ml Eppendorf® microtube. The weight of the collected water was measured using a using an analytical balance (weighing to the nearest 0.001 g)

4.3.3 Targeting accuracy test

The effect of pressure and distance from the target were tested on the targeting accuracy of the applicator when wind was applied. Tests were performed when the applicator was moving and when it was static. The target was an A4 uncoated paper attached to a wooden board. The level of the surface was checked with a spirit level each time the height of the board was adjusted. Wind was applied using an 12" floor fan and it was measured using a digital anemometer (HP-866B, HoldPeak®, Yongtian Road, ZHUHAI, China) at three places: at the nozzle outlet, halfway between the nozzle and the target and at the level of the target. A plastic honeycomb structure was used as an air flow straightener to minimize the turbulence of the air generated by the fan.

Twelve treatments applied comprising of three distances from the target (15 cm, 30 cm and 50 cm), at each four pressures (69 kPa, 138 kPa, 207 kPa and 276 kPa). A constant wind speed of 10 km h^{-1} was applied with the wind coming from four directions when the applicator was static and three directions when it was moving. Wind directions were defined relative to the direction of travel of the applicator, that is head (0°), cross (90°) and tail (180°). Control treatments involved the use of pressure and distance at zero speed and were applied first before any wind was introduced. The factorial experimental design became unbalanced because displacement could not be measured for the 69 kPa pressure and 50 cm from the target, treatments. For that treatment combination, emitted droplets fragmented were scattered with no clear indication of the point of application was even at 0 km h^{-1} wind speed (Supplementary figure 4.1). Overall 55 and 44 treatments were applied when the applicator was static and moving, respectively and the experiment was replicated four times. For each treatment ten and five droplets were applied when the applicator was static and when it was moving, respectively. When the applicator was moving droplets were emitted every 3.9 cm over a distance of 19.2 cm. Displacement of droplets was not measured at the first and last point of application because the applicator was speeding and slowing down at these places, respectively (Figure 4.2). After allowing some time for the blue dye to dry on the A4 paper, a circle was drawn using a plastic geometric designer. A digital calliper (iGaging®, Granada Hills CA, U.S.A) was used to measure the distance between the outer sides of the circles containing droplets applied with the zero wind, with those when different directions of wind were applied. Results were expressed as displacement (mm) relative to zero wind.

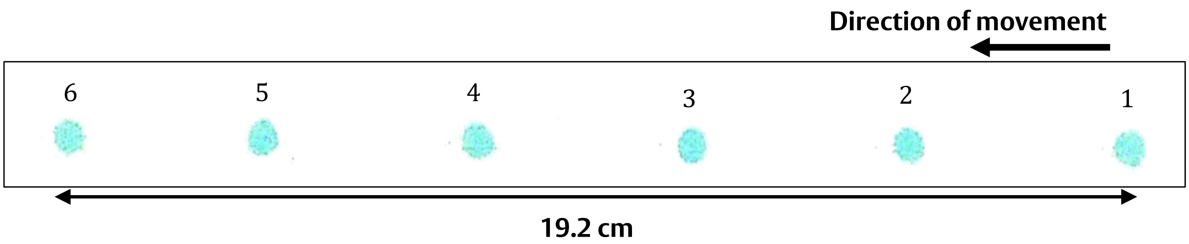


Figure 4.2. Droplets of 1 µl coloured with blue dye emitted from a moving applicator after traversing one time. Target was an A4 sheet of uncoated paper which was placed 15 cm from the applicator. The applicator was operating at 138 kPa and droplets were applied with 0 km h^{-1} wind. Droplet displacement was not measured at points 1 and 6 of application. No spatter was observed. Experimental setup is described in Figure 4.1.

4.3.4 Statistical analysis

Results from the calibration test were analysed with linear regression using R version 3.2.1 (R Development Core Team, 2014). Unbalanced ANOVA was carried out using GenStat (17^{th} Version) (VSN International Ltd., Hemel Hempstead, UK) to analyse the effects of pressure, distance from the target, wind direction and motion of the applicator on the displacement of droplets.

4.4 Results

Results from the calibration test showed that the mean weight of the water when 1000 droplets were applied was 1 g, when the applicator was operating for 6 ms, 4 ms, 3 ms and 2.5 ms at 69 kPa, 138 kPa, 207 kPa and 276 kPa pressure, respectively (Figure 4.3). Therefore, the average volume of the droplets coming from this pressure and time combinations was 1 µl. Furthermore, linear models were fitted to each pressure in order to be able to predict the time needed to apply droplets of different volumes (Figure 4.3). Also, it was proven that there was significant effect of time and pressure interaction to the weight of distilled water for the calibration test ($P<0.001$) (Supplementary table 4.2). To be able to predict other operating times and pressures of the applicator to apply a droplet of 1 µl, a logarithmic model was fitted when the time was plotted against the pressure required (Figure 4.4). Also, for each operating time of the applicator, the higher the pressure applied it increased the volume of the droplets (Figure 4.3).

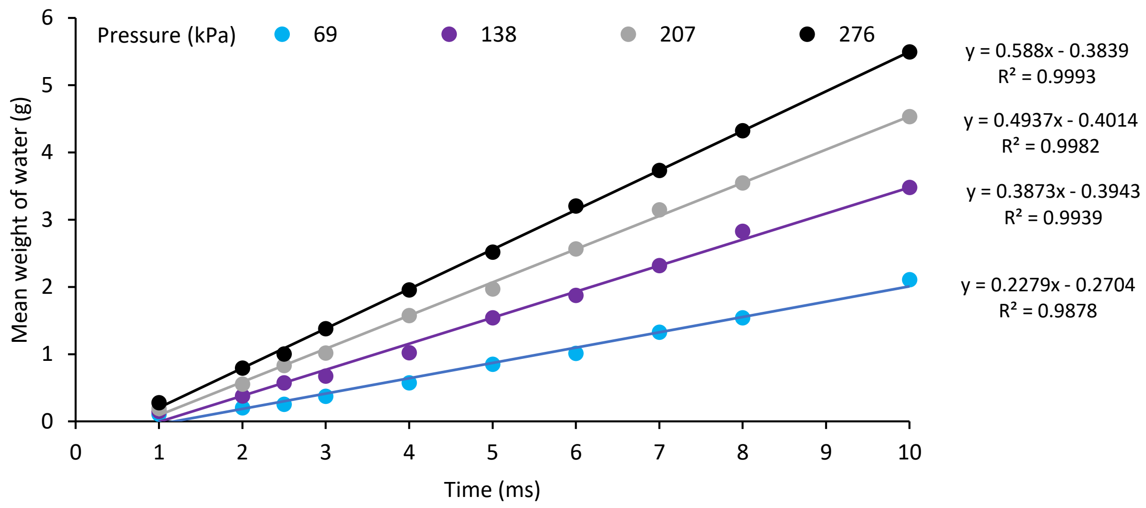


Figure 4.3. Mean values and fitted linear models of the mean weight of water after dispensing 1000 droplets plotted against the time it took the applicator to dispense a single droplet for the four different pressures. Coefficients (±SE) of the linear models are presented in Supplementary table 4.1.

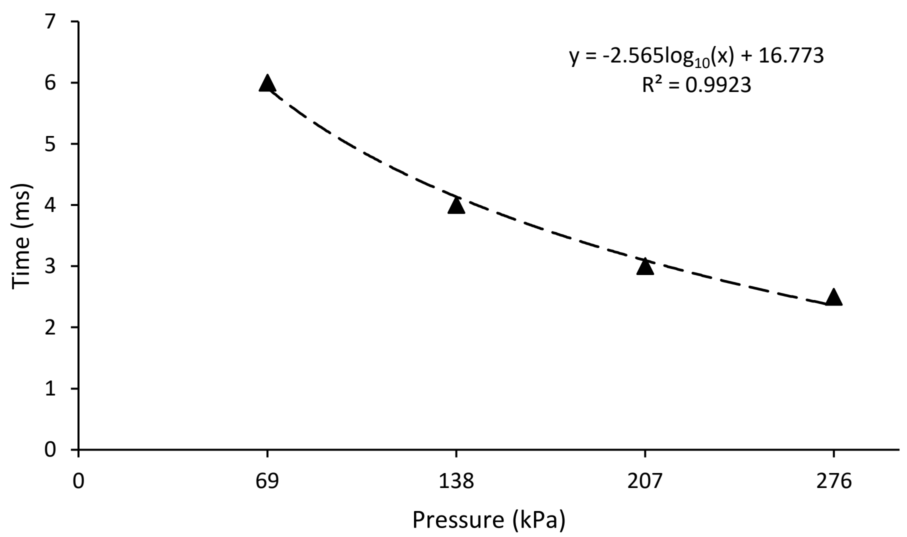


Figure 4.4. Mean values and fitted logarithmic model for the pressure and time it took the applicator to dispense 1 µl droplets. Coefficients (±SE) of the logarithmic model are presented in Supplementary table 4.3.

For the moving and the static applicator, the main effect of time, pressure and wind direction on the displacement of the droplets was significant (P<0.001) (Supplementary table 4.4). However, motion of the applicator (moving or static) did not have a significant effect on droplet displacement (P=0.38). The interaction of distance from the target and operating pressure of the applicator on the displacement of the droplets was, however significant (P<0.001). The smallest droplet displacement which, ranged from 1.8 to 4 mm and was observed when the applicator was operating at 15 cm above the target (Figure 4.5, Figure 4.6 (A)). Also, for this distance the displacements associated with pressures of 138, 207 and 276 kPa were not significantly different (Figure 4.5). Furthermore, when the applicator was operating at higher pressures, lower relative displacement of droplets was observed. Although, displacement was significantly smaller for the 276 kPa when compared with 138 kPa, split droplets were observed for the 276 kPa and the 207 kPa pressure (Supplementary figure 4.2). Moreover, the higher the distance from the target, the droplets tended to displace further. However, the largest displacement was observed when droplets were applied at 30 cm from the target and using 69 kPa pressure (Figure 4.5 (A)).

The interaction of wind direction and distance from the target on the displacement of droplets was significant (P<0.001). A tail wind caused a 12.5 mm displacement of droplets emitted 30 cm from the target which was significantly higher than the displacement caused by head and cross winds for that distance. When the target was 50 cm from the nozzle, the displacement was not significantly different between tail and head winds (Figure 4.5 (B)). A significant interaction was observed between the wind direction, distance from the target and motion of the applicator on the displacement of droplets (P<0.001). The droplets were not displaced significantly between the three wind directions and the two motions of the applicator, when the system was operating at 15 cm distance from the target (Figure 4.6 (A)). At 30 cm distance from the target however, a tail wind resulted in a significantly higher droplet displacement compared to a head wind for both the moving and the static applicator (Figure 4.6 (B)). However, when emitting droplets 50 cm from the target there were no significant differences between the displacements caused by a head and a tail wind, for the moving and the static applicator (Figure 4.6 (C)).

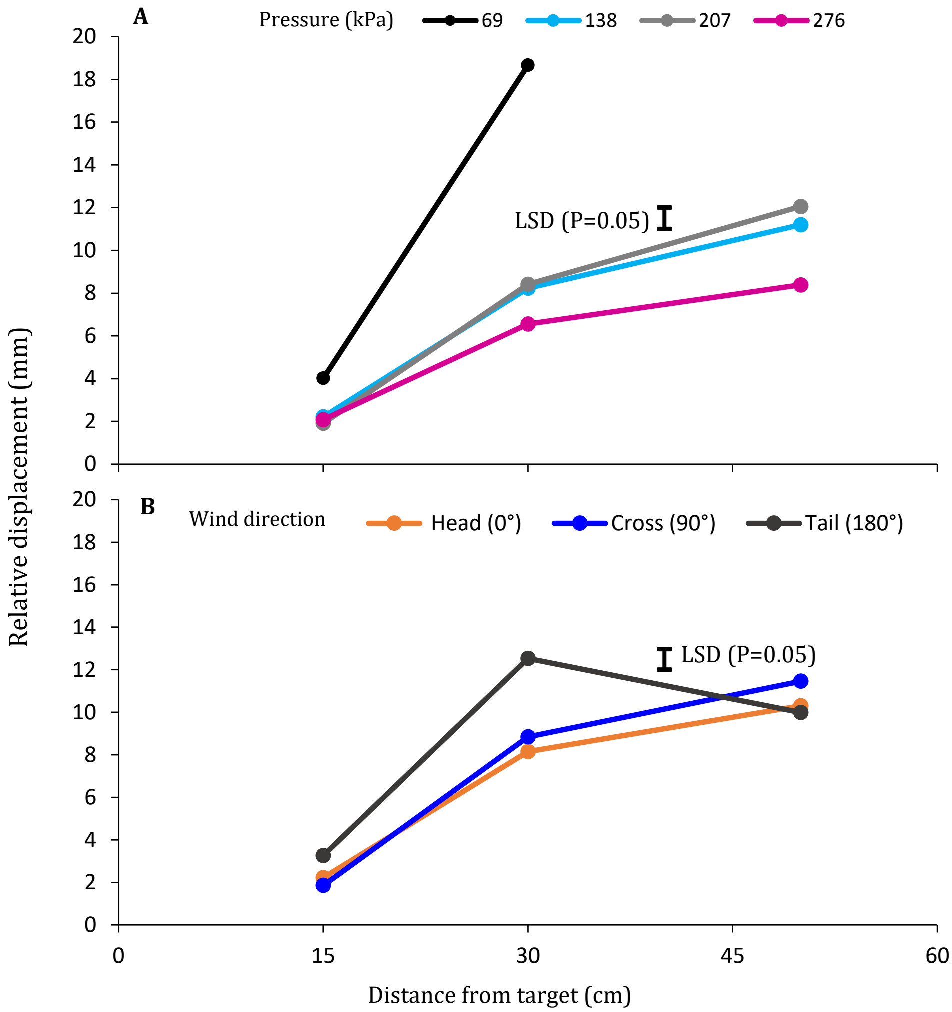


Figure 4.5. Effect of distance from target (15, 30 and 50 cm) on the relative displacement of droplets relative to the zero-wind control, as a result of application of (A) four pressures (69, 138, 207 and 276 kPa) and (B) three wind directions (0°, 90° and 180°) regardless of the motion of the applicator. Maximum l.s.d. was 0.99 and 0.97 for A and B respectively (P=0.05).

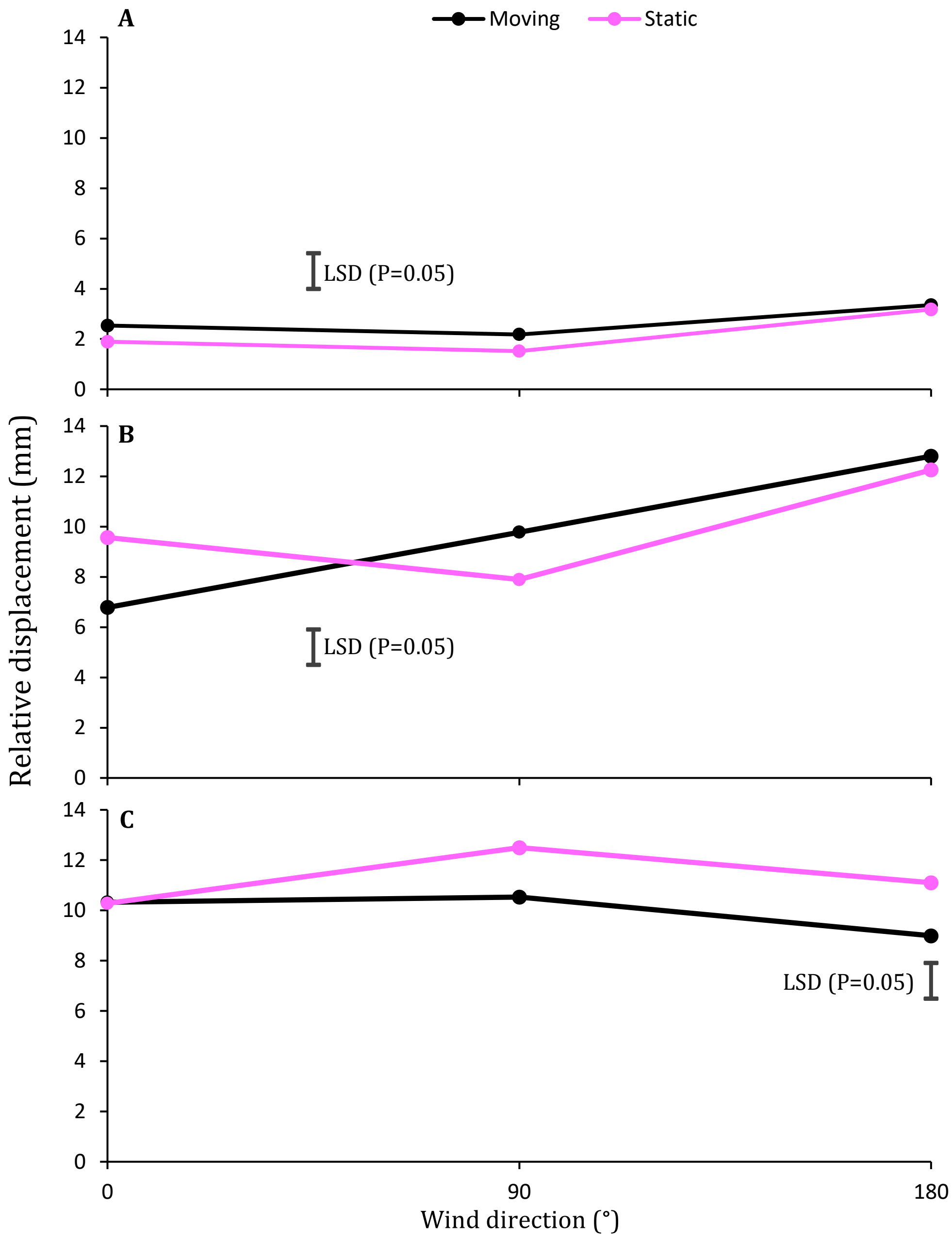


Figure 4.6. Effect of 10 km h-1 wind speed when wind was applied from three different directions of 0° (head), 90° (cross) and 180° (tail) and distance from the target at (A) 15 cm, (B) 30 cm and (C) 50 cm to the displacement of the droplets when applicator was static and moving across the four pressures. Maximum l.s.d. is 1.41 (P=0.05).

When the applicator was operating at 138 kPa, the interactions of motion and wind direction (P=0.047) and motion and distance from the target (P<0.001), had a significant effect on the displacement of droplets (Supplementary table 4.5). The displacement caused by the three wind directions did not differ significantly when the applicator was moving. However, when it was static the 7.3 mm mean displacement caused by a 180° (tail wind) was significantly higher than the 6.04 mm displacement caused by the wind coming from the 0° (head wind) (P<0.05, Figure 4.7 (A)). When the applicator was operating at 138 kPa and applying droplets at 15 cm from target there were no significant differences on the displacements associated with the moving and static applicator. For both motions of the applicator, at 138 kPa pressure, the droplets were displaced significantly further from the 0 km h^{-1} control, for higher distances from the target (30 and 50 cm) (Figure 4.7 (B))

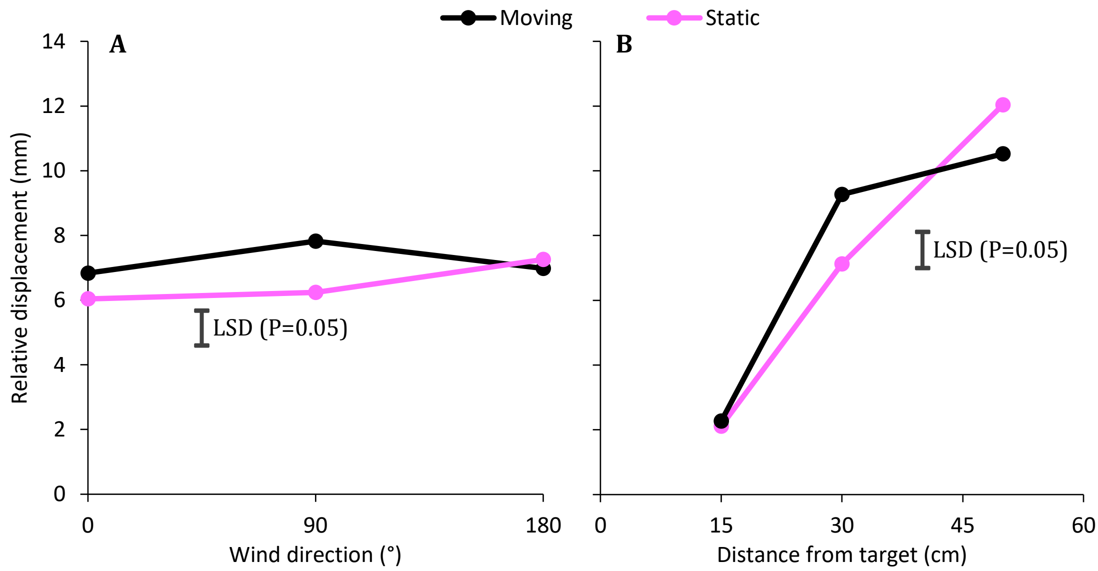


Figure 4.7. Effect of (A) wind direction and (B) distance from the target on the displacement droplets from the no wind control, when applicator is operating at 138 kPa. Maximum l.s.d was 1.07 and 1.11 for (A) and (B), respectively.

4.5 Discussion

Results presented in this paper estimated the time taken for the prototype applicator to dispense droplets of the size required for a DoD application system. The hypothesis that the time would not exceed 10 ms was accepted for the four operating pressures tested. The time taken to dispense a droplet of 1 μl ranged from 2.5 to 6 ms. Similar times (10 ms) were reported for the micro-spray systems of Lee *et al*. (1999) and Lamm *et al*. (2002). Also, Søgaard and Lund (2007) etsimated that to apply a droplet of 2.5 μl at 40 kPa, the time needed was 10 ms. Giles *et al*. (2004) however, used pulse durations ranging from 6 to 10 ms to apply dose rates of 37 μl. Furthermore, the hypothesis that when higher pressures will be used this will increase the volume of the droplets was also accepted and this may partly account for the higher volumes achieved by Giles *et al*. (2004), although nozzle aperture is also an important factor in relation to droplet sizes and flow rates.

The hypothesis that when the applicator operates at higher pressures the displacement of droplets will tend to be smaller, was accepted. The lowest operating pressure of 69 kPa could not be recommended for the nozzle tested as that was when the droplets were displaced further from the target. Higher pressures of 207 and 276 kPa were also unsatisfactory despite their potential for smaller displacements because the droplets were splitting into smaller ones which were deviating from the target especially at 30 cm and 50 cm distances. As it described by Vadillo *et al*. (2010), the main parts of a droplet generated by a DoD inkjet system are the tail, the filament and the main drop (Figure 4.8). When the droplet is falling, the tail and filament may be absorbed into the main droplet or, the filament can break up and create a separate droplet or multiple satellite droplets, if the Plateau–Rayleigh instability occurs (Vadillo *et al*., 2010). Without the effect of wind or any other disturbance which can compromise the stability of the system, satellites and main droplets should reach the same target and coalesce to become as a single droplet (Dong *et al*., 2006). Although, Dong and Vardillo's studies describe the droplets generated by DoD inkjet printers, which are much smaller than those used in this study, Castrejón-Pita *et al*. (2008) confirmed that the same principle applies to larger droplets as well. Interesting, in this study droplets disintegrated into smaller satellites even when no wind treatments were applied with a static applicator at 207 and 276 kPa pressure and further work to optimize nozzle aperture is recommended. Satellite droplets also occurred in the micro-sprayer system developed by Nieuwenhuizen *et al*. (2010). When it was tested in the field for control of volunteer potato, 1% of crop plants showed herbicide damage which may have been caused by these satellite droplets. It is important that droplets do not disintegrate, especially when they are travelling long distances. Therefore, for future usage of the applicator system presented here, in order to avoid formation of satellite droplets which might end up damaging the crop it is concluded and recommended that the operating pressure should be set at 138 kPa, since no satellites occurred and the system operated satisfactorily at this pressure.

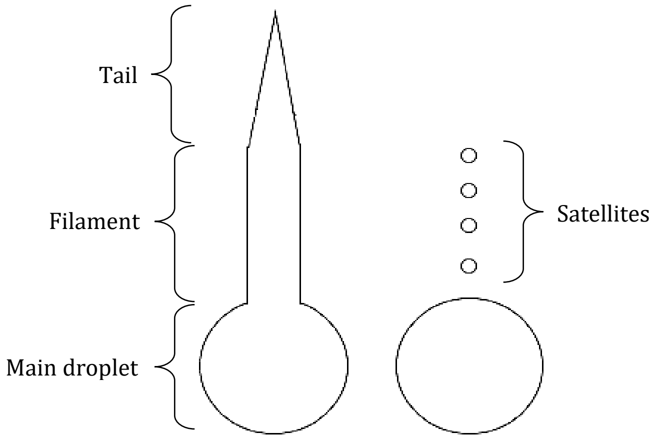


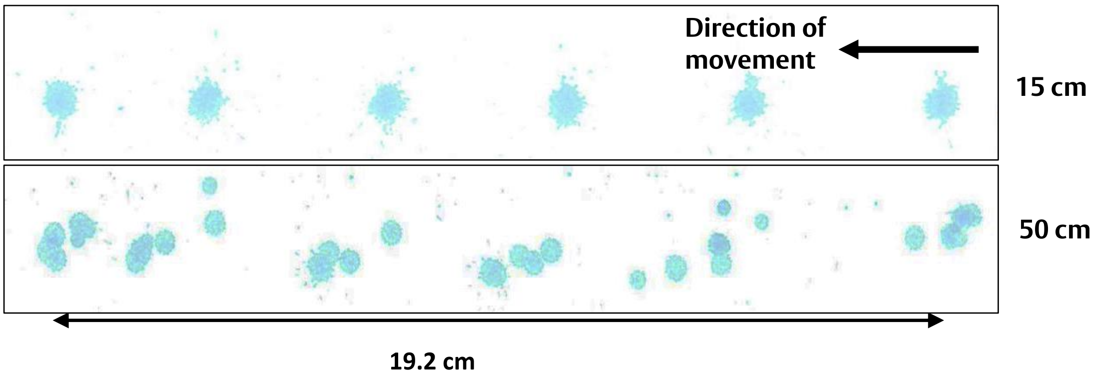
Figure 4.8. Parts of a droplet as they were described by Vadillo *et al.* (2010) and generated by a DoD inkjet.

The hypothesis that the displacement of droplets increases with distance from the applicator, was accepted and the smallest droplet displacement was observed when application was carried out at 15 cm distance from the target (the closest distance tested). Similar results were observed by Nieuwenhuizen *et al*. (2010) when evaluating the precision of an automated micro-spraying system. Although larger droplets were applied in that study (20 µl) the deviation of droplets was higher at 30 cm height when compared to 10 cm for both, the longitudinal and transverse deviation relative to the direction of the micro-spray system. Therefore, based on the present and Nieuwenhuizen's results, it is recommended that the nozzle outlet should be at 15 cm from the target, especially for small seedlings (leaf area=100 mm^2). If there is a need for the applicator to be higher above the crop (e.g. leek seedlings at planting are approximately 20 cm tall), then larger areas than 100 mm^2 could be targeted to account for increase in the displacement of droplets.

When the applicator was moving in a tail wind of 10 km h^{-1}, the droplets tended to be displaced further compared with the effect of a head wind, for the same wind speed. However, that was observed for 15 and 30 cm distance from the target only. When droplets were emitted at a height of 50 cm, the displacement caused by a tail wind was either smaller than what was caused by a head wind or not significantly different (latter observed at 138 kPa pressure). Therefore, the hypothesis regarding the displacement caused by the two wind directions for the moving applicator was accepted only for 15 and 30 cm distance from target. Also, from the tests with the static applicator, when the wind was coming at 180° (same as tail wind when moving) droplets were displaced further when compared with a 90° (head wind). This suggests that there might be an issue in the design of the gantry system or a wind turbulence causing the droplets to displace further, with a tail wind. With regards to the droplet displacement at 50 cm from the target, there is a certain level of uncertainty especially in the cases of 207 and 276 kPa pressures, because of the amount of satellite droplets not hitting the target. Although the number of split droplets was not quantified for these pressures at 50 cm distance, estimating where they would have landed and their deviation from the zero-wind control was trivial.

Based on these results it is recommended that for safe use of the applicator in field conditions, with minimal droplet displacement, the operating pressure should be set at 138 kPa and the distance from the weeds should be fixed at 15 cm. Although, there may be an increased risk of droplets hitting the ground and/or the crop if a 30 cm nozzle height is required, the effect of wind might be minimized with the use of wind baffles.

4.6 Supplementary material



Supplementary figure 4.1. Droplets applied from the moving applicator when operating at 69 kPa at 15 and 50 cm from the target with 0 km h^{-1} wind speed. Applicator traversed five times. Satellite droplets are evident in the 50 cm distance. Apparent spattering in the 15 cm and 50 cm is because gantry traversed five times over a short period of time and applied to the wet surface before previous application had dried. No spattering is observed from a single traverse of the gantry (Figure 4.2).

Supplementary table 4.1. Coefficients (±SE) of the linear models fitted to the time and weight of water data for each pressure for the calibration test.

Pressure (kPa)	Coefficients	Estimate	Standard error	*P*-value
69	Intercept	-0.2704	0.024	<0.001
	Time	0.2279	0.004	<0.001
138	Intercept	-0.3943	0.03	<0.001
	Time	0.3873	0.005	<0.001
207	Intercept	-0.4014	0.02	<0.001
	Time	0.4937	0.003	<0.001
276	Intercept	-0.3839	0.01	<0.001
	Time	0.5880	0.003	<0.001

Supplementary table 4.2. ANOVA table of the linear models fitted to the time and pressure data for the calibration test. Model coefficients are presented in

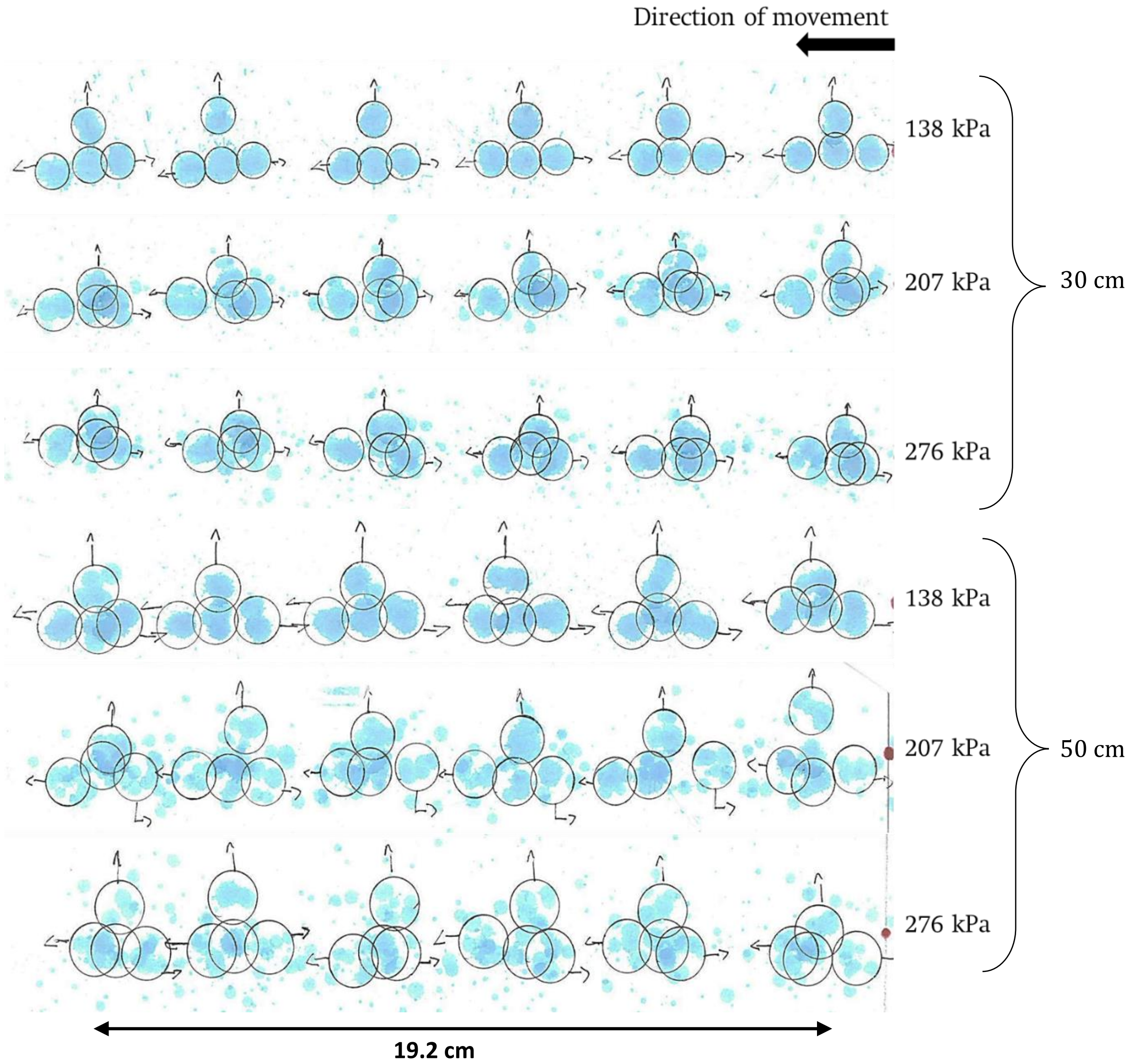
Source of variation	d.f.	Sum of Squares	Mean Sum of Squares	*P*-value
Time	1	216.024	216.024	<0.001
Pressure	1	58.523	58.523	<0.001
Time.Pressure	1	21.141	21.141	<0.001
Residuals	156	1.377	0.009	

Supplementary table 4.3. Coefficients (±SE) of the logarithmic model fitted to the pressure and time data presented in Figure 4.4 for the calibration test.

Coefficients	Estimate	Standard error	*P*-value
Intercept	16.773	0.81	0.002
log(Pressure)	-2.565	0.16	0.004

Supplementary table 4.4. Unbalanced ANOVA of the relative displacement of droplets due to effects of distance from the target, pressure, wind direction and motion of the applicator. Design was unbalanced because displacement could not be measured for the combination of pressure of 69 kPa and 50 cm distance from the target. Overall factorial design is for three treatments x four pressures x three wind directions x 2 motions with four repetitions.

Source of variation	d.f.	Sum of Squares	Mean Sum of Squares	*P*-value
Replication	3	19.714	6.571	0.04
Distance	2	3721.49	1860.74	<0.001
Pressure	3	1592.65	530.882	<0.001
Wind Direction	2	230.866	115.433	<0.001
Motion	1	1.791	1.791	0.38
Distance.Pressure	5	806.937	161.387	<0.001
Distance.Wind Direction	4	259.085	64.771	<0.001
Pressure.Wind Direction	6	161.368	26.895	<0.001
Distance.Motion	2	33.866	16.933	<0.001
Pressure.Motion	3	33.981	11.327	0.003
Wind Direction.Motion	2	22.196	11.098	0.01
Distance.Pressure.Wind Direction	10	143.456	14.346	<0.001
Distance.Pressure.Motion	5	72.065	14.413	<0.001
Distance.Wind Direction.Motion	4	92.699	23.175	<0.001
Pressure.Wind Direction.Motion	6	26.737	4.456	0.08
Distance.Pressure.Wind Direction.Motion	10	45.319	4.532	0.04
Residual	180	414.915	2.305	
Total	248	7679.13	30.964	



Supplementary figure 4.2. Results from moving applicator operating at 138, 207 and 276 kPa dispensing droplets from 30 and 50 cm from the target. Area marked with a circle indicates where five droplets were applied. Arrows points the displacement of the droplets when wind blowing at 10 km h^{-1}. Apparent spattering is because gantry traversed five times over a short period of time and applied to a wet surface before previous application had dried.

Supplementary table 4.5. Anova table of the relative displacement of droplets due to effects of the distance from the target, wind direction and motion of the applicator. The applicator was operating at 138 kPa pressure.

Source of variation	**d.f.**	**Sum of Squares**	**Mean Sum of Squares**	***P*-value**
Replication	3	2.869	0.956	0.612
Distance	2	961.333	480.667	**<0.001**
Wind Direction	2	6.107	3.054	0.154
Motion	1	1.499	1.499	0.333
Distance.Wind Direction	4	3.235	0.809	0.725
Distance.Motion	2	41.519	20.76	**<0.001**
Wind Direction.Motion	2	10.213	5.107	**0.047**
Distance.Wind Direction.Motion	4	5.816	1.454	0.457
Residual	48	75.362	1.57	
Total	68	1107.954	16.293	

Chapter 5
Discussion and conclusions

Throughout the thesis the main objective was to evaluate the efficacy of leaf-specific weed control for field vegetables in the UK. Work presented here is part of a project developing a robotic weeder for vegetable crops. The robotic platform will use an image analysis system to distinguish the crop from the weed and then a novel applicator will apply a dose as a single droplet.

Efficacy of herbicide droplet application for the leaf-specific weed control was tested in the glasshouse and the field. Amounts of glyphosate and glufosinate-ammonium, needed to control individual weed species at different growth stages, were quantified in the glasshouse (Chapter 2). Also, efficacy of weed control using droplets of 2,4-D, alone and mixed with glyphosate and glufosinate-ammonium was demonstrated. The efficacy of weed control was also replicated in the fields with cabbages and leeks, using manual droplet application. Application of droplets containing known amounts of herbicides was carried out on a per plant and individual leaf basis (Chapter 3). Cabbage and leek yield and quality was compared with current spraying methods and hand-weeding. Based on the yields produced, preliminary economic analysis was carried out in order to determine the profit or loss likely to be associated with the use of an automated platform when compared with pre and post-emergence sprays or with hand-weeding (Chapter 3). Finally, droplets appropriate for Drop-on-Demand herbicide application were applied using a prototype applicator and the displacement of droplets was estimated. Recommendations for future applications in the field were made.

Hypothesis 1: Leaf-specific applications of droplets of a systemic, translocated herbicide at the recommended dose for conventional spraying achieves at least 90% efficacy of weed control in both glasshouse and field.

There is evidence in the literature that weeds can be controlled using micro-doses of herbicides when they are applied as single droplets per weed, with DoD applicators (Giles *et al*., 2004; Graglia, 2004; Lund *et al*., 2006; Søgaard *et al*., 2006; Nieuwenhuizen *et al.*, 2010; Basi *et al.*, 2012; Mathiassen *et al.*, 2016; Utstumo *et al.*, 2018). The volume of droplets applied in these studies ranged from 0.8 µl to 37 µl with respective amounts of herbicide in the range of 1 µg to 450 µg of active ingredient per plant. Although efficacy of weed control of 80% and higher is reported, there is no indication how these dose rates relate to the label recommendation if the herbicide was sprayed. Furthermore, no other broad-spectrum herbicide than glyphosate was tested.

To test the hypothesis glasshouse and field experiments were conducted. In the glasshouse, dose-response trials were carried out using commercial herbicides containing glyphosate, glufosinate-ammonium and 2,4-D. Doses applied were relative to the minimum label recommendations of the herbicides and were calculated based on the mean ground cover of the seedlings, for each trial. This resulted in application of specific amounts of herbicide (µg) as a single droplet to a leaf. In the field, known amounts of glyphosate (9 or 36 µg) and glufosinate-ammonium (7.5 or 60 µg) were applied as single droplets per plant of per leaf. The hypothesis was accepted for both the glasshouse and field trials. In the glasshouse application of the minimum label recommendation controlled most of the weed species as described in Chapter 2. However, in the cases of *Poa annua* and the perennial *Rumex crispus* higher doses were needed. For *R. crispus* the ED_{90} dose was equivalent to 10-times the recommended rate of 540 g of glyphosate ha^{-1}. Although the ED_{90} the value was imprecisely estimated and not significant, when the weed was tested in a glasshouse study, a dose of 7200 g of glyphosate ha^{-1} was having an effect before 100% mortality (Boutin *et al.*, 2004). For *P. annua* however, lower doses than those reported here are needed for effective weed control when glyphosate (Binkholder *et al.*, 2011) and glufosinate-ammonium (Riemens *et al.*, 2008) are applied as a broadcast spray to the weed. The approximately five-times the recommended rate of glyphosate and glufosinate-ammonium dose needed for 90% control of *P. annua* could be a result of limited translocation of the herbicides. The herbicide droplets were applied at the growing point of the weed, which sometimes was not clear.

Efficacy of weed control from herbicide droplets applied to a single leaf was if not better, at least as good as what is expected if the herbicides were applied using a conventional spray. Mixtures of glyphosate or glufosinate-ammonium with 2,4-D have also been identified as potential alternatives for droplet applications but this conclusion needs to be tested in the field. A major improvement in application technology is that the amount of herbicide applied to each plant is known whereas, when spraying, only the average per unit land area is known. In the glasshouse, annual weeds that were up to the 4-leaf stage were controlled with a dose of 32 μg of glyphosate and 28 μg of glufosinate-ammonium per seedling.

In the field a dose of 36 μg of glyphosate applied as a single droplet per weed and a dose of 9 μg applied as multiple droplets per weed based on the size of individual leaves, provided over 90% levels of weed control. Application of glufosinate-ammonium in the field, as a single droplet per weed with a dose of 60 μg failed to provide a good level of weed control (~ 80%). Phytotoxic effects were observed after the application of a single droplet of glufosinate-ammonium. The 20% concentration used to apply the 60 μg dose appeared to be causing necrotic spots on the leaves, which might have limited the uptake of the herbicide by the plant. However, when the lower dose of 7.5 μg of glufosinate-ammonium applied on a per leaf basis caused high levels of weed control. Although some small phytotoxic effects were observed, the lower herbicide concentration used (2.5%) and applications of droplets to more than one leaf, might have resulted to better uptake and translocation of the herbicide, which resulted to plant death.

Hypothesis 2: The yield, quality and economic value of cabbage and leek crops, where weeds are controlled leaf-specifically, are not significantly lower than for either conventional pre- and post-emergence herbicide treatments or for hand-weeding.

The automated systems for weed control already described in the literature, the research reports are limited to assessing the efficacy of weed control (Giles *et al*., 2004; Søgaard *et al*., 2006; Nieuwenhuizen *et al*., 2010; Utstumo *et al*., 2018); impacts on crop yield and profitability were not estimated. Furthermore, these studies were limited to single weeding treatments and do not make compare efficacy with current spraying methods of conventional herbicides.

The main hypothesis presented in Chapter 3 was accepted for both cabbages and leeks, since the efficacy of weed control and the yield produced and economic value of the crops achieved from the droplet treatments were not statistically significantly lower than the weed-free controls and current spraying methods. Efficacy of weed control from the pre and post-emergence sprays of commercial herbicides ranged at the low levels of 30% to 50% when they were applied in fields with leeks. Probable explanations for the low efficacy are that these treatments were applied early in the growing season and the soil seed bank appears to have contained several weed species (~10) which are not common in fields where vegetables are grown and they occurred in high numbers (~ 400 to 500 weeds m^{-2}). The pre-emergence herbicide pendimethalin, which was applied five days before planting, is a selective herbicide controlling most annual grass weeds and a limited number of broad-leaf weeds (Pal *et al*., 1991). According to the label of Stomp Aqua® weeds like groundsel (*S. vulgaris*), black nightshade (*S. nigrum*) and common yarrow (*A. millefolium*) are not controlled and mayweed (*M. recutita*) and shepherd's purse (*Capsella bursa-pastoris*) are only moderately susceptible to pre-emergence applications of pendimethalin. All these species were, however, abundant in plots where the pre-emergence spray was applied which probably accounts for the poor levels of weed control and significant yield losses from that treatment. For the post-emergence spray in leeks bromoxynil was used (Buctril®), which is a selective herbicide not used for control of grass weeds (Culpepper *et al*., 1999, Corbett *et al*., 2004). Although broad-leaf weeds were controlled, grass weeds were not affected and indeed spread in the plots where bromoxynil was applied. Furthermore, the mandatory eight-week interval from the post-emergence spray to harvest was sufficient to allow re-growth of some broad-leaf weeds, which were observed during harvest. Because of failure to control the weeds, growers would have been well-advised to combine the pre- and post-emergence sprays with at least one intra-row cultivation and/or inter-row hand weeding.

Trimmed yield and marketable yield of cabbages produced from the droplet treatments were not significantly different than the weed-free controls for both years. What was observed when harvesting cabbages was the big variation in head size, especially for the savoy during the 2016 trial. In this study both cabbage varieties were harvested once, when the majority of heads had reached maturity. However, when harvesting cabbages, the common agricultural practice is harvesting the crop in different times to ensure that all heads have reached a marketable size (e.g. savoy: 500 g per head) (Stoffella & Fleming, 1990). The primary focus of the field trials here was the effect of herbicide treatments on the efficacy of weed control so that residual weed infestations at crop harvest had to be assessed at the same time. Harvesting cabbage at different times according to size and postponing weed harvesting until all the cabbages in a plot had been harvested, would have allowed differential weed regrowth until all heads had been harvested and the relative effect of droplet treatments to weeds would have been confounded with the time of harvesting the cabbages.

Going forward, a critical question is: would the reductions in amounts of herbicides applied justify the investment in an automated platform? A preliminary economic analysis was based on the yields produced from the field trials. For leeks, a very high increase in crop profitability associated with droplet applications of both glyphosate (£22,255 ha^{-1}) and glufosinate-ammonium (£20,617 ha^{-1}) compared with spraying commercial herbicides (pre-emergence). For cabbages however, only the adjusted glyphosate treatment appeared to be profitable for both years when compared with pre-emergence spraying. Making the questionable assumption that the hand-weeded, weed-free treatment in the field experiments was equivalent to a commercially hand-weeded crop, controlling weeds by leaf-specific droplet application would be less profitable than hand-weeding for leeks and savoy cabbages. It is of course, recognized that commercial hand-weeding would not need to achieve a weed-free condition and it is also highly unlikely that sufficient labour would be available especially given the expected immigration controls after Brexit.

Hypothesis 3: In the field, herbicide inputs per unit land area are significantly lower when droplet applications are compared with spray applications of commercial herbicides and do not exceed the label recommendations for the products used.

When weeds are treated leaf-specifically, the predictions on potential herbicide savings are expected to be at least 94% (Christensen *et al*., 2009; Zijlstra *et al.*, 2011; Blackmore, 2014). The studies from Søgaard *et al*. (2006) and Mathiassen *et al*. (2016) have quantified the amounts of glyphosate needed for weed control and in order to demonstrate herbicide savings, weed densities from 100 m^{-2} to 300 m^{-2} were assumed. Only in the study by Utstumo *et al.* (2018) the application rate was estimated (191 g of glyphosate ha^{-1}) when the Asterix robotic platform was tested in field with a carrot crop. However according to the authors two to three treatments will be needed for effective weed control which reduces further the potential herbicide savings. Also, reductions in herbicide use were expressed relative to broadcast spraying of glyphosate only and not to any other commercial herbicides. In this study droplets applied per plot were counted for the individual droplet treatments, throughout the growing season of the crops and therefore, allowing the overall application rate to be calculated.

The hypothesis regarding significant reductions in herbicide use was accepted. In cabbages the reductions in herbicide inputs when glyphosate droplets were used in three occasions, were from 91% to 98% when compared to the conventional pre-emergence spray of pendimethalin. The amounts of 119 and 28 g of glyphosate ha^{-1} applied for the 2016 and 2017 trials, respectively is also lower than the label recommendation for spraying glyphosate (540 - 2160 g ha^{-1}). Leeks are known to be weak competitors against weeds (Baumann *et al.*, 2001) and because of the long critical period for weed cotnrol (Tursun *et al.*, 2007) more droplet treatments were needed, to achieve a 'satisfactory' (say over 95%) level of weed control. Applications of droplets containing glyphosate or glufosinate-ammonium demonstrated that herbicide inputs can be reduced by up to 82% and 74% respectively in leeks when compared with the combined treatment of a pre and a post-emergence spray. Although more droplets were applied to the leek trials, amounts of herbicide actives were within the label recommendations, if not lower. Even in the very weedy field of the 2017 trial, label recommendations for a single treatment with glyphosate were not even exceeded when droplets were applied on a weekly basis.

The critical period for weed control, is the period during the crop growth when weeds must be controlled to prevent yield losses (Nieto *et al*., 1968). Studies with cabbages have demonstrated that the crop does not have a critical period for weed control, and a single weeding treatment between three and five weeks after planting is all that is needed to avoid yield loss (Weaver, 1984; Roberts *et al*., 1976). However in this study three droplet treatments were needed to produce yields with no signifncalt yield penalty and a single treatment gave poor levels of weed control and significantly lower yields. The reasons why more than one droplet treatment was needed were that during the first visit to the field very small seedlings (leaf area <1 cm^2) were left untreated to avoid accidental applications to soil, on the basis that they might not be targeted accurately enough by an automated system. Also, since the aim of applying droplets to weed leaves is that no herbicide will end up on the crop, some weeds very close to the crop were deemed unsafe to treat due to the risk of collateral damage – the intention being that if such weeds were allowed to grow larger, some individual leaves could be targeted without risking crop damage. A third reason was to ensure effective weed control if weeds were poorly controlled or emerged after previous applications.

Unlike cabbages, leeks have a clear critical weed-free period and in order to avoid yield losses of over 5% in leeks, the crop needs to remain weed-free from 1 to 12 weeks after planting (Tursun *et al.* 2007). Consistent with Tursun *et al.*'s research, multiple treatments using droplets of glyphosate and glufosinate-ammonium were found necessary to keep the crop largely weed-free. Leek yields, produced from the weekly applications (10-times) of glyphosate droplet starting from 3 up to 12 weeks after planting, produced yields which were not significantly lower than the weed-free controls. Although fortnightly (5-times) glyphosate droplet applications achieved high levels of weed control when glyphosate was applied to each leaf (over 100 mm^2), it yielded significantly lower than the hand-weeded controls. A possible reason for that could be an infestation with leek miner which resulted in overall lower leek yields for the 2018 trial. For weed control in leeks using an automated platform, it is envisaged that fortnightly treatments will be needed.

The prototype DoD applicator developed by Concurrent Solutions LLC was tested under indoor conditions to study the effect of wind, hydraulic pressure and distance from the target on the displacement of droplets. The time taken for the applicator to dispense a droplet of 1 µl ranged from 2.5 to 6 ms, with similar times (up to 10 ms) reported in the literature when micro-dosing systems were tested (Lee *et al*., 1999: Lamm *et al*., 2002; Søgaard & Lund, 2007; Giles *et al*., 2004; Urdal *et al*., 2014). The hypothesis regarding the effects hydraulic pressure, distance from the target and wind speed was accepted.

Although with the use of higher pressures of 207 and 276 kPa the displacement of droplets was smaller, satellite droplets were observed which deviated unacceptably from the target especially when the nozzle was 30 or 50 cm above the target. Since this effect of a single droplet splitting into smaller ones was observed when no wind was applied, it is recommended that the operating pressure should be set at 138 kPa as at that pressure droplets did not disintegrate. When the applicator was operating at

138kPa, the largest droplet displacement (12 mm) was observed when a constant wind of 10 km h^{-1} was applied and droplets were emitted from a 50 cm height above the target. The smallest droplet displacement, which ranged from 1.8 to 4 mm was observed when the applicator was operating at 15 cm above the target with similar results reported by Nieuwenhuizen *et al*. (2010). Therefore, it is recommended that the nozzle outlet should be at 15 cm from the target, especially for small seedlings (leaf area ≤100 mm^2). If there is a need for the applicator to be higher above the crop (e.g. a leek seedling at planting is approximately 20 cm tall), then larger areas than 100 mm^2 could be targeted to account for increase in the displacement of droplets.

5.1 Automated platform recommendations

Based on the results presented in Chapters 2, 3 and 4 recommendations regarding the use of an automated platform in field conditions are made. Provided that the onboard machine vision system would be able to identify and target weed leaves of approximately 100 mm^2, droplets containing 9 µg of glyphosate or 7.5 µg of glufosinate-ammonium are recommended. If the crop/weed classification system would be able to identify individual weeds, then a single droplet containing 36 µg of glyphosate would be appropriate for weed control. Because of the herbicide's limited translocation, glufosinate-ammonium is not recommended to be applied as a single droplet per weed.

In fields with transplanted cabbages, for effective weed control droplets containing glyphosate should be applied on three occasions starting at approximately three weeks after planting (or with weed emergence) with the last application carried out at five or six weeks after planting. For the last droplet application, crop canopy closure must be taken into consideration to allow for the platform to navigate through the field without damaging the crop. Furthermore, since the detrimental effect of accidental droplet application has been demonstrated, weeds very close to the crop should be avoided. Although there is a level of uncertainty regarding the profitability associated with the use of an automated platform for cabbages however, there are environmental benefits associated with high reductions in herbicide use. For robotic weed control in leeks, weekly applications using glyphosate or glufosinate-ammonium droplets, is recommended.

Regarding the safe use of the applicator it is recommended that the operating pressure should be set at 138 kPa and the distance from the weeds should be fixed at 15 cm. Although, there may be an increased risk of droplets hitting the ground and/or the crop if a 30 cm nozzle height is required, the effect of wind might be minimized with the use of wind baffles.

5.2 Recommendations for further research

From the dose-response studies in the glasshouse, the potential of using mixtures of glyphosate or glufosinate-ammonium with 2,4-D was demonstrated. However, the efficacies of the mixtures for weed control need to be tested in the field. Since only one adjuvant was used further work is needed to test different compounds which are commercially available in the UK and are used for spraying glyphosate (e.g. Silwet L-77). Furthermore, higher concentrated glyphosate formulations which contain wetting agents (e.g. Roundup® Flex and Roundup® Ultimate), could be tested for leaf-specific droplet applications. To be able to compare efficacy of weed control by droplet application to that achieved by broadcast spraying the herbicide, a dose-response trial is suggested where doses relative to recommend rates for spraying are applied to the same species, both as single droplets per plant and overall spraying. This will test the hypothesis regarding the potential of better weed control by droplet application. Apart from comparing the efficacy of weed control, an experiment like that can compare the translocation and absorption of herbicide between the two methods application if, for example, a C14-labelled herbicide is used. A detailed protocol is described by Nandula and Vencill (2015).

Furthermore, since the only perennial weed tested in this study was not effectively controlled, dose-response trials using other perennial weed species are suggested. Also, an experiment where droplets are applied under different environmental conditions is recommended. Drought and heat stresses are known to make post-emergence herbicides less effective, because they reduce the absorption, translocation and metabolism (Zhou *et al.*, 2007).

Over the last 20 years, a lot of research has focused on different machine vision techniques in order to improve the weed/crop classification (Lee *et al*., 1999, Utstumo *et al*., 2018). The classification accuracies ranged from 65% to 95% however, most have been conducted under ideal conditions and are not suited for real field conditions. Challenges such as occlusion between crop and weed plants, leaves overlapping, varying lighting conditions and different growth stages of the plants need to be overcome for a robust automated weeding machine (Wang *et al*., 2019). Although it was not an objective of this thesis, the machine vision which is developed by Concurrent Solutions LLC needs to be tested under field conditions with vegetable crops. Before any herbicide applications are carried out in the field, the applicator needs to be tested under indoor conditions. An experiment is recommeded to test the retention and efficacy of droplets containing the suggested doses of 9 μg of glyphosate and 7.5 μg glufosinate-ammonium, with easy-to-wet species like *Stellaria media* or *Solanum nigrum* and difficult-to-wet species like *Chenopodium album*. An overall final experiment of the robotic platform in field conditions with cabbages and leeks, should test the crop/weed recognition system, the targeting accuracy and the biological efficacy of droplet application with different speeds of the plaform.

5.3 Concluding remarks

Weed control is one of the most important factors in agricultural and horticultural production. Broadcast spraying of herbicides has been one of the major tools for effective weed control. Vegetable crops represent a small market compared to other major agronomic crops, which does not make them a priority for herbicide development from the agrochemical industry. With no new herbicide mode of action in over 30 years and legislations from policy makers that limit the use of pesticides, herbicide-based weed control is becoming increasingly challenging. Meanwhile, shortages in farm labour and higher costs associated with manual weeding has left vegetable farmers with only few weed control options available.

These pressures have resulted in a significant amount of research, developing new methods for weed control. One example is the use of automated platforms which merge traditional herbicide chemistry with robotics. These robotic weeders are a promising new tool for weed control for vegetable crops as they can be cheaper to develop and safety concerns and environmental impacts are much less than for herbicides.

Work presented in this thesis was designed to contribute to the development of an automated platform for robotic weed control in vegetable crops. The essence of the platform is to detect the weeds and then apply droplets of herbicides on the leaves. Efficacy of weed control using leaf-specific droplet application of broad-spectrum herbicides has been demonstrated in the glasshouse and replicated in fields with cabbages and leeks. Efficacy of weed control and crop yield and value did not differ significantly from hand-weeded controls and current spraying methods. Over 90% and 75% reductions in herbicide inputs have been achieved for cabbages and leeks respectively when droplet treatments were compared with commercial herbicide spraying. Engineering-related work with a prototype applicator demonstrated that droplets appropriate for leaf-specific application were emitted at 138kPa pressure and 15 cm distance from target with minimal displacement when constant wind of 10 km h^{-1} was applied.

These results prove the concept of leaf-specific weed control which represents a paradigm shift to current spraying methods and when commercialized, will provide an alternative approach to weed management for vegetable growers.

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Appendix

Aspects of Applied Biology 132, 2017
International Advances in Pesticide Application

Dose response relationship of droplet applications for the leaf-specific weed control in vegetable crops

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Summary

As a prelude to leaf-specific weed control using droplets targeted by a robotic weeder, amounts of herbicide required to control individual weed seedlings were estimated. Roundup Biactive was applied at doses equivalent to 1/128th to four times the recommended rate in addition to undiluted Roundup and water controls. Based on the mean ground cover of the seedlings, the recommended dose (1.5 l ha^{-1}) was estimated and droplets were applied to individual plants by micropipette. All treatments contained 1% AS 500 SL, Agromix (adjuvant). Three weeks after application dry weights (DW) of each seedling was recorded. DW reductions of 50% were achieved in the five species tested at less than the recommended rate whereas only in one species was a 90% reduction obtained at that rate. In *Galium aparine* for example, 19.3 µg of glyphosate reduced DW per plant by 90% compared to the recommended dose of 8.4 µg.

Key words: leaf-specific weed control, droplet application, dose-response, glyphosate, vegetables

Introduction

Weeds pose a major threat to agriculture and can severely impair crop yield and/or quality. They can cause 30 – 45% yield losses among the major crops worldwide, and 100% loss occurs in extreme cases due to the inability to achieve effective control (Christensen *et al.*, 2009; Singh *et al.*, 2014). Weed control is therefore vital for achieving high yields and good quality crops. But with fewer herbicide active ingredients available, increasing resistance to herbicides and the risk of water contamination, new approaches to weed control are needed.

While it might be nice to ban the use of pesticides, food security is linked to the availability of adequate supplies of good quality food at affordable prices. Ignoring price, it is well-established that organically grown crops consistently yield less (by 5-34%) than conventionally-grown ones (Seufert *et al.*, 2012). Another policy issue is that the use of genetically-modified, herbicide-tolerant crops (GM-HT) is not currently permitted in the UK. This paper therefore relates to an engineering solution to the problem of weed control as an alternative to either selective chemistry or GM-HT crops.

Miller *et al.* (2010) developed an image analysis-based weed detection and spot spraying system which achieved 90% control of volunteer potatoes in carrots and onions. However, some collateral damage to the crop is inevitable with this system. Christensen *et al*. (2009) mentioned "drop on demand technology" which is a leaf-specific herbicide application system using gravity-fed droplets is mounted on the BoniRob field robot. Bonirob was developed for phenotyping crop varieties in field trials by Ruckelshausen (2009) and a smaller version of that, "Weedy" (Klose *et al*., 2008) was described for plant specific weed control using directed sprays. Blackmore (2013) predicted that such systems might reduce herbicide inputs by up to 99.9%.The study reported in this paper is part of a project developing a system for herbicide droplet applications to individual leaves of weeds in field vegetables. The objectives of the research were (1) to estimate the amount of herbicide needed to control individual weed seedlings using leaf-specific droplet applications and (2) to assess the consequences of accidental damage to the crop. Experiments have been carried out on four weeds and on Savoy cabbage as a crop. Detailed results are presented here for *Galium aparine* and the cabbage.

Materials & Methods

Glasshouse trials were carried out at Reading during summer 2015 in order to quantify the volume and number of herbicide droplets required to control the annual weeds *Galium aparine, Chenopodium album, Matricaria recutita,* the perennial weed *Rumex crispus* and also to Savoy cabbage seedlings (*Brassica oleracea* var. *sabauda*). Dose-response relationships were analyzed to estimate the amount of herbicide required to reduce biomass by 50 and 90% (the ED50 and ED90 values respectively).

G. aparine and *M. recutita* seeds were provided by the Herbiseed Ltd and *C. album* and *R. crispus* by the University of Reading. The cabbage seedlings were supplied by Hammond Produce. The seeds were sown on the surface of J. Arthur Bower's multi-purpose compost in multicell plastic trays. After the seeds had germinated they were thinned to one seedling per cell. 180 seedlings of *G. aparine* were treated at the 4-leaf stage. The cabbage seedlings were transplanted at the 2-leaf stage into individual pots (9 cm diameter) and left to grow until they reached the 4-leaf stage when they were treated. Treatments were complete randomized blocks with 15-30 replications according to the number of seedlings available.

Roundup Biactive (Monsanto, 360 g l^{-1} glyphosate) was used at different rates related to the recommended dose rate of 1.5 l ha^{-1}. Based on visual inspection, all treatments, except for the pure water controls included 1% of the adjuvant AS 500 SL, Agromix (Woznica *et al*., *2015)* (Fig. 1). Based on the mean leaf area of plants, the recommended dose was estimated in µg of glyphosate per plant. Leaf area as viewed from above was estimated using photographs which were analysed using the WinDias software (Fig. 2). The recommended rates applied were 8.44 µg and 112.32 µg of glyphosate per seedling for *G. aparine* and cabbage, respectively.

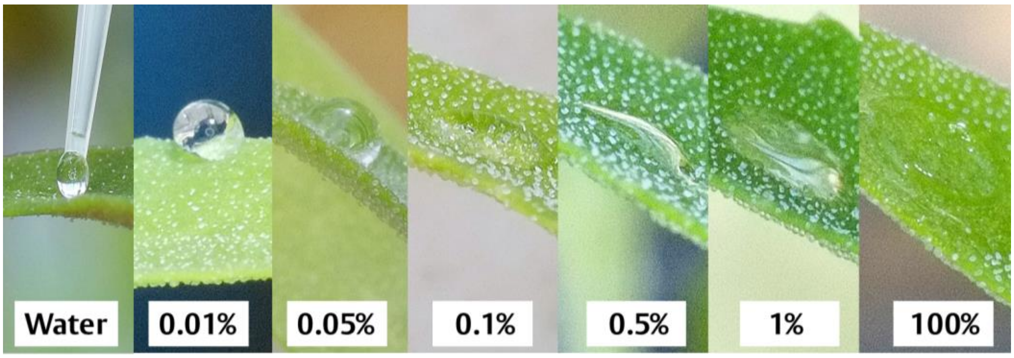

Fig. 1. Images show 1 µl droplets of water with different concentrations of AS 500 SL applied to *Chenopodium album* leaves with a micropipette.

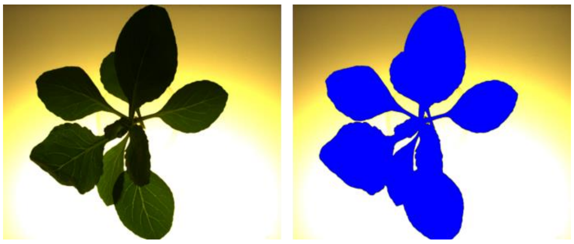

Fig. 2. Images of savoy cabbage seedlings before (left) and after (right) image analysis to assess ground cover using Windias software

Two droplets of 1.56 µl were applied to one leaf on the cabbage seedlings and one droplet of 1 µl was applied to the *G. aparine*. The rates used ranged from 1/128 to four times the recommended dose in addition to undiluted Roundup Biactive, water and adjuvant controls. Dilutions were prepared with deionized water. Three weeks after the application fresh and dry weights of the seedlings were assessed using an analytical balance (weighing to the nearest 0.0001 g). To produce the dose response curves the biomass data were fitted to the four parameter logistic model using R software as described by Ritz & Streibig (2007):

$$f(x,(b,c,d,e)) = c + (d - c) / (1 + \exp\{b(\log(x) - \log(e))\})$$

Where b is the relative slope around parameter e, c is the lower limit of dose response curve, d is the upper limit of dose response curve and e is the ED_{50}.

The ED_{90} value was calculated as follows (Knezevic *et al*., 2007):

$$ED_{90} = e(90 / (100 - 90))^{(1/b)}.$$

Results

For *G. aparine* the recommended dose was estimated at 8.44 µg per plant and reduced the biomass of the seedlings by 43% (Fig. 3). Similarly in the case of the cabbage seedlings, the biomass data were able to fit in a dose response curve with all of the parameters being statistically significant (Fig 5, Table 1). The cabbage crop was susceptible to the herbicide because of the ED50 value was less than half of the recommended dose (Table 1). DW reductions of 50% were achieved in the five species tested at less than the recommended rate whereas only in *M. recutita* was a 90% reduction obtained at that rate (Table 2).

Table 1. *Parameters (±se) of the logistic dose-response model and ED90 value for the dry weight data of the* Galium aparine *(df: 161) and cabbage (df: 386) seedlings.*

	Galium aparine		***Brassica oleracea var. sabauda***	
Parameters	**Estimate**	***P*-value**	**Estimate**	***P*-value**
b	1.8710 ± 0.8001	0.0207	0.9702 ± 0.1664	<0.0001
c (g)	0.0088 ± 0.0024	0.0003	0.1681 ± 0.0308	<0.0001
d (g)	0.0315 ± 0.0013	<0.0001	0.6650 ± 0.0179	<0.0001
e (ED50) (µg)	5.9526 ± 1.5439	0.0002	35.9184 ± 7.9870	<0.0001
ED90 (µg)	19.26 ± 11.78	NA	345.83 ± 171.37	NA

NA: not applicable

Table 2. *Recommended dose rates and estimated doses to reduce dry weight by 50% (ED50) and 90% (ED90) in µg of glyphosate per seedling (± se) for each species tested.*

Species	**Recommended dose* (µg)**	**ED90 (µg)**	**ED50 (µg)**
Brassica oleracea var. sabauda	112	346 ± 171	35.9 ± 8.0
Galium aparine	8.44	19.3 ± 11.8	5.95 ± 1.5
Matricaria recutita	16.9	10.2 ± 6.5	2.22 ± 0.7
Chenopodium album	5.83	31.8 ± 18.6	3.54 ± 1.0
Rumex crispus	17.7	322 ± 639	5.30 ± 4.9

* based on seedling ground cover and recommended rate of 1.5 litres Roundup Biactive per ha

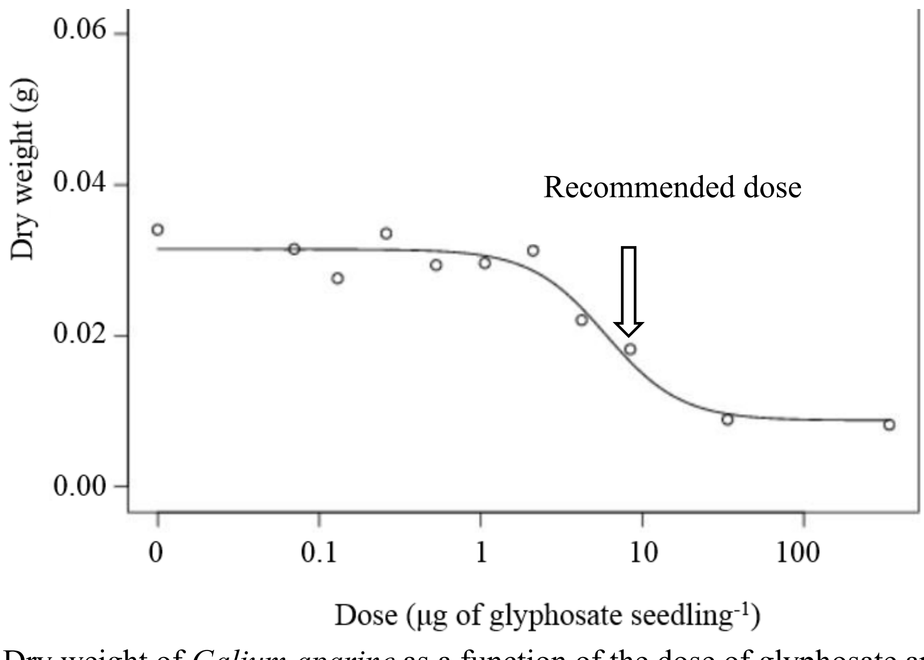

Figure 3. Dry weight of *Galium aparine* as a function of the dose of glyphosate applied per seedling. Parameters of the fitted line are presented in Table 1.

Figure 4. *Galium aparine* seedlings, three weeks after application of different concentrations of glyphosate relative to the recommended dose (1x). The controls were one droplet of water and one droplet of 1% adjuvant (ConAdj). Seedlings were treated at the four-leaf stage.

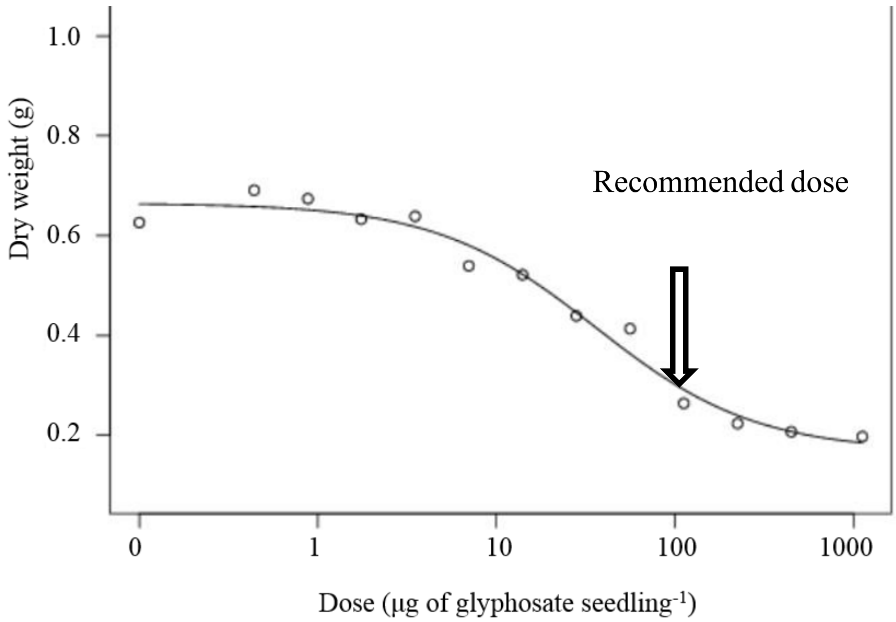

Figure 5. Dry weight of Savoy cabbage as a function of the dose of glyphosate applied per seedling. Parameters of the fitted line are presented in Table 1.

Discussion

The ED90 values showed that if 19.3 µg of glyphosate were applied to a seedling of the weed *G. aparine* this amount would effectively control the weed however, if this amount of herbicide was accidentally applied on the seedling of a vegetable crop it would reduce its biomass by 27%. A good targeting system is therefore essential in order to avoid crop damage. The results presented here are compatible with a field test with *Solanum nigrum* seedlings where an efficacy of 82% was achieved with 22.6 µg of glyphosate applied per plant using 2.5 µl droplets using a drop on demand system (Lund *et al*., 2006; Urdal *et al.*, 2014). These results are of general interest in terms of dose-response relationships. Most studies report effects of plot spraying where the actual doses received per plant are not known or are estimated by chemical analysis. In this study the amount of herbicide applied to individual leaves of plants is known precisely and shows an interesting variability in responsiveness of weed species to a broad-spectrum herbicide like glyphosate. Among the weed species tested *Matricaria recutita* was the most susceptible and *Rumex crispus* was the least. For the latter the high variability in the ED50 and ED90 values is currently being investigated further (Table 2).

It is clear that weeds can be controlled leaf-specifically by droplet application to one leaf providing a non-selective, systemic, broad spectrum herbicide like glyphosate is used. The detrimental effect of accidentally misplacing droplets on leaves of a vegetable crop is also equally evident. Research is in progress developing a robotic applicator for autonomous use.

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Precision Systems in Agricultural and Horticultural Production

Precision targeting of herbicide droplets potentially reduces herbicide inputs by at least 90%

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Summary

Weed control in field vegetables in the UK is increasingly challenging due to the loss of herbicide actives and demands by policy makers and consumers for lower pesticide use. Research at Reading in conjunction with Concurrent Solutions LLC in the USA, is developing a robotic weeder for field vegetables in the UK using image analysis to locate weed leaves and a novel applicator to apply droplets of herbicides to these leaves. No chemical is applied to the crop and none directly to the soil.

In glasshouse trials, efficacy of applying one droplet of herbicide per weed was determined. Dose-response relationships for control of *Stellaria media* L. Vill. with glyphosate and of *Chenopodium album* L. with glufosinate-ammonium showed ED50s of 3.0 and 4.4 µg per seedling compared to the calculated manufacturers' recommended doses of 48.8 and 21.9 µg, respectively, for weed seedlings of the sizes treated. The question remains: is this efficacy reproducible in the field?

Manually applied droplets of glyphosate were made to the naturally occurring weed population in a transplanted cabbage crop in summer 2016. Efficacy of droplet applications to control weeds and to prevent crop yield loss were assessed in comparison to weed-free (hand-weeded), and weedy controls. Reductions in herbicide were compared with use of the pre-emergence herbicide, pendimethalin, and inter-row glyphosate sprays.

Droplet applications 3, 5 and 7 weeks after transplanting reduced residual weed biomass at harvest by 92% compared to the weedy control and gave a crop yield, which did not differ significantly from the weed-free control. At the same time, the total amount of herbicide active ingredient applied was 94% lower than the recommended rate for pendimethalin.

Key words: Leaf-specific weed control, cabbage, herbicide dose-response, critical weed-free period, glyphosate, glufosinate-ammonium, EC Regulations

Introduction

Weed control in field vegetables in the UK is increasingly challenging due to the loss of herbicide actives and demands by policy makers and consumers for lower pesticide use. Examples include potential and actual losses of approval for some herbicide actives and the increasing difficulty of gaining approvals for new compounds under Regulation (EC) No 1107/2009, potential losses under the EU Water Framework Directive (2000/60/EC), potential restrictions on pesticide use under the EU Directive on sustainable use of pesticides (2009/128/EC) and the UK National Action Plan together with the need for EAMUs.

Current spraying technology also constrains the Maximum Residue Levels (MRLs) under EC Regulation 396/2005. While MRLs are not safety limits, they reflect "the largest amount of pesticide which the regulatory body setting the MRL would expect to find in that crop when it has been treated in line with good agricultural practice". Leaf-specific weed control, in which herbicide is never deliberately applied to the crop is clearly expected to lower herbicide residues achievable "by good agricultural practice". Similar benefits could accrue in run-off water to satisfy the Water Framework Directive.

These pressures act as a driver towards a paradigm shift in approaches to weed control. Genetically-modified, herbicide-tolerant (GM-HT) crops are a paradigm shift for weed control but may still involve spraying whole fields on several occasions. Environmental impacts were not greatly different in the UK farm-scale evaluations between GM-HT and conventional crops (Hawes *et al.*, 2003). So while the biotechnological solution may have a place, it may not solve the underlying policy issues already highlighted and GM-HT crops are not currently permitted in the UK. Moreover, even if GM crops are ultimately approved, GM-HT vegetable crops will not be the first choice for development by seed companies as the market will be small compared to broad-acre crops.

This paper explores an engineering solution to the problem of weed control as an alternative to either selective chemistry or biotechnology. If successful, it is likely to tick all the policy boxes mentioned here; it also reduces the risk of the evolution of weeds tolerant to glyphosate, which is in an increasing problem in the USA, and at the same time offers a potential reduction in herbicide inputs per hectare compared to GM-HT and selective chemistry options.

Miller *et al.* (2010) developed an engineering solution comprising a novel image analysis-based weed detection and spot spraying system which achieved 90% control of volunteer potatoes in carrots and onions and further research is in progress to assess its use in legumes (Scrimshaw, 2014). Some crop damage occurred due to the use of sprays, but any yield losses were deemed "acceptable" and the system is commercially available. Closer to the approach adopted here, Christensen *et al.* (2009) mentioned “drop on demand technology" using gravity-fed droplets. Ruckelshausen (2009) stated an intention to apply chemicals using ink-jet printer technology, which would apply microdots of pesticide directly to the leaves of weeds (Crow, 2012). No successful systems have been developed although Blackmore (2013) predicted a microdot system could reduce herbicide inputs by up to 99.9%.

The robotic weeder being developed by Reading and Concurrent Solutions LLC in the USA uses an alternative method of herbicide application from spot spraying or microdot technology, using actively-targetted droplets. Individual weed leaves are located and dosage rates are controlled to ensure the most effective control based on weed type and its growth stage. In this paper we present results of glasshouse studies exploring dose-response relationships for leaf-specific droplet applications and carry out a proof of the concept in the field by manually applying droplets to the weeds in a cabbage crop.

The underlying hypothesis of the research in this paper is that herbicide inputs may be over 90% lower using targetted droplets. Simplistically, if there is 10% weed ground cover, 90% of a correctly applied herbicide could be causing collateral damage and is certainly off-target. Even if this hypothesis is accepted, it is unlikely to be of commercial interest unless two further hypotheses are accepted, namely, that the weed control efficacy and crop yield achieved are not significantly lower than that achieved either by hand-weeding or current methods. A further technical hypothesis is that, because non-residual herbicides are used and weed seedlings are controlled individually post-emergence, multiple treatments may be required so that late-emerging weeds are controlled during the critical weed-free period for the crop (Nieto *et al.* 1968). Finally, to alleviate concerns that the approach overly depends on glyphosate, a glasshouse trial using glufosinate-ammonium is also reported.

Materials and Methods

Glasshouse trials were carried out with glyphosate (Roundup® Biactive GL, 360 g/L, SL, Monsanto (UK) Ltd.) and glufosinate-ammonium (Harvest®, 150 g /L, SL, Bayer CropScience Ltd.) on seedlings of *Stellaria media* and *Chenopodium album*, respectively. The adjuvant, AS 500 SL (Z.P.H Agromix, Niepołomice, Poland), was included in all herbicide treatments. All trials were randomized complete blocks with a range of herbicide doses and 11 or 13 replicates for *S. media* and *C. album*, respectively. There were three controls: water only, water with adjuvant (1%) and undiluted product. All solutions were prepared with deionised water. One droplet was applied to a single leaf of each seedling. Dilution series were prepared so that one droplet could be applied to achieve the correct amount of product per seedling. Droplets were applied when seedlings had a mean ground covers of 9 cm^2 (6-8 leaves) and 4.8 cm^2 (4-6 leaves) for *S. media* and *C. album*, respectively.

Fresh and dry weights of the weed seedlings were estimated three weeks after droplet application. The dry weights were estimated after oven-drying fresh seedlings for 48h at 80 °C.

To fit the dose-response curves, biomass data were analysed using the open source statistical software R, version 3.2.1 and the add-on package DRC (Ritz *et al.*, 2015). A four-parameter log-logistic model was fitted by non-linear regression:

$$y = \frac{c + (d - c)}{[1 + \exp[b\,(\log x - \log \mathrm{ED50})]} \qquad (1)$$

where *y* is the biomass, *c* and *d* are the lower and upper limits of *y*, respectively, *b* is the relative slope, *x* is herbicide dose and ED_{50} is the dose for a 50% reduction of *y*. The dose reducing biomass by 90% (ED_{90}) was estimated from the model.

For the field experiment, seedlings of Savoy cabbage (*Brassica oleracea* var. *capitata*) were transplanted to the field at Sonning Farm, Reading, at the 3 to 4 leaf stage with 50 cm row spacing and 30 cm between plants within the rows. Fertilizer application was carried out one week after transplanting using sulfur (SO_3) and nitrogen (N) at the rates of 50 kg/ha and 100 kg/ha respectively. Plants were individually watered using an automated drip irrigation system. The natural weed infestation comprised *Chenopodium album, Senecio vulgaris*, *Matricaria recutita*, *Spergula arvensis* and *Poa annua*. *Equisetum* also occurred but was removed by hand as this is not a typical weed of field vegetables. The experimental design was a randomized complete block with four replications of eight treatments (Table 1), including weedy and weed-free (hand-weeded) controls. Other controls were either an overall spray of pre-emergence (pre-planting) herbicide (2.9 l/ha, Stomp Aqua®, 455 g/l pendimethalin) or an inter-row post-emergence spray (1.5 l/ha, Roundup® Biactive, 360 g/l glyphosate, three weeks after planting). Droplets containing 36 μg of glyphosate were applied using a micropipette and the number of droplets applied per unit area was counted allowing the equivalent application rate per hectare to be calculated. Droplets were either applied three weeks after planting as a single treatment or after 3, 5 and 7 weeks for a triple treatment. The latter was included to catch late emerging weeds or those which were inadequately controlled in earlier treatments. Cabbages and weeds were harvested from the treated area of the plots, 18 weeks after transplanting. Cabbages were trimmed and weighed as for commercial sale and the dry biomass of weeds was determined as above. Data were analysed by ANOVA using GenStat 16th Edition.

Results

For *S. media*, the quarter rate dose, in which 12.6 µg of glyphosate was applied as a single droplet to each seedling, controlled the weeds satisfactorily (Fig. 1A). The recommended rate of glufosinate-ammonium appeared to be needed to kill the *C. album* seedlings (Fig. 1B).

As fitted by Eqn (1), dry weights of untreated seedlings were 0.23 g (±0.01, SE) for *S. media* and 0.10 g (±0.005, SE) for the smaller *C. album* plants. No herbicide effects appeared to occur until treatments exceeded c. 1.5 µg of glyphosate (a.i.) per seedling (the 1/32 treatment in Fig. 1A was 1.52 µg per seedling) or 1.4 µg of glufosinate-ammonium (a.i.) per seedling (the 1/16 treatment in Fig. 1B was 1.36 µg per seedling). Above these levels, efficacy increased rapidly with ED50s of 3.04 µg (±1.1, SE) and 4.43 µg (±1.22, SE) and ED90s of 6.3 µg (±7.8, SE) and 8.9 µg (±6.1, SE), respectively. Note that the ED90s are very imprecisely estimated, the standard errors being similar to the parameter estimates, so considerable uncertainty exists in that parameter.

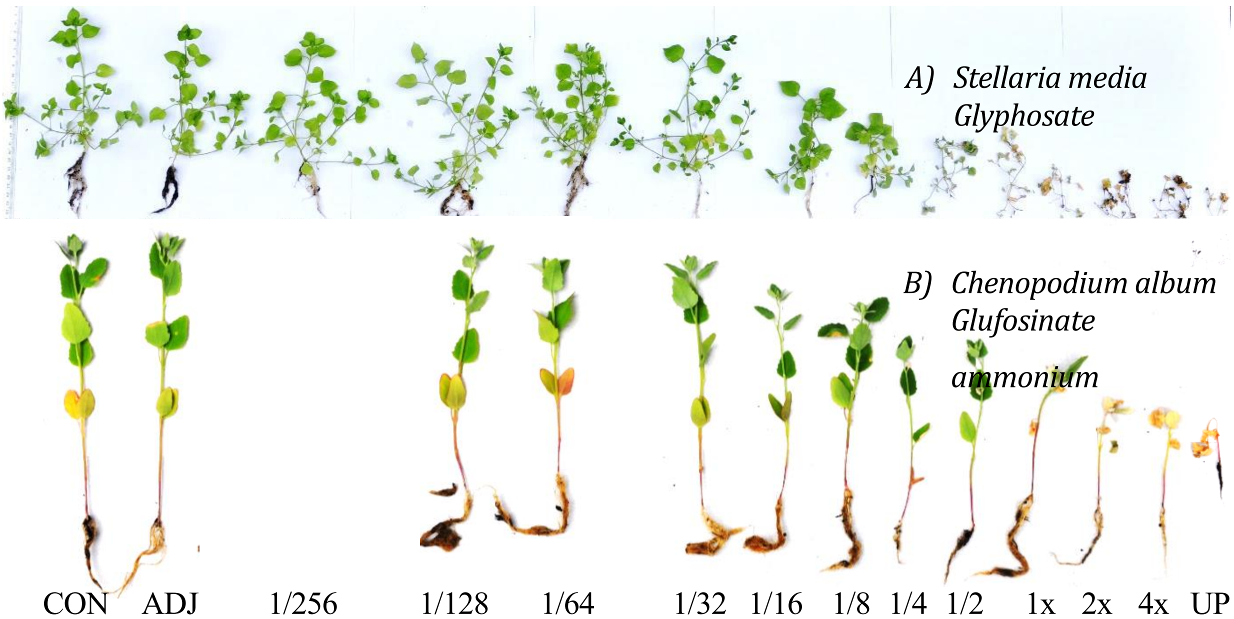

Fig. 1. Seedlings of A) *Stellaria media* three weeks after glyphosate treatment and B) *Chenopodium album* two weeks after glufosinate-ammonium treatment. Chemicals were applied as droplets to one leaf per seedling. Droplets contained different concentrations of a.i. relative to the recommended dose (1x). The recommended amounts of a.i. per seedling were (A) 48.8 µg for *S.media* seedlings (6 to 8-leaf stage; mean ground cover: 9 cm^2) and

(B) 21.8 μg for *C. album* seedlings (4 to 6-leaf stage; mean ground cover: 4.8 cm^2). Control treatments were treated with purified water (CON) or with 1% adjuvant (ADJ) or with undiluted formulated products (UP), Roundup Biactive (A) and Harvest® (B). The 1/256 treatment was not applied in (B).

In the field, the treatment where droplets containing 36 μg glyphosate were applied to each surviving weed 3, 5 and 7 weeks after transplanting reduced dry weed biomass by 92%, and produced the highest yield among herbicide treated plots. Moreover, the yield in this treatment was not significantly lower than the 241 t/ha fresh weight achieved in hand-weeded, weed-free plots. A single droplet application was, however, inadequate to achieve a satisfactory level of control and the yield was only half that of the weed-free control (data not shown). Weed control in the pre-emergence treatment was also significantly lower with dry weed biomass only reduced by about 60% compared to the weedy plots (P=0.05, LSD 20%). Pre-emergence and inter-row spray treatments also yielded significantly less than the weed-free plots.

With respect to the total amounts of herbicide applied, the triple droplet treatment received the equivalent of 83.3 g glyphosate ha^{-1} compared to the manufacturers' recommended rates of 1320 and 540 g ha^{-1} for pendimethalin and glyphosate in the pre-emergence and inter-row spray treatments, respectively. The droplet treatment therefore reduced herbicide a.i. applications per hectare by 94% and 85%, respectively.

Discussion

In the glasshouse studies, when droplets of both herbicides were applied to one leaf of each weed seedling, the efficacy of weed control was as good if not better than what would be expected were the same doses of herbicide applied using a conventional spray. One difference is that it is known that all plants were treated as is the exact dose of herbicide applied to each individual plant. When spraying, only the average per unit land area is known. Encouragingly, the efficacy of glyphosate droplets seen in the glasshouse was repeated in the field trials where cabbages were grown and harvested under conditions designed to replicate typical UK commercial practice. The field trials reported here were limited to Savoy cabbages and the application of glyphosate. Clearly relying on a single herbicide a.i. is unsustainable due to risks of loss of approval and herbicide resistance. The glasshouse trial reported here with glufosinate-ammonium achieved satisfactory efficacy even though the product has only limited systemic action particularly in *C.album* (Pline *et al.*, 1999). More extensive field trials were carried out in the 2017 growing season and including both glyphosate and glufosinate-ammonium and leeks in addition to cabbages. The 2017 trials are showing similarly promising results for both crops (data not shown).

The hypothesis that multiple treatments with a herbicide lacking residual activity like glyphosate would be necessary was accepted since a single glyphosate treatment gave a very poor weed control and returned the lowest yield of all weed control treatments. Multiple treatments were needed to keep the crop largely weed free and to achieve a yield which did not differ significantly from the weed-free control. For cabbages however, the literature suggests that a single weed control treatment should have been sufficient. For example, Weaver (1984) found a single inter-row spray treatment after 3-5 weeks was sufficient in Canada, while for drilled summer cabbage in the UK, Roberts *et al.* (1976) also found a single weeding three weeks after 50% crop emergence was adequate and there was no critical weed-free period. UK organic farmers are similarly advised to carry out a single "thorough weeding 3-8 weeks after planting" (Bond and Grundy, 2001), but these authors also cite French studies where several inter-row tine operations were employed. If there is indeed no critical weed free period, the triple droplet treatment could be argued to be "overkill", but results in this experiment showed that the triple treatment was required to ensure weeds caused no significant reduction in crop yield and achieved satisfactory weed control. In reconciling the results, it is relevant to highlight Bond and Grundy's (2001) use of the word "thorough". Leaf-specific applications are, by definition, not designed to be "thorough". Since a broad-spectrum, non-selective herbicide is being applied, some plants may be left untreated on the first treatment if they are deemed too close to the crop to treat without risk of collateral crop damage. Moreover, since the aim is not to apply herbicide to the soil, very small weed seedlings were left if their leaf area might be too small (<1 cm^2) to target accurately by an automated system on the basis that if such weeds were growing, they would be larger and could be treated safely on a later visit to the field. Multiple treatments are also an insurance against poor weed control – failure of control for whatever reason during a first application could be remedied on a subsequent occasion. So, even if a crop such as cabbage does not have a critical weed-free period, more than one treatment is likely to be necessary as demonstrated here.

In conclusion, droplet applications with glyphosate achieved satisfactory weed control without a significant yield penalty and reduced amounts of herbicide active ingredients applied to field grown cabbages by 94% compared to a pre-planting spray with pendimethalin and by 85% compared to an inter-row post-emergence spray with glyphosate. Glufosinate-ammonium has also been identified as a potential alternative to glyphosate for droplet applications but this conclusion needs to be tested in the field.

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Implications of dose-response relationships of herbicide droplet applications for leaf-specific weed control in leeks

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Abstract

Weed control in UK field vegetables faces the problem of producing and maintaining high yields while herbicide active ingredients (a.i.) and amounts applied are reducing. These problems, which are unlikely to be solved with new herbicides or the use of GM crops, call for a paradigm shift in weed control. In this study, the concept of leaf-specific weed control was tested. Individual weed seedlings were controlled in the glasshouse by applying a single droplet of herbicide and dose-response relationships were quantified. In the field, manually-applied droplets achieved high levels of weed control in leeks, prevented yield loss and reduced herbicide inputs by up to 77%. Because of the high value of the crop and the higher yields associated with ultra-precise droplet application, it would appear to be economical to apply these droplets using a robotic weeder.

Keywords: glyphosate; glufosinate-ammonium; 2,4-D; droplet application; leaf-specific weed control.

Introduction

Weeds and their control is one of the most important factors in maintaining yields and quality of field vegetable crops. Typical weed control methods for field vegetables include use of pre-planting tillage followed by pre-emergence herbicide and one or more of post-emergence selective herbicide, inter-row cultivation and hand-weeding (Slaughter et al, 2008). Herbicides are an efficient and economic method of weed control. However, improper or inappropriate use of herbicides, when they are applied as an overall spray, risks adverse effects on human health and the environment. These impacts along with pressures from consumers and policy makers to lower pesticide inputs, have resulted in strict regulation including the EC Regulation No. 1107/2009, the Water Framework Directive (2000/60/EC) and the Sustainable Use Directive (2009/128/EC). As a result, several herbicides have been withdrawn and it is much more difficult for new chemicals to gain approval (Hillocks, 2012). The predicament is worse for vegetable growers because they rely on a limited and old range of herbicides that require a lot of funding and effort in order to keep them in the market (Fennimore et al., 2014).
Weed control of vegetables, therefore, needs a paradigm shift from conventional spraying. Efficacy needs to be maintained or improved while reducing herbicide usage. Genetically-modified, herbicide-tolerant crops (GM-HT) may offer an alternative, but whole fields still need to be sprayed. Moreover, growing GM-HT crops is not currently permitted in the UK and, even if it were, seed companies would focus on broad-acre crops rather than vegetables because of the relatively small market for vegetable seeds. Other approaches include site-specific weed control for example by spraying weed patches or, plant-specific weed control for example by identifying individual weed plants in the field and then spot-spraying them using precisely targeted applications (Christensen et al., 2009). Drop-On-Demand (DOD)

application to individual weed plants is close to the leaf-specific approach to weed control described here. Although no DOD systems are commercially available, efficacy of weed control by DOD treatment with glyphosate in research trials in fields of carrots, was 73% - 95% (Utstumo et al., 2018).

The study presented in this paper is part of a project which explores an engineering solution to weed control as an alternative to spraying whole fields with selective herbicides, spot-spraying or using GM crops. The system, which is under development by Reading University, UK and Concurrent Solutions LLC in the USA, is designed to apply single herbicide droplets (1-2 µl) at an appropriate concentration to the leaves of unwanted plants (Pilgrim & Murdoch, 2008). The concept is that a machine vision system locates individual green leaves, classifies each leaf as being crop or weed. Individual weed leaves are then targeted and treated with a single droplet of a broad-spectrum, translocated herbicide (e.g. glyphosate). No herbicide should, therefore, directly hit the soil and none should be applied to the crop. The use of much larger droplets than for conventional spraying avoids the risk of drift and appropriate formulation should minimise spatter.

In this paper, dose-response studies are reported in which doses relative to commercial rates were applied as single droplets to weed leaves. For proof of concept in the field, herbicide droplets were applied manually to weed leaves in a field growing leeks. The hypothesis tested for is that the recommended rate of herbicide applied as a single droplet will reduce weed biomass by over 90%. In the field, the hypotheses tested were that (i) the efficacy of leaf-specific weed control by herbicide droplets is not significantly lower than that achieved from the hand-weeding or current herbicide spraying methods, (ii) crop yield is not significantly less than for handweeding, (iii) leaf-specific weed control reduces the amount of herbicide applied by around 80% and (iv) a sequence of droplet applications is required over the growing season to control late emerging weeds to ensure that the crop remains weed-free during the critical period for weed control.

Materials and methods

Glasshouse trials

Dose-response trials were carried out under glasshouse conditions at the facilities of the Crop and Environment Laboratory at Reading University during summer 2018. Droplets containing glyphosate, glufosinate-ammonium or 2,4-D were applied to seedlings of *Chenopodium album*, *Senecio vulgaris* and *Poa annua*. In addition to the herbicides described in Table 1, a combined treatment of 2,4-D and glufosinate-ammonium was applied as two droplets on the same leaf. For the other herbicides, one droplet (1-2 µl) was applied to a single leaf except in the case of *P. annua* where droplets were applied at the growing point of the weed. The volume of droplets and amount of herbicide was calculated according to the ground cover of the seedlings and the minimum label recommendation for spray application. The adjuvant AS 500 SL (Z.P.H Agromix, Niepołomice, Poland), was included in all treatments at 1% concentration. All trials were randomized complete blocks with ten replicates and herbicide doses ranged from 1/128 to 4-times the recommended rates. Application of the three control treatments included the use of droplets containing water only, water with adjuvant (1%) and undiluted product. Deionised water was used to prepare all solutions. Serial dilutions were prepared so that the correct amount of herbicide per seedling could be applied.

Table 0.1. Details of herbicides used for the dose-response trials. The 'recommended' dose is for a targeted droplet application to a *C. album* seedling with 721 mm^2 ground cover.

Active ingredient (a.i.)	Product	A.i. concentration in product ($g\ l^{-1}$)	Recommended product dose ($l\ ha^{-1}$)	'Recommended' dose (μg a.i. $seedling^{-1}$)
glyphosate	Roundup® Biactive GL	360	1.5	38.9
glufosinate-ammonium	Harvest®	150	3	32.5
2,4-D	Depitox®	500	1.4	50.5
2,4-D & glyphosate	Kyleo®	160 & 240	3	86.5

Seedling mean ground covers when treated were 721 mm^2 (4-6 leaves), 223 mm^2 (2-3 leaves) and 1054 mm^2 (2-3 tillers visible) for *C. album*, *S. vulgaris* and *P. annua*, respectively. Fresh and dry weights of the weed seedlings were estimated three weeks after droplet application. In order to estimate dry weights, fresh seedlings were oven-dried for 48h at 80°C. Biomass data were fitted to the four-parameter log-logistic model which can be specified as a non-linear function given by:

$$y = \frac{c+(d-c)}{[1+\exp[b\ (logx-loge)]]} \qquad (1)$$

where *y* is the biomass, *b* is the relative slope around parameter *e*, *e* is the ED_{50} which corresponds to the dose causing 50% reduction of biomass and *c* and *d* are the lower and upper limits of the curve respectively. Analyses were carried out using the open source statistical software R, version 3.3.2 and the add-on package 'drc' (Ritz et al, 2015).

Field trials

Field experiments were carried out with leeks during spring/summer of 2017 and 2018 at Sonning Farm near Reading, UK. Leek seedlings were transplanted to the field at the three-leaf stage with 0.4 m between rows and 0.2 m between plants within the row. Fertilizer was applied on the day of transplanting using sulfur (SO_3) and nitrogen (N) at the rates of 50 kg ha^{-1} and 100 kg ha^{-1} respectively. An automated drip irrigation system was used in order to water plants individually. The natural weed infestation comprised *Senecio vulgaris*, *Matricaria recutita*, *Chenopodium album*, *Poa annua*, *Capsella bursa-pastoris* and *Solanum nigrum*. The experimental design was a randomised complete block with three replications of nine treatments for the 2017 trial and ten treatments in 2018. In addition to weedy (unweeded) and weed-free (hand-weeded) control plots, other controls included a broadcast spray of a pre-emergence herbicide (Stomp Aqua®, 455 g l^{-1}, pendimethalin) at a rate of 2.9 l ha^{-1} or a post-emergence herbicide (Buctril®, 255 g l^{-1}, bromoxynil) at 1.5 l ha^{-1}. The pre-emergence treatment was applied five days before planting and the post-emergence four weeks after planting the crop. In 2018, a further treatment received both pre- and post-emergence herbicides. Droplets containing 9 µg of glyphosate or 7.5 µg of glufosinate-ammonium were applied manually to every weed leaf bigger than 100 mm^2, using a pipette. Droplet applications commenced 2 and 3 weeks after planting the leeks for the 2017 and 2018 trials, respectively, with the last application taking place 12 weeks after planting. Droplet treatments were applied either five or ten times over the 12-week period. Glufosinate-ammonium droplets were only applied weekly because of the herbicide's limited translocation. Leeks and weeds were harvested from the treated central area of the plots 14 weeks after planting. Leeks were trimmed to a height of 0.34 m and then weighed as for commercial sale. Weed dry weights (above ground fresh biomass only) was determined as above. Yield and weed control data were analysed by ANOVA using GenStat 17th Edition.

Preliminary economic analysis was carried out in which the value of the crop and the cost of weed control (material + application cost) were estimated for each herbicide treatment for both years. The economic value of the crop was based on the average wholesale market prices for 2018 (Brigham, 2018). Information regarding the material cost of herbicides came from personal communications with industry representatives. Application cost for spraying herbicides was assumed to be £12.50 ha^{-1} (Redman, 2018). The gross margin was estimated by subtracting the weed control cost from the crop value. Further assumptions were: (i) the cost of an applicator platform including a maintenance contract would be £25,000 over five years, costed at £5,000 per year (S. Sanford, personal communication, 13 December 2018), (ii) an area of 4 ha could be treated per day and would need to be retreated every 14 days, (iii) a platform could operate for 10 days every fortnight and a platform could, therefore, treat a maximum of 40 ha over 14 days, and, to build in a safety margin, (iv) the platform is only used for 40 ha per year. The platform cost per hectare was, therefore, assumed to be £125 ha^{-1} $year^{-1}$ (i.e. £5000/40 ha).

Results

In the glasshouse, *C. album* seedlings were effectively controlled when the recommended rates of the herbicides used in this study were applied as a single droplet to a single leaf, producing increasingly phytotoxic effects with increase of dose (Fig. 1). These effects were quantified by fitting Eq. 1 to the dry weight data to produce dose-response curves as exemplified for *C. album* (Fig. 2). Interestingly, the same relative levels of efficacy were observed for both glyphosate and glufosinate-ammonium, the ED_{50} values for each herbicide corresponding to 1/6th of their respective recommended doses (Fig. 1B, C). A dose of 36.6 μg (±19.9, SE) per seedling was needed to achieve a 90% level of control (ED_{90}) for both herbicides (Fig. 2). A slightly lower ED_{50} was observed when the mixture of 2,4-D and glyphosate was applied in comparison compared to the application of glyphosate on its own (Fig. 1D). Glyphosate was more potent than the other herbicides when applied to seedlings of *Senecio vulgaris* (ground cover: 223 mm^2) with an ED_{90} of 0.24 μg (±0.04, SE). The second-best efficacy was achieved from the mixture of 2,4-D and glyphosate with a dose of only 1.33 μg (±0.30, SE; 1/20th of the recommended dose) being required to achieve 90% control of *S. vulgaris*. The larger *P. annua* seedlings needed higher doses than *S. vulgaris* and *C. album* to achieve the same efficacy. Efficacies of glyphosate and glufosinate-ammonium were also the same for *P. annua*, a dose of 79.9 μg (±15.5, SE) being needed to reduce weed biomass by 50%.

In the field in both years, the treatment where droplets containing 9 μg of glyphosate were applied approximately weekly (i.e. ten times over 12 weeks) reduced weed dry biomass by 99%. The same levels of weed control were achieved when this treatment was applied fortnightly (i.e. only 5 times) in 2018. Yields achieved from the weekly glyphosate droplet treatment were the highest among the plots treated with herbicides (either as droplets or overall spray) and were not significantly lower than the yields from the hand- weeded controls. However, yields for the fortnightly application of glyphosate droplets were significantly lower than the yield from the weed-free control (63% of weed-free).

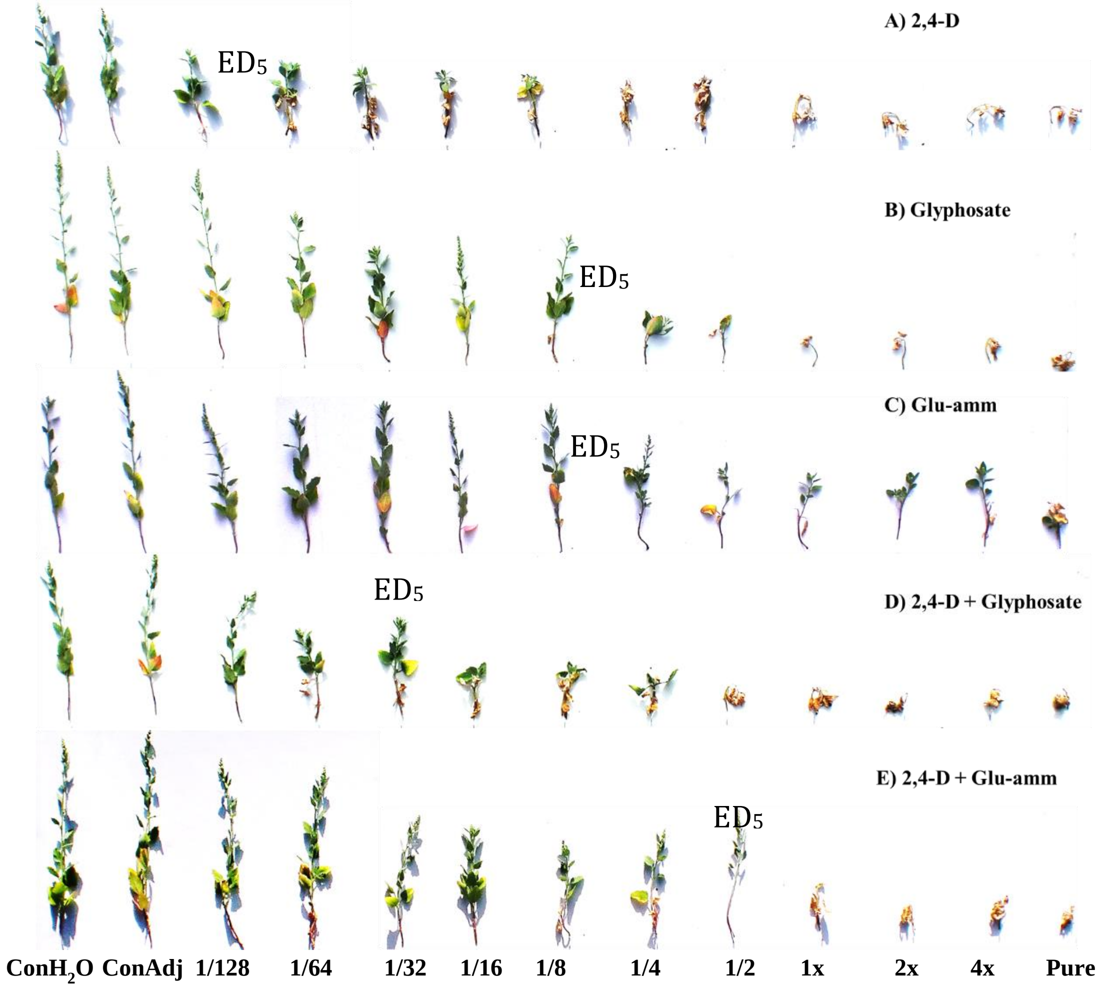

Figure 0.1. *Chenopodium album* seedlings three weeks after applying droplets containing different concentrations of A) 2,4-D, B) glyphosate, C) glufosinate-ammonium, D) 2,4-D plus glyphosate and E) 2,4-D with glufosinate-ammonium. Doses of a.i. are expressed relative to the recommended (1x) rate (Table 1). In (E), separate droplets of the two products were applied to the same leaf. For the controls, droplets of deionized water (ConH_2O), 1% adjuvant (ConAdj) or undiluted herbicide (Pure) were applied. Concentrations giving 50% reduction in dry biomass (ED_{50}) are shown.

The weekly glufosinate-ammonium droplet treatment reduced weed biomass by 91% or more in both years. Trimmed leek yields from these glufosinate-ammonium treated plots were not significantly lower than the 39 t ha^{-1} in the hand-weeded control in 2018. In 2017, however, yields in this treatment were only 65% of the 42.2 t ha^{-1} hand-weeded control yield. A pre-emergence spray of pendimethalin reduced weed dry biomass by 42% and 47% in the 2017 and 2018 trials, respectively and crop yields were significantly lower in both years. When both pre and post-emergence herbicides were applied in 2018, the efficacy of weed control was still only 64%.

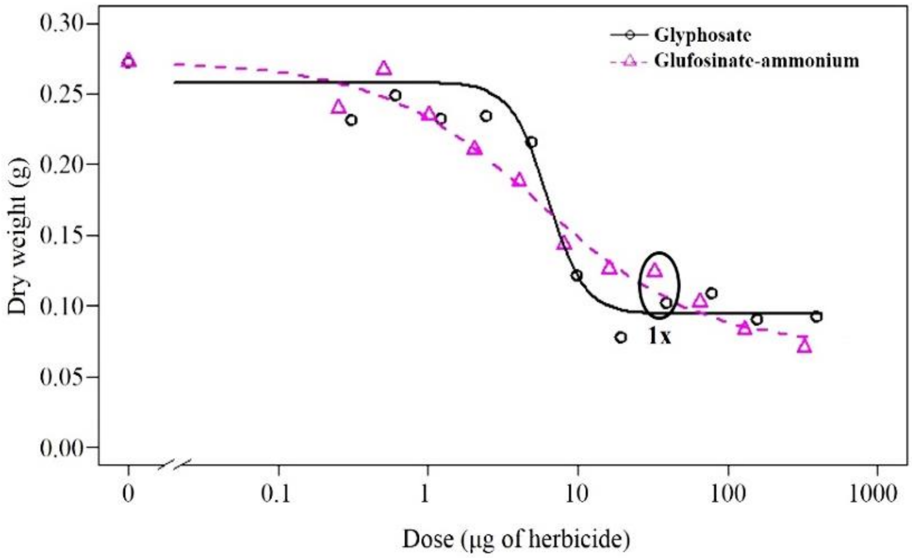

Figure 0.2. Dry weight of *C. album* seedlings as a function of herbicide dose, applied as one droplet per seedling. Recommended rates (1x) were 38.9 µg for glyphosate and 32.5 µg for glufosinate-ammonium. Parameters of lines fitted by Eq. 1 are in Table 2.

Table 2. Parameters of herbicide dose-response curves (Eq. 1) for *C. album* in Fig. 2.

Herbicide active	Parameter (±SE)			
	b (slope)	c (lower limit)	d (upper limit)	e (ED_{50})
Glyphosate	3.54 (±1.59)	0.09 (±0.01)	0.26 (±0.01)	6.29 (±0.85)
Glufosinate-ammonium	0.81 (±0.23)	0.07 (±0.02)	0.27 (±0.01)	5.66 (±2.41)

Regarding the amounts of herbicide applied over the whole growing season, the weekly glyphosate droplet treatment was the equivalent of 340 g and 299 g glyphosate ha^{-1} in 2017 and 2018, respectively, approximately 75% less in weight than spraying with pendimethalin (1320 g ha^{-1}). The fortnightly application of glyphosate droplets in 2018 reduced the herbicide inputs by 71% and 77% relative to pre-emergence and the pre+post-emergence sprays, respectively. Weekly application of glufosinate-ammonium droplets reduced total herbicide usage by 51 and 68% in 2017 and 2018 trials, respectively.

The preliminary economic analysis suggested that the droplet treatments could be highly profitable. The average gross margin of the fortnightly droplet treatments was estimated at £30,246 ha^{-1}, much more than the combined pre+post-emergence treatment (£18,502 ha^{-1}). Allowing for the platform cost of £125 ha^{-1} y^{-1} for the platform, then the estimated additional profit of the droplet treatments above that for the combined pre- and post-emergence treatment would be £11,619 ha^{-1} for the 2018 trial.

Discussion

In this study, the concept of weed control by applying a single droplet of herbicide to weed leaves was proven in the glasshouse and in the field in a leek crop. Dose-response studies have quantified the amount of herbicides needed to control *C. album, S. vulgaris* and *P. annua* seedlings. The relative efficacy of control of *C. album* (4-6 leaves) when droplets of either glyphosate or glufosinate-ammonium were applied was the same, the ED_{50} for both being about 1/6th of the recommended dose, equivalent to 90 and 75 g a.i. ha^{-1}, respectively.

In glasshouse spraying trials with *C. album* plants about twice the size of those here (i.e. 8-10 leaves), Tharp et al. (1999) found the same ED_{50} for glyphosate and glufosinate although slightly over twice as much was needed (200 g ha^{-1}). As regards, the amount required for each plant for droplet applications, Mathiassen et al. (2016) reported that 3.7 μg of glyphosate per seedling reduced the fresh weight of *C. album* seedlings (2-leaf stage) by 50%, which compares well with the 5.1 μg here at the ED_{50}.The efficacy of weed control reported here is, therefore, at least as good as that expected if the same doses were applied using a conventional spray. Moreover, a major improvement in application technology is that the amount of herbicide applied to each plant is known whereas, when spraying, only the average per unit land area is known.

In the field, the crop must be kept weed-free during the critical period for weed control, which, for transplanted leeks, is from 1 to 12 weeks after planting (Tursun et al., 2007). Not surprisingly, therefore, a sequence of droplet applications was needed to keep the crop free of weeds and achieve yields that did not differ significantly from the hand-weeded controls. Multiple treatments are desirable to control weeds which emerge later in the season, but especially for any larger weeds which might not be controlled satisfactorily by droplets. Moreover, since one aim is to avoid applying herbicide to soil, seedlings too small to target accurately (say <100 mm^2) could be treated on a subsequent visit to the field. In this study, it was found that fortnightly applications during the critical period achieved high levels of weed control provided a systemic herbicide like glyphosate is used and if one droplet is applied to each leaf over 100 mm^2. This overall concept has also been shown to be successful in fields with cabbages (Murdoch et al., 2017) although because cabbage is a more weed suppressive crop without a clear critical period only three treatments were needed to achieve high yields and approximately 95% control of weeds.

The reductions in herbicides used per unit area are an important environmental consideration and the droplet treatments reduced the a.i. required by 68-82% ha^{-1} compared with pre-emergence and combined pre- and post-emergence sprays. Although much lower amounts of herbicides are being applied per hectare, the amounts applied to individual weeds were within the range of label recommendations. Concerns about individual plants surviving low doses and herbicide resistance are not, therefore, relevant.

The preliminary economic analysis calculated the net profitability of an automated droplet applicator platform for weed control in leeks. The very high profitability (£11,619 ha^{-1}) compared with spray applications using commercial herbicides derives from the high value of the crop. In this study, a single value of the crop has been used (£1.22 kg^{-1}). However, leek prices change seasonally and are categorized according to stem diameter and then sold as pre-packed, loose product or discarded as non-marketable.

Finally, development of software and hardware (including a prototype applicator) have been in progress for several years and are funded in the UK by AHDB (Murdoch, 2018).

Conclusions

Dose-response studies in the glasshouse proved that single droplet application on to small seedlings was a highly efficient method of weed control. Droplets containing 36.6 μg of glyphosate or glufosinate-ammonium controlled *C. album* seedlings. In leek crops, a sequence of applications of glyphosate or glufosinate-ammonium achieved high levels of weed control, no yield penalty and reduced herbicide applications by over 70% compared to conventional pre- and post-emergence sprays. Economic analysis demonstrated that because of the high crop value, droplet applications could increase profits by over £11,000 ha^{-1} per year, even after allowing £5000 per year for the cost of the application system.

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