University of Reading

Leaf-specific weed control in vegetable crops

Research Student Confirmation of Registration Report

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School of Agriculture, Policy and Development

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Declaration of Own Work

I declare that this confirmation of registration report `Leaf-specific weed control in vegetable crops` is entirely my own work and that where any material could be construed as the work of others, is fully cited and referenced, and/or with appropriate acknowledgement given.

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Curriculum vitae

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PUBLICATIONS IN SCIENTIFIC JOURNALS AND CONFERENCES

- **Koukiasas, N., Yu, T. and Murdoch, A. J. (2016)**
  Dose response relationship of droplet applications for the leaf-specific weed control in vegetable crops. *Aspects of Applied Biology* 132: 343-348.

- **Koukiasas, N., Pembroke, B., El-Hassan, S., Vagelas, I. and Crump, D. (2013)**
  The potential of a novel formulation of the fungus *Purpureocillium lilacinum* to regulate sugar beet cyst nematode. Proceedings of *Advances in Nematology Conference, Linnean Society of London, Piccadilly, UK.* [Poster and abstract.]

- **Malandrakis, AA., Koukiasas, N., Veloukas, Th., Karaoglanidis, GS. and Markoglou, AN. (2013)**
  Baseline sensitivity of *Monilinia laxa* from Greece to fenhexamid and analysis of fenhexamid-resistant mutants. *Crop Protection* 46: 13-17.

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Table of Contents

List of figures

List of Tables

List of Appendices

Appendix 6. Parameters estimates (±se), T-values and P-values of the dose-response model and ED90 value for the dry weight data of the *Urtica urens* drc trial.

Appendix 7. Anova table after one-way analysis of variance of the dry weight of the *U. urens* seedlings and the doses of glyphosate applied.

Appendix 8. Anova table after one-way analysis of variance of the leaf area of the *U. urens* seedlings and the doses of glyphosate applied.

Appendix 9. Means and standard errors of leaf area (cm^2) data of *U. urens* seedlings 3 weeks after application of different number of droplets containing 25% concentration of Roundup Biactive.

Appendix 10. Parameters estimates (±se), T-values and P-values of the dose-response model and ED90 value for the dry weight data of *Brassica oleracea var. sabauda* drc trial.

Appendix 11. Parameters estimates (±se), T-values and P-values of the dose-response model and ED90 value for the dry weight data for each of the times of droplet application of *Brassica oleracea var. sabauda* sequential trial.

Appendix 12. *Brassica oleracea var. sabauda* dry weight as a function of the dose of glyphosate (µg) applied per seedling, after 1, 2, 3 and 4 times of droplets application. Parameters estimates are presented in Appendix 11.

Appendix 13. Anova table after two-way analysis of variance between doses of glyphosate applied and times of application in response of the dry weight data of the *Brassica oleracea var. sabauda* seedlings.

Appendix 14. Parameters estimates (±se), T-values and P-values of the dose-response model and ED90 value for the dry weight data of *Brassica oleracea var. sabauda* sequential trial.

1.Introduction

1.1 Background

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; Jabran *et al*., 2015). Therefore, weed control plays an essential role in maintaining agricultural productivity, with herbicide application being the most common and efficient method of control (Urdal *et al*., 2014). However, herbicide-based weed control is receiving a lot of negative attention because of human health and environmental issues. These issues have led to new regulations and directives (EC Regulation No. 1107/2009, Water Framework Directive (2000/60/EC), Sustainable Use Directive (2009/128/EC) which could lead to actual or potential losses of herbicide actives and make it more difficult for new compounds to gain approvals (Murdoch *et al*., 2010). This predicament is worse for vegetable growers because of their reliance in a limited and old range of herbicides (first released in 1960s and 1970s) which require a lot of funding and effort in order to keep them in the market (Fennimore *et al*., 2014).

There is, therefore, a need to change current weed control methods due to legislation and to meet demand for more sustainable crop production. Instead of controlling weeds with whole-field methods, managing them in a precise way can minimize the herbicide inputs and environmental contamination and allow growers to produce vegetables in a more sustainable way. It has been proven that using site-specific weed control can reduce the herbicide use by up to 90% (Gerhards and Oebel, 2006) with Blackmore (2013) predicting a reduction of 99.9% if a microdot technology is used which applies amount of herbicide directly onto the weed leaf. While a lot of research has been carried out for targeted applications of herbicides little is known about the exact dose rates that are needed to control individual weed seedlings plant specifically (Young and Giles, 2014). Furthermore, although several systems have been developed for plant specific weed control using directed sprays (Klose *et al*., 2008; Midtiby *et al*., 2011; Miller *et al*., 2011), no successful, commercial system has yet been developed which applies micro-doses of herbicide directly to the leaves of the weeds.

1.2 Project Overview

In order to prove the concept of leaf-specific weed control this PhD project aims to perform glasshouse and field trials so as to evaluate and model the responses of individual weed and crop plants to discrete herbicide droplets. This research is part of a larger overall robotic weeding project. Dose-response relationships of glyphosate and glufosinate droplet application will be studied for the most common weed species found in vegetable crops. Also herbicide droplets will be applied to crop seedlings so as to record susceptibility of the vegetable to accidental droplet application. In addition to that, trials with the prototype robotic applicator will take place under glasshouse and field conditions. Prior to that images will be captured from fields with vegetables in order for collaborators in the overall project, to develop an image analysis algorithm to distinguish weeds from the crop.

1.3 Aims and Objectives

For field vegetables growing in rows, this project aims to prove the concept of weed control by leaf-specific herbicide droplet application. A successful outcome of the overall project aims to:

- minimize herbicide inputs and meet demand for more sustainable crop production, providing an efficient and effective means of controlling weeds in vegetables where few post-emergence herbicide options are allowed or available.
- eliminate herbicide drift and reduce run-off to the soil, crop and non-target organisms.
- provide an engineering alternative to the biotechnological option of genetically-modified herbicide tolerant crops.

The objectives that will be addressed during the course of this PhD are to:

- model dose-response relationships of herbicide droplet application to the common weed species in vegetable crops. Trials will be carried out in glasshouse and field conditions.
- capture images of field vegetables in order to develop image analysis algorithms to distinguish weeds from the crop.
- test an autonomous platform with a novel leaf-specific herbicide applicator in glasshouse and field conditions.

2.Research in Current Literature

According to FAO (2009), weeds should be considered from the famers as the No. 1 natural enemy, causing $ 95 billion losses per year in food production which can be even higher if the time and effort which is devoted from the farmers to weed control is taken into account. Depending on the infestation level, these unwanted plants can cause yield losses from 30% to 100% if weed control is not implemented (Christensen *et al*., 2009; Singh *et al*., 2014). 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 and O'Donovan, 2013). It is worth mentioning that more than 45% of the pesticides currently available in the market are herbicides (Chan Cupul *et al*., 2014). However, the over-reliance on these chemicals and use of a broadcast-type of equipment has raised public awareness because of reports where residues have been found in fruits, vegetables and drinking water and their effect on the ecosystem and non-target organisms (Power *et al*., 2013). On the other hand, the introduction of new legislation and directives (EC Regulation No. 1107/2009, Water Framework Directive (2000/60/EC), Sustainable Use Directive (2009/128/EC) can limit the availability of herbicides and the introduction of new chemicals in the market (Clarke *et al*., 2009). This will have an immediate impact on vegetable growers as they rely on a limited herbicide market with most these chemicals first released in the 60's and 70's (Fennimore *et al*., 2014). Therefore, it is essential to balance the weed control needed to maintain and increase productivity with the need to minimise the negative effects of herbicides.

As stated by Young *et al*. (2014) precision weed management "places the right amount of inputs on the right target at the right time". Precision weed control methods have a great potential as an alternative to overall-spray applications as they reduce the amount of herbicide that is applied without reducing crop yield, which is better for both the environment and the producer. Drop-on-demand technology has also been investigated, which is closer to the concept studied in this project, as it uses very low volume droplets of herbicide which are applied directly to weeds using a weed detection and herbicide application system (Christensen *et al*., 2009; Basi *et al*., 2012; Urdal *et al*., 2014).

Such systems comprise an automated method for weed detection, the output from which is then passed to a decision algorithm that manages a spraying system (Christensen *et al*., 2009). The first report of a robotic weed control system was published by Lee *et al*. (1999) who developed a spraying system for selective herbicide application to in-row weeds in tomato crops, based on a weed map generated by a machine vision system. Gerhards and Oebel (2006) developed a patch-spraying system which treats weeds according to a GIS that consists of three maps. After carrying out a series of experiments, 60% herbicide savings were achieved for annual broad-leaf weeds and 90% for annual grass weeds. Ruckelshausen *et al*. (2009) developed 'BoniRob' for phenotyping crop varieties in field trials and, based on that, Klose *et al*. (2008) developed 'Weedy' an autonomous field robot for selective weed control in maize, spraying inter-row and intra-row (close to crop) weeds. Miller *et al*. (2011) achieved 95% control of volunteer potatoes in onion, leek and sugar beet crops after developing a spot-spaying system based on a real-time weed detection system. However, because of the need to use a non-selective herbicide the crop could be accidentally damaged, but at a commercially acceptable level. Furthermore, other non-herbicide based weed control methods have been studied for use in conjunction with robots to control weeds using thermal, chemical (organic oils) and electrical agents (Slaughter *et al*., 2008).

Although a lot of research has been carried out using targeted applications by a spraying device, research quantifying the rates of herbicide needed to control weed seedlings after small droplets have been applied onto weed leaves. Therefore, further research is needed to evaluate the efficacy of droplet applications to facilitate development and optimisation of the equipment used for making these targeted applications (Søgaard and Lund, 2007; Young and Giles, 2014).

3.Materials and Methods

Several trials were carried out under glasshouse conditions in order to quantify the volume and number of droplets needed to effectively control weed and crop species in different growth stages. The trials were designed in such a way that the treatments applied would fit to a dose-response curve and then the values for 50 and 90% reduction in biomass would be estimated (ED50 and ED90 respectively). In order to describe plants' growth stages the BBCH scale was used (Meier *et al*., 2009). Also phytotoxicity symptoms (yellowing and stunting) were recorded using the European Weed Research Council (EWRC) scoring system (Ciba-Geigy, 1975). Air and soil temperature as well as relative humidity data were recorded using the General Thermocron temperature loggers.

3.1 Materials

All trials took place at the University of Reading's glasshouse facilities. Seeds of *Chenopodium album* and *Rumex crispus* were provided by Dr. Alistair Murdoch, University of Reading and *Matricaria recutita, Galium aparine, Stellaria media* and *Urtica urens* by Herbiseed Ltd. All seeds were sown on J. Arthur Bower's multi-purpose compost surface. The cabbage seedlings, savoy variety, were supplied by Hammond Produce. Depending on the size of the seedlings and the duration of the trial a variety of pot sizes was used.

Roundup® Biactive® (Monsanto®, 360 g/l glyphosate) was used as a source of glyphosate and was provided by the technical staff from the Sonning Farm, University of Reading. In order to decrease the surface tension and increase the retention of the droplets the adjuvant AS 500 SL, Agromix was used (Woznica *et al*., 2015). For the preparation of the glyphosate solutions the ErgoOne 1000μl pipette (100 μl – 1,000 μl, Starlab®, Milton Keynes, United Kingdom) was used whereas for the application of the droplets the ErgoOne 2.5μl (0.1 μl - 2.5 μl, Starlab®**,**Milton Keynes, United Kingdom) was more appropriate. A Nikon D90 Digital SLR Camera with 18-105 mm VR Lens Kit and a ManFrotto Compact Action tripod were used to take photographs of the seedlings.

3.2 Leaf area estimation

In order to be able to calculate the recommended rate of Roundup (1.5 l/ha) as μg of glyphosate per seedling, the WinDias Leaf Area Meter System (Delta-T Devices Ltd, Cambridge, United Kingdom) was used to estimate the ground cover of individual weed plants. Individual photos of a representative sample of seedlings of the same age and size as the ones that were treated, were taken and they were analysed using the leaf area meter. Photographs were taken from above (Figure.2) and ground cover was estimated in cm^2 by the proportion of green pixels in an image of known area. The software then estimates leaf area was able to transform the greenness of the seedling (Figure. 1).

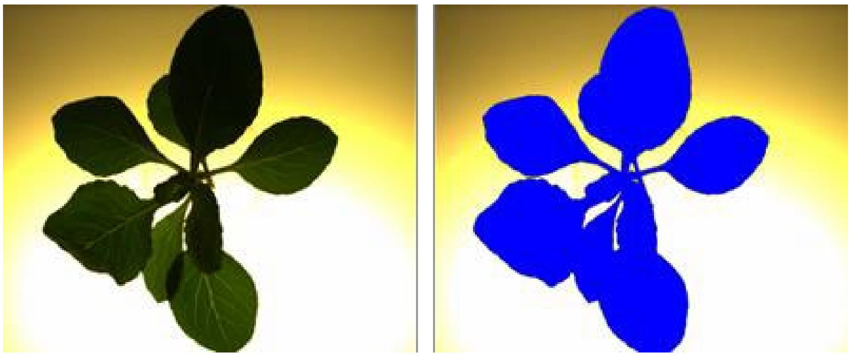


Figure 1. Images of savoy cabbage seedlings before (left) and after (right) image analysis to assess ground cover using WinDias software.

Furthermore, the same software was used to monitor the reduction of the leaf area after the seedlings had been treated with glyphosate (Fig. 2).

Figure 2. Method for taking photographs for leaf area determination. An untreated *Chenopodium album* seedling is shown, 3 weeks after the application of glyphosate treatments using a Nikon D90 Digital SLR Camera with 18-105 mm VR Lens Kit and a ManFrotto Compact Action tripod. Ruler was set at the height of the seedling in order to calibrate the WinDias software.

3.3 Calculations

The recommended dose rate of Roundup Biactive (360 g/l glyphosate) was 1.5 l/ha for annual weeds, i.e. 540 g glyphosate /ha or 5.4 µg glyphosate /cm^2. According to the ground covered by the seedling, the recommended dose for an individual seedling can be calculated is as follows:

$$\text{Glyphosate (µg/seedling)} = \text{ground cover of seedling (cm}^2\text{)} \times 5.4$$

In most experiments, the concentration of herbicide was varied according to species so that a droplet of around 1 µl of glyphosate could be applied to achieve the recommended rate. Calculations of the concentration needed were carried out according to Table 1.

Table 1. Calculation for volume of droplets according to the µg of glyphosate per seedling (Glyphosate µg) and the % of solution.

Percentage solution	Volume of droplet (µl)
20%	Glyphosate (µg)/72
10%	Glyphosate (µg)/36
5%	Glyphosate (µg)/18
2.5%	Glyphosate (µg)/9

3.4 Preparation of glyphosate solutions

Depending on the leaf area of each weed species tested, the initial dilution for the recommended dose was between 2.5% and 10% so that each droplet would have a volume of approximately 1 µl as will ultimately be required by the robotic applicator. The range of the doses to be tested was based on preliminary trials so that it would be possible to model the dose-response relationship of droplet application to leaves of *C. album* and *R. cripsus* (Yu, 2015). The applied doses of glyphosate were 1/256, 1/128, 1/64, 1/32, 1/16, 1/8, 1/4, 1/2, 1/1, 2/1, 4/1 of the recommended rate (compare Christensen, 1994 for similar dose ranges). In addition to these treatments, adjuvant only and pure product controls were applied. In order to produce the series of dilutions the highest concentration was prepared at first and then the rest were half diluted using deionized water and 1% of the adjuvant AS 500 SL (Fig. 3). The dilution procedures which were followed for producing the different concentrations of the glyphosate are illustrated in Fig. 3 and described in Appendix 1.

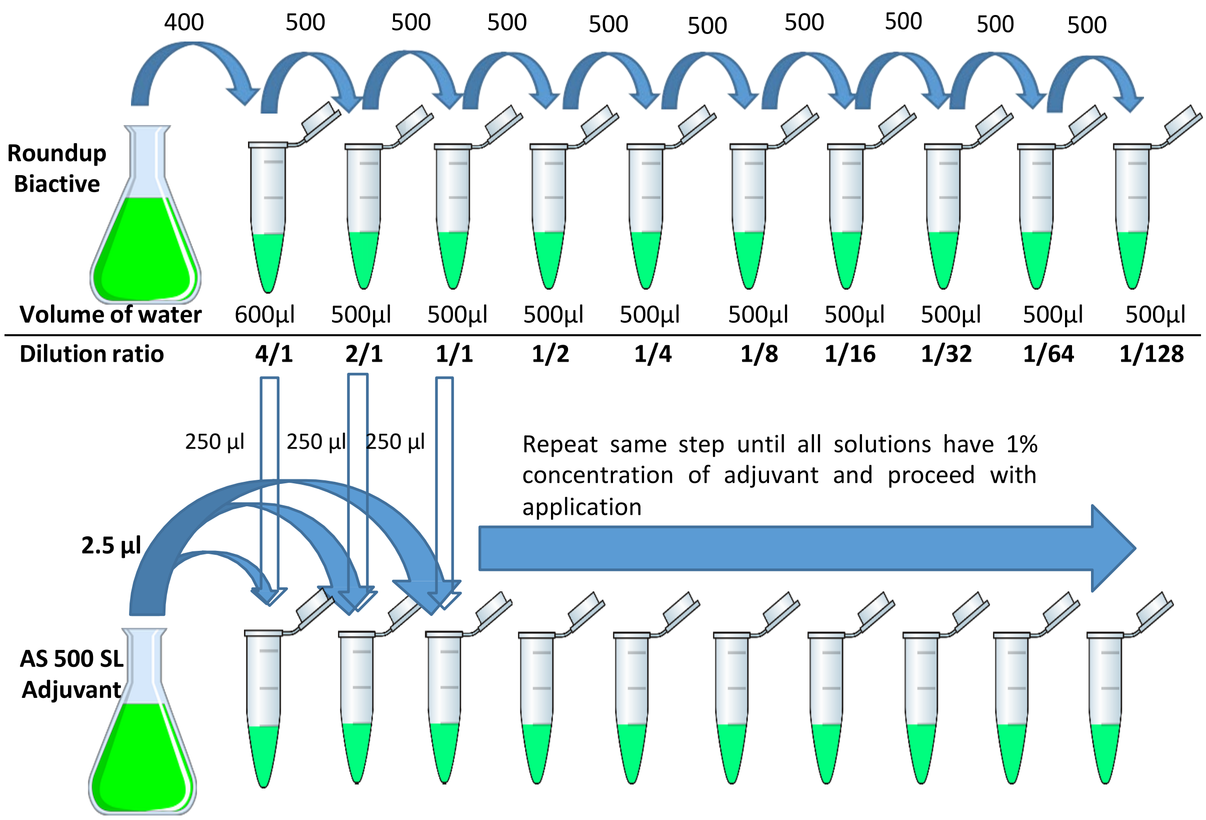


Figure 3. Preparation of a two-fold serial dilution with deionized water, starting from undiluted Roundup Biactive and making a concentration of 1/128. Then 250 µl were removed from its solution and 2.5 µl of the adjuvant AS 500 SL were added, making a 1% concentration of the adjuvant.

3.4 Adjuvant concentration test

In this preliminary trial, the adjuvant AS 500 SL was applied to the waxy leaves of seedlings of *Chenopodium album* and *Brassica oleracea* (Savoy variety) in order to record any phytotoxicity symptoms and also to observe concentration needed to spread the droplets on the leaves of these seedlings.

To germinate the *C. album* seeds, they were placed in a Petri dish (25 seeds per dish) with filter paper and 2 ml deionized water. The Petri dish was then left in an incubator with constant light and alternating temperatures 10/25 °C. After ten days, when the seeds had reached the cotyledon stage, they were transplanted into flowerpots with compost and left to grow in the glasshouse. Two seedlings were growing in each pot and they were treated with adjuvant droplets when they reached at BBCH stage 14-16. *B. oleracea* seedlings were growing one seedling per pot and were treated with adjuvant at the BBCH growth stage 13-15.

Although, according to the manufacturer the recommended concentration of the adjuvant for overall spray application is at 1% v/v (Woznica Z., personal communication, July, 2015), in this trial the concentrations of the adjuvant applied were at 0%, 0.01%, 0.05%, 0.1%, 0.25% 0.5%, 1%, 2% and 100%. Deionized water was used to prepare the solutions of the adjuvant. One droplet of 1 µl volume per treatment was applied to one leaf of each seedling. Overall seven treatments were applied which were replicated four times and overall 56 seedlings were treated. After seven days visual assessment was carried out using the EWRC scoring system, recording phytotoxicity and stunting symptoms.

3.5 Dose response trials (DRC)

The trials which have taken place so far were designed in such a way to test the initial hypothesis that plants can be controlled leaf-specifically by using glyphosate droplet application to one leaf. In order to achieve that, dose-response relationships of the droplet applications to weeds were studied. Glyphosate droplets were also applied manually to cabbage plants to record the effects of accidental herbicide application by a robotic weeder.

In this section the dose response trials will be described in further detail for each of the weed and crop species tested. Weed seeds were sown on multi-purpose compost surface in multi-cell plastic trays with 84 cells. After germination seedlings were thinned down to one seedling per cell.

Treatments for all the trials were randomized complete blocks and replicated according to the number of seedlings available. Based on the preliminary trial, all treatment solutions included 1% of adjuvant. In addition to the application rates of glyphosate, undiluted Roundup Biactive, water and adjuvant control treatments were applied to all of the experiments. Coloured pot labels were used for each treatment. A representative sample of seedlings was used in order to estimate the ground cover using the WinDias leaf area meter. Then the recommended dose rate was estimated in µg of glyphosate per seedling.

Finally, three weeks after the application fresh and dry weights of the seedlings were recorded using an analytical balance (weighing to the nearest 0.0001 g). The dry weights were estimated after oven-drying fresh seedlings for 48h at 80 °C. In order to produce the dose response curves the biomass and leaf area data were fitted to the four parameter log-logistic model using the 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 x is the dose of glyphosate, b is the relative slope around parameter e, c is the lower limit of dose response curve, d is the upper limit and e is the ED50 which generates response halfway between d and c. The ED90 value was calculated as follows (Knezevic *et al*., 2007):

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

3.5.1 *Chenopodium album*

in this trial, *C. album* seedlings were treated at the BBCH growth stage 14-16 (Hess *et al*., 1997). The application rates of glyphosate ranged from 1/128 to two times of the recommended dose. Twelve treatments were applied which were replicated 39 times. The mean ground cover was estimated at 1.08 cm^2 from a sample of ten seedlings, which meant that the recommended dose for this trial was 5.83 µg per seedling. In order to achieve that amount of glyphosate a 2.5% solution of Roundup Biactive was prepared and one droplet of 0.66 µl was applied per seedling from that solution.

3.5.2 *Rumex crispus*

In this trial 325 *R. crispus* seedlings were treated at the BBCH growth stage 12-13. Glyphosate treatments started from 1/64 and reached up to four times the (assumed) recommended dose rate of 1.5 l/ha. Overall 13 treatments were applied which were replicated 25 times. The mean ground cover of a sample of eleven *R. crispus* seedlings was estimated at 3.3 cm^2, meaning that the recommended rate was 17.8 µg of glyphosate per seedling. In order to apply this amount of glyphosate a 5% solution of Roundup was prepared and one droplet of 1.0 µl volume was applied.

3.5.3 *Matricaria recutita*

For this trial 187 *M. recutita* seedlings were treated at the BBCH growth stage 12-14. The glyphosate treatments applied started from 1/128 reaching up to four times of the recommended dose rate of Roundup Biactive. The treatments were replicated 17 times. A sample of seventeen seedlings was used in order to assess the ground cover, which was estimated at 3.1 cm^2. Then the recommended rate of glyphosate was calculated at 16.7 µg and in order to achieve this amount of herbicide one droplet of 0.93 µl was applied in the centre of each seedling from a 5% solution of Roundup Biactive.

3.5.4 *Galium aparine*

In order to test the dose response of *G. aparine* to glyphosate droplet application, 180 seedlings were treated at the BBCH growth stage 13-14. In total 12 treatments were applied which were replicated 15 times. The glyphosate treatments started from 1/64 to four times of the recommended dose rate. The ground cover of a sample of ten seedlings was found to be 1.56 cm^2 with the recommended dose of glyphosate per seedling being 8.44 µg. One droplet of 1.0 µl volume was applied from a 2.5% solution of Roundup Biactive.

3.5.5 *Urtica urens*

3.5.5.1 Dose response trial

For this trial the weed seedlings were treated in a more mature stage (BBCH: 17-18) as they had eight leaves fully developed. The glyphosate treatments started from 1/64 to four times the recommended dose rate. In total 14 treatments were applied which were replicated 15 times. The ground cover of a sample of nine *U. urens* seedlings was estimated at 25.4 cm^2 meaning that the recommended rate of Roundup was at 137.16 µg per seedling. So as to achieve this amount of glyphosate two droplets of 1.905 µl were applied from a 10% solution of Roundup Biactive.

3.5.5.2 Number of droplets trial

Urtica urens seedlings, same as the ones from the drc trial, were used for this test (BBCH: 18, Ground cover: 25.4 cm^2). Seven glyphosate treatments were applied, starting from one droplet per leaf which corresponded to the recommended rate and reached the eight droplets treatment which was equal to eight-times the recommended dose (8Drop treatment or 8-times dose). Droplet application started from the top, younger leaves until all leaves have been treated. In order to apply the recommended dose of 137.16 µg from a 25% solution of herbicide one droplet of 1.524 µl was used.

3.5.6 *Brassica oleracea var. sabauda*

3.5.6.1 Dose response trial

Cabbage seedlings, savoy variety, were transplanted into flowerpots (9 cm diameter) at the 2-3 leaf stage (BBCH: 12-13) and left to grow in glasshouse conditions until they reached 4-5 leaf stage (BBCH: 14-15) when they were treated. In total 12 treatments were applied which were replicated 35 times. Glyphosate treatments started from 1/256, reaching 4-times the recommended dose of 1.5 l/ha. 420 seedlings were used for this trial and the ground cover was estimated from a representative sample of ten seedlings and was found to be 20.8 cm^2. The recommended rate of glyphosate was 112.2 µg per seedling and in order to achieve this amount of herbicide 2 droplets of 1.56 µl were applied from a 10% solution of Roundup Biactive.

3.5.6.2 Sequential trial

Savoy cabbage seedlings were used for this trial and they were treated at the 8 to 10-leaf stage. After they were received from Hammond Produce, they were transplanted in pots and they were fertilized using a controlled release fertilizer containing NPK and magnesium (17-11-10+2). As this experiment took place in winter, artificial lighting was provided from 5 am to 7 am and from 5 pm to 7 pm in the evening using mercury vapour bulbs (HLRG, 400W) in order to extend the length of the photoperiod to 14 hours. Also to make sure the uptake and translocation of glyphosate, heating was applied to achieve a minimum of 15 °C. Five treatments were applied which included the untreated control, control treated with 1% adjuvant, 1/16, 1/4 of the recommended rate and finally the recommended dose rate (1x) of Roundup Biactive (Table 2). The experiment was set out in randomized complete blocks with five replicates and treatments were applied four times to the same plants at two-week intervals. In this case the cabbages were treated as an annual weed hence, 1.5 l/ha of herbicide was used as the recommended dose. The mean ground cover of a representative sample of seven cabbages seedlings was estimated at 246.42 cm^2 giving a recommended dose rate of 1330.67 µg of glyphosate per plant. In order for the seedlings to receive this dose, two droplets of 1.85 µl of undiluted Roundup Biactive were applied on one leaf. Each time the droplets were applied onto healthy leaves of the plants and on both sides of the centre nerve of the leaf. Two weeks after the last application leaf area, fresh and dry weight and shoot length data were recorded. In addition to the biomass data phytotoxicity symptoms were recorded on a weekly basis.

Table 2. Dates of applications of the sequential herbicide trial treatments with the number of the plants treated each time. Plants were harvested 2 weeks after the last application on the 17 of November 2015.

Dates of applications	**Treatments**					**Plants treated**
29/10/15	Control(untreated)	Control+Adjuvantx1	1/16 x 1	1/4 x 1	1x	85
06/10/15	Control (untreated)	Control+Adjuvantx2	1/16 x 2	1/4 x 2	2x	68
20/10/15	Control (untreated)	Control+Adjuvantx3	1/16 x 3	1/4 x 3	3x	34
03/11/15	Control (untreated)	Control+Adjuvantx4	1/16 x 4	1/4 x 4	4x	17

4.Results & Discussion

4.1 Adjuvant concentration test

In order to improve the performance characteristics of glyphosate, it is a common agricultural practice to add an adjuvant either to the original formulation of the herbicide or by mixing it before the application (Pacanoski, 2015). Seeing that without an adjuvant it was not able to achieve adequate wetting of waxy leaf surfaces using droplet application, the adjuvant AS 500 SL was used (Figure 4). The formulation of AS 500 SL contains a build-in multifunctional adjuvant mixture comprising of non-ionic surfactants, ammonium salts,

Figure 4. Droplet of 1 µl of deionized water applied on the waxy surface of a *Chenopodium album* leaf without an adjuvant (left) and with 0.1% AS 500 SL adjuvant (left).

organic acid, pH buffer and humectant (Woznica *et al*., 2015).

After the use of the adjuvant it was observed that droplets containing 0.01% and 0.05% were difficult to apply and did not provide sufficient wetting of the leaf whereas, droplets with 0.1% adjuvant concentration and higher were easier to apply and resulted in better coverage of the leaf surface (Figure 5). Furthermore, seven days after the application of adjuvant treatments, phytotoxicity symptoms were observed on the *C. album leaves* from the treatments containing 100% adjuvant concentration (Figure 6). No yellowing or stunting symptoms were observed for the cabbage seedlings (Table 3). After the test it was decided that all the glyphosate solutions would contain 1% adjuvant concentration.

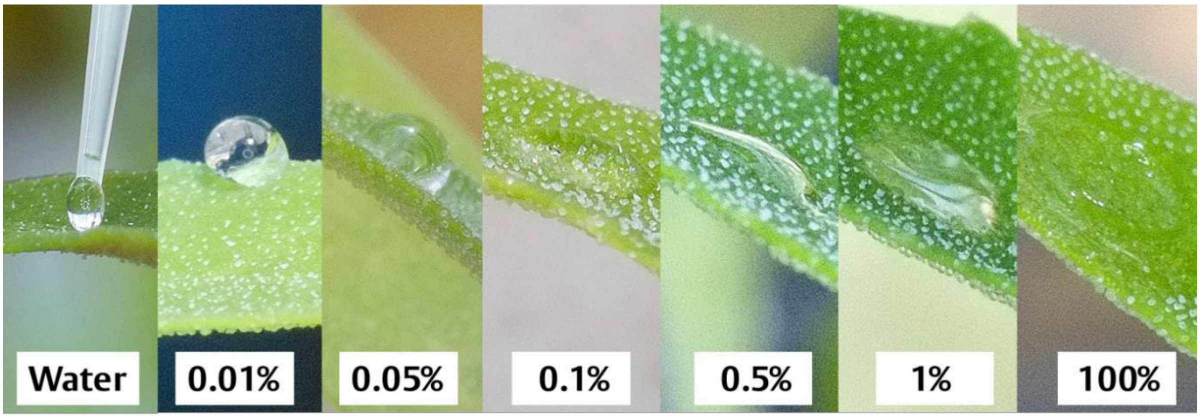


Figure 5. Images show 1µl droplets of different concentrations of AS 500 SL in deionized water applied to *Chenopodium album* leaves with a micropipette.

Table 3. Median EWRC yellowing and stunting symptoms (1-9) for the *C. album* (BBCH: 13-14) and *B. oleracea* (BBCH: 13-15) seedlings seven days after application of adjuvant concentrations.

	Chenopodium album		***Brassica oleracea var. sabauda***	
Adjuvant Concentration	**Yellowing**	**Stunting**	**Yellowing**	**Stunting**
0%	1	1	1	1
0.01%	1	1	1	1
0.05%	1	1	1	1
0.1%	1	1	1	1
0.25%	1	1	1	1
0.5%	1	1	1	1
1%	1	1	1	1
2%	1	1	1	1
100%	5.5	4.5	1	1

Figure 6. *Chenopodium album* seedlings untreated (left) and treated with one droplet of 1 µl containing 100% adjuvant concentration (right) seven days after application of adjuvant treatments

4.2 Dose response trials (DRC)

In order to estimate the effective dose of a herbicide, the reduction it causes to the dry weight matter of the weed has to be assessed (Knezevic *et al*., 1998). So in order to estimate values for the 10%, 50% and 90% reduction in the biomass of the seedlings (ED10, ED50 and ED90) dry weight data have to fit to the four parameter logistic model (LL.4) (Ritz, 2010). However, in the case of the *Chenopodium album* and *Rumex cripsus*, dry weight data were not able to fit the model hence, fresh weights were analysed for these two weed species (Figure 7).

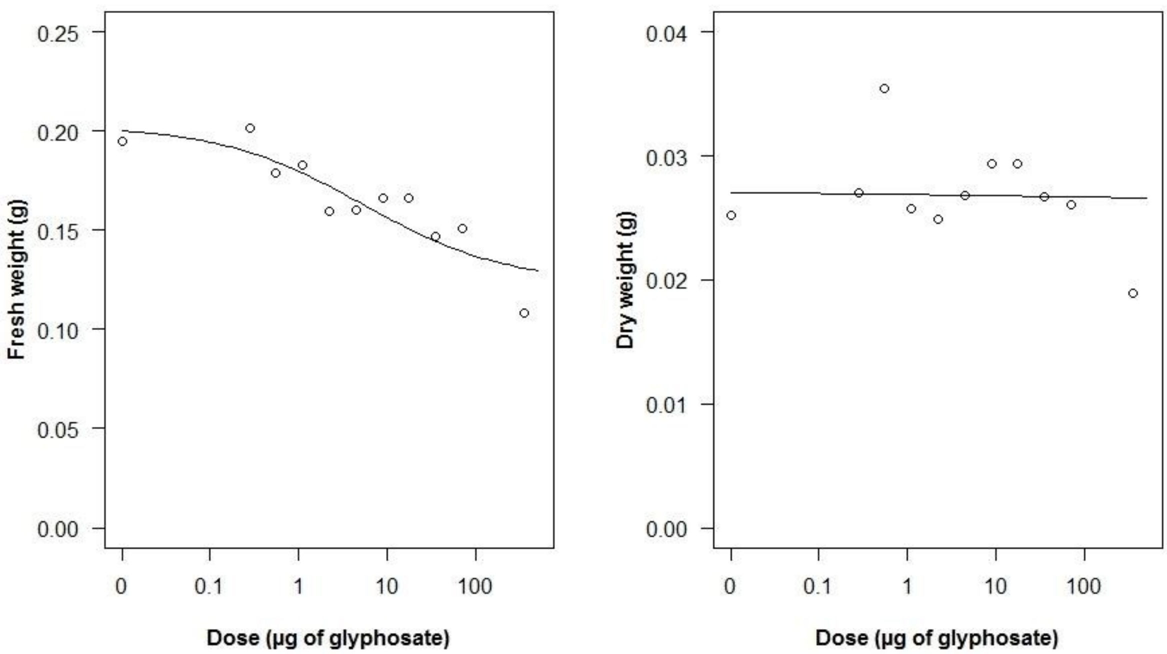


Figure 7. *R. crispus* dose response curve using µg of glyphosate per seedling and fresh weight data (left) and dry weight data (right) which failed to fit the four parameter logistic model.

4.2.1 *Chenopodium album*

C. album seedlings were controlled leaf-specifically after applying droplets containing a series of glyphosate solutions (Figure 8). After fitting the fresh weight data to the four parameter logistic model it was found that all of the parameters were statically significant (Appendix 2) and a dose-response curve was generated (Figure 8). Although the recommended dose was estimated at 5.83 µg, a 90% reduction in the fresh biomass was achieved at 32 µg of glyphosate.

Figure 8. *C. album* seedlings three weeks after application of droplets containing different concentrations of glyphosate relative to the recommended dose (1/1). Control treatments were completely untreated and treated with 1% adjuvant. Seedlings were treated at the BBCH stage:12-14.

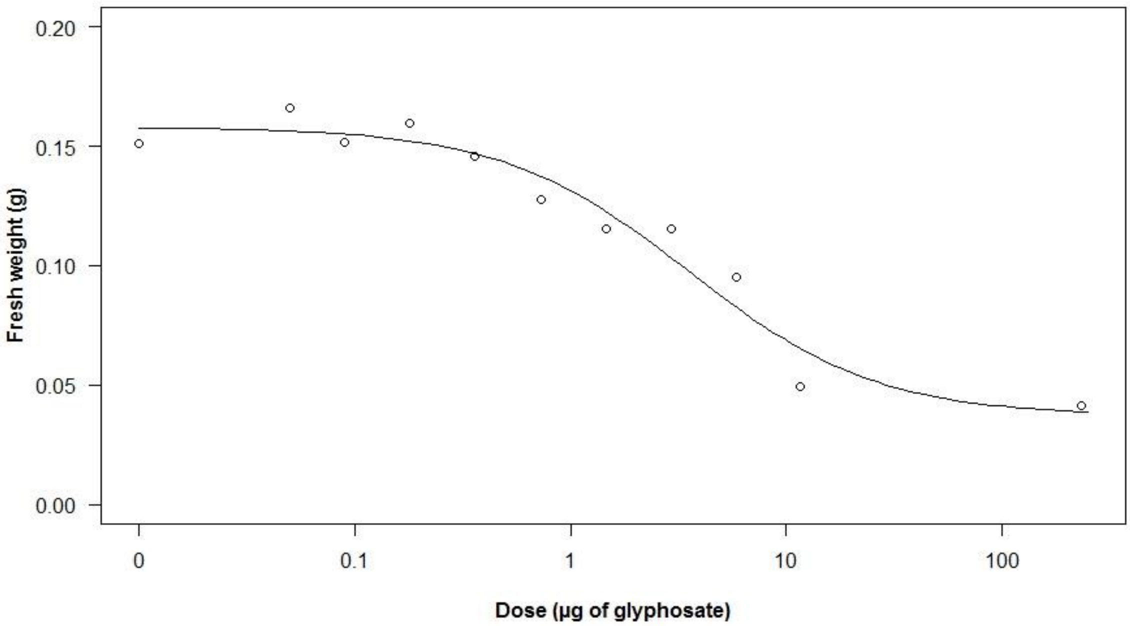


Figure 9. Fresh weight of *Chenopodium album* as a function of µg of glyphosate applied per seedling.

4.2.2 *Rumex crispus*

Trying to imitate what the robotic herbicide applicator will do in a field situation, 1.5 l/ha of Roundup Biactive was applied as the recommended dose to all of the weed and crop species. However and according to the herbicide's label instructions this dose is recommended for annual weeds whereas for a perennial weed like *R. crispus* an application rate of 5 l/ha is recommended (Monsanto®, 2011). This may partly explain why after applying glyphosate treatments the recommended rate of 17.82 µg of glyphosate was insufficient to control the weed (Figure 10). Furthermore, the ED90 value was estimated at 321 µg of glyphosate per seedling with the ED50 value of 5.30 not being statistically significant (Figure 11, Appendix 3).

Figure 10. *R. crispus* seedlings three weeks after application of droplets containing different concentrations of glyphosate relative to the recommended dose (1x assumed to be 1.5 l/ha of Roundup Biactive). Control treatments contained one droplet of water and one droplet of 1% adjuvant (ConAdj). Seedlings were treated at the BBCH stage:12-13.

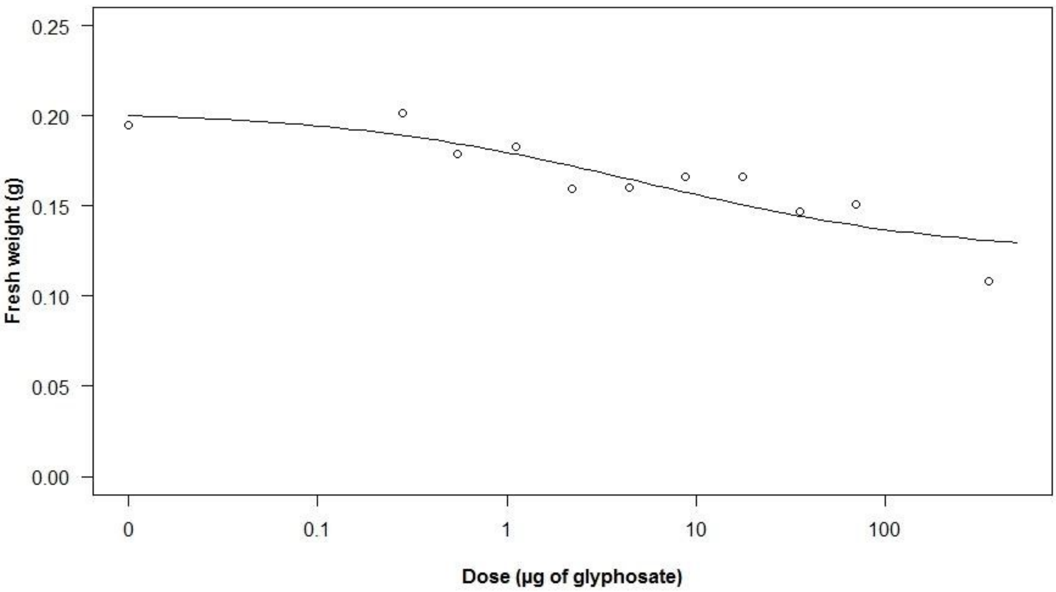


Figure 11. Fresh weight of *Rumex cripus* as a function of µg of glyphosate applied per seedling.

4.2.3 *Galium aparine*

G. aparine seedlings were controlled at the four-leaf stage after the application of herbicide droplets containing a series of glyphosate solutions (Figure 13). Dry weight data were able to fit in a dose-response curve with all of the parameters being statistically significant (Appendix 4). The recommended dose was estimated at 8.44 µg per plant and was able to reduce biomass of the seedlings by 43% (Figure 12).

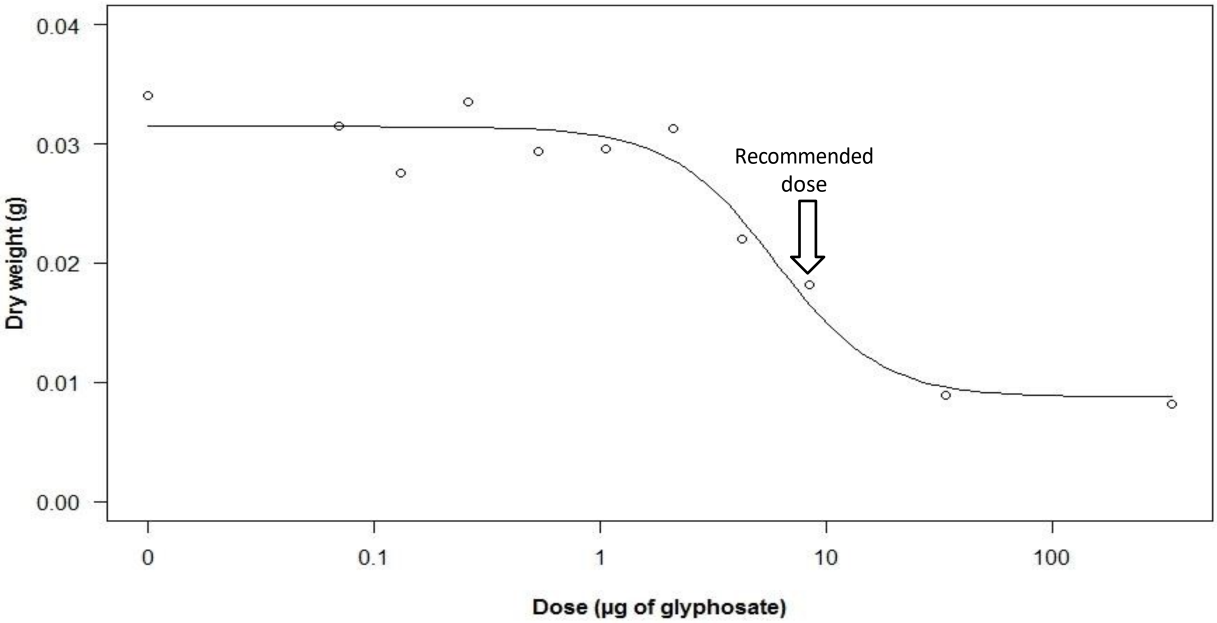


Figure 12. Dry weight of *Galium aparine* as a function of the dose of glyphosate (µg) applied per seedling.

Figure 13. *G. aparine* seedlings three weeks after application of droplets containing different concentrations of glyphosate relative to the recommended dose (1x). Control treatments contained one droplet of water and one droplet of 1% adjuvant (ConAdj). Seedlings were treated at the BBCH stage:14.

4.2.4 *Matricaria recutita*

The biomass of *M. recutita* seedlings was reduced after droplet application with different concentrations of glyphosate (Figure 14). Dry weight data showed a typical dose-response relationship as a function of the µg of glyphosate (Figure 15.) The recommended dose of 16.7 µg achieved almost 90% reduction of the dry weight of the seedlings (Appendix 5).

Figure 14. *M. recutita* seedlings three weeks after application of droplets containing different concentrations of glyphosate relative to the recommended dose (1x). Control treatments contained one droplet of 1% adjuvant (ConAdj). Seedlings were treated at the BBCH stage:12-14.

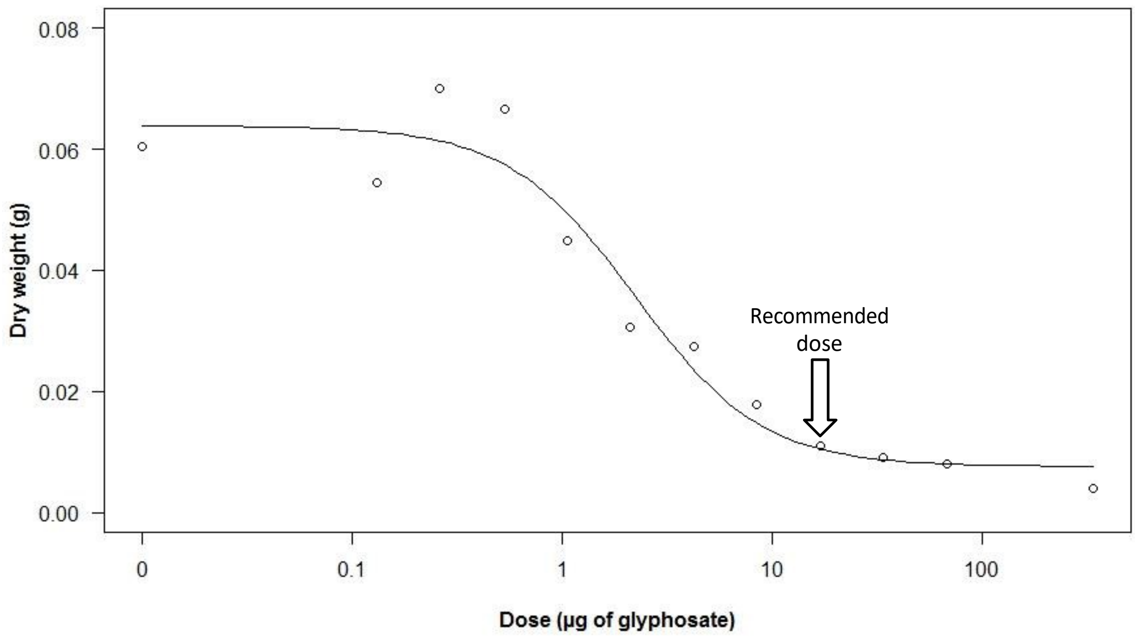


Figure 15. Dry weight of *M. recutita* as a function of the dose of glyphosate (µg) applied per seedling.

4.2.6 *Urtica Urens*

4.2.6.1 Dose response trial

After applying droplets containing different concentrations of glyphosate to *U. urens* seedlings at the 6 to 8-leaf stage, biomass of the plants was successfully reduced compared to the control treatments (Figure 16). Dry weight data were fitted to the dose-response curve with all of the parameters being statistically significant (Figure 17, Appendix 6). A 50% reduction in the biomass was achieved with concentrations of glyphosate containing 3/8 of the recommended dose.

Figure 16. *U. urens* seedlings three weeks after application of droplets containing different concentrations of glyphosate relative to the recommended dose (1x). Control treatments contained one droplet of water and one of 1% adjuvant (ConAdj). Seedlings were treated at the BBCH stage:16-18.

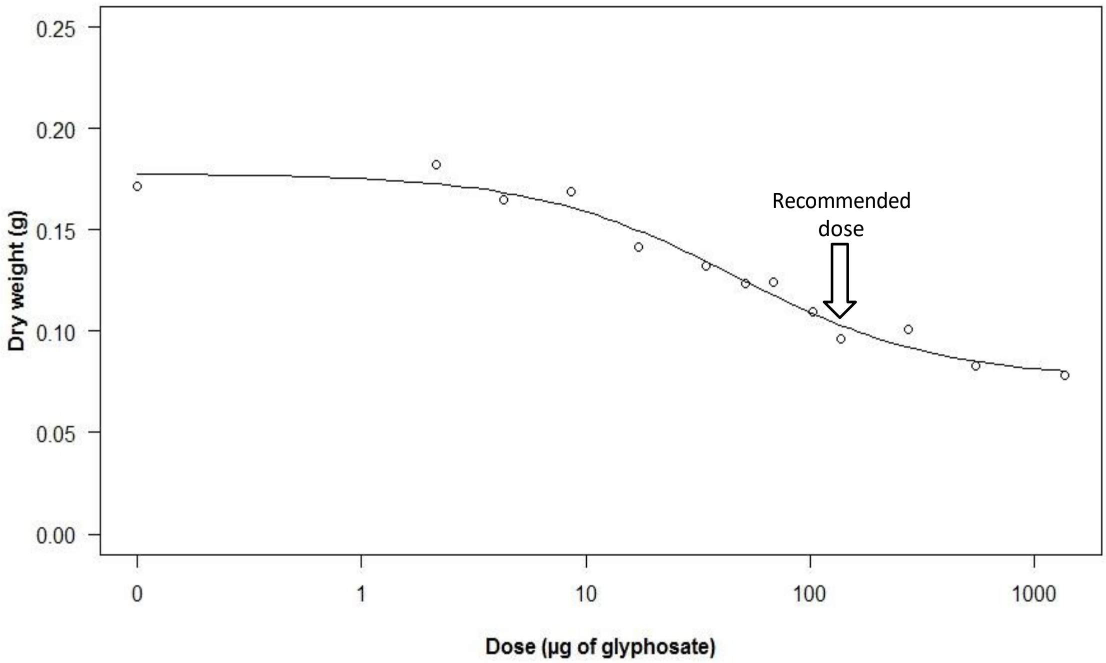


Figure 17. *Urtica urens* dry weight *as* a function of the dose of glyphosate (µg) applied per seedling

4.2.6.2Number of droplets trial

After a suggestion from the manufacturer of the robotic applicator, a trial was designed in which the recommended dose rate was applied from a 25% solution of Roundup Biactive (Pilgrim B., personal communication, September, 2015). Glyphosate treatments started from the recommended dose (1x) and ended up to 8-times the recommended rate (8x) (Figure 18). GenStat 17th version and one-way analysis of variance was used to analyse the data. Results showed that the glyphosate treatments caused significant differences for the dry weight and leaf area data (Appendix 7, Appendix 8). However, the effect on the biomass did not differ significantly when 5, 6 and 8 droplets per seedling were applied and also among the treatments which contained 3 and 4 droplets (Figure 20) Same non-significant differences were observed for the leaf area data (Appendix 9). This could be explained because for some seedlings it was observed that when droplets were applied to top, younger leaves (1Drop to 4Drop) they would burn the leaf resulting to no translocation of the herbicide. Whereas to treatments with the five and more droplets no such burning symptoms were observed because of droplets were applied to lower, more mature leaves with glyphosate being translocated and resulting to complete kill of the weed (Figure 19).

Figure 19. Burning of leaves for *U. urens* seedlings three weeks after application of 1, 3 and 4 droplets per each leaf per seedling containing 25% concentration of glyphosate resulting to stunting symptoms. Complete control was achieved when 5 and 8 droplets were applied.

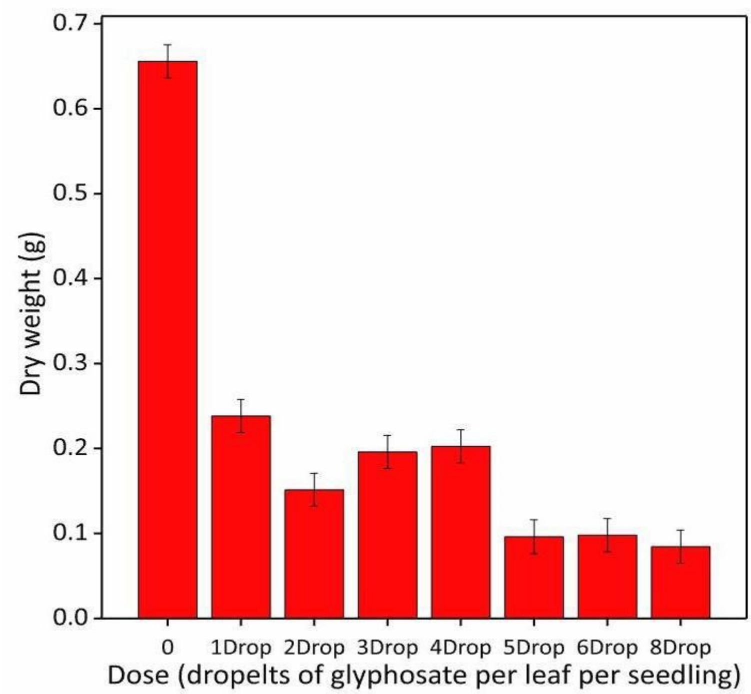


Figure 20. Means and standard errors of dry weight data of *U. urens* seedlings 3 weeks after application of different number of droplets containing 25% concentration of Roundup Biactive.

4.2.7 *Brassica oleracea var. sabauda*

4.2.7.1Dose response trial

Droplets containing series of glyphosate concentrations were applied to cabbage seedlings, savoy variety, in order to record phytotoxicity symptoms, to assess the tolerance of the crop to droplet application (ED10) and the effective dose to reduce 50% and 90% the dry weight of the plant (ED50 and ED90). After fitting the dry weight data to the logistic model it was found that all of the parameters were statistically significant and the response of the biomass data to the doses of herbicide fitted to a dose-response curve (Figure 21). The ED values showed that cabbages were susceptible to glyphosate droplet application as the

ED50 was less than half the recommended dose. Also the crop can tolerate a 10% reduction in the biomass when 1/32 of the recommended dose (3.5 μg) of herbicide is applied (Appendix 10).

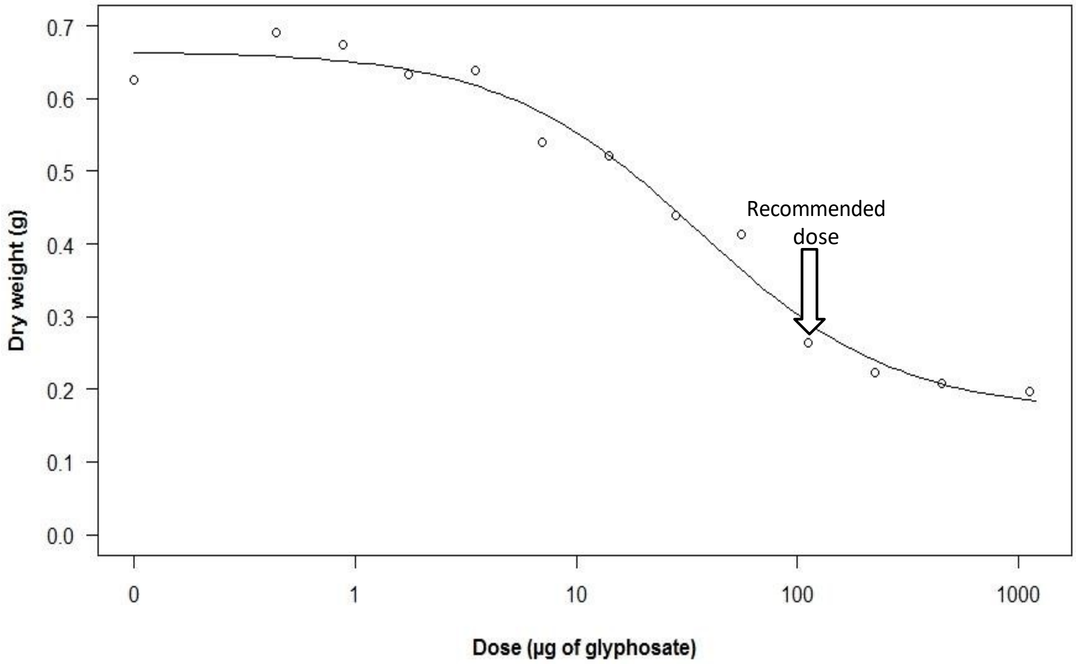


Figure 21. *Brassica oleracea var. sabauda* dry weight as a function of the dose of glyphosate (μg) applied per seedling

4.2.7.2 Sequential trial

In September 2015 a sequential herbicide trial was established at the glasshouse facilities of the University of Reading. The objective of this trial was to test the hypothesis that when the same amount of herbicide will be applied at different times, a difference in the response of the biomass is expected. The doses of glyphosate applied were 1/16, 1/4 of the recommended dose rate according to ground cover of the plants with the highest being the recommended rate (Figure 22). The biomass data fitted in the log-logistic model for each one of times of the herbicide applications and presented typical dose-response relationships (Appendix 11, 12). Although there were differences when the same amount of glyphosate was applied and the time of application, the interaction of time and dose was not found significant (Appendix 13). Therefore, all the data were analysed without taking into consideration the time of the application and were able to fit in a single dose-response curve (Figure 23).

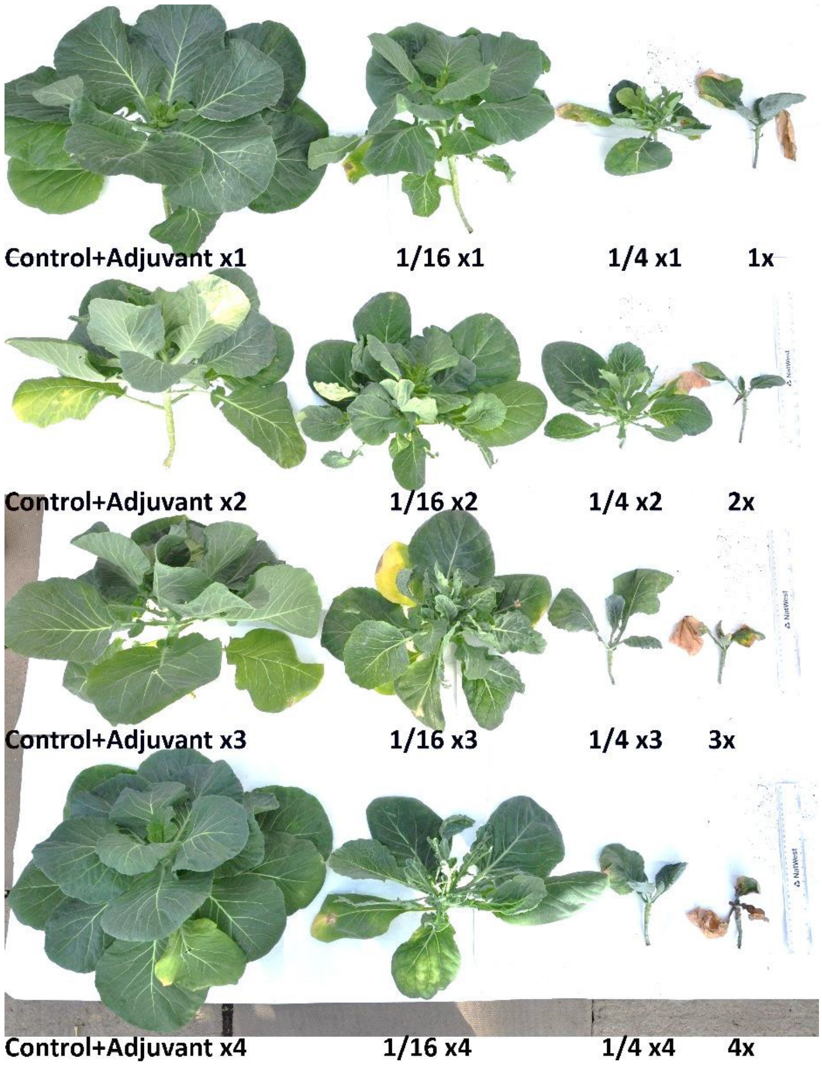


Figure 22. *B. oleracea var. sabauda* plants 10 weeks after the first application of different concentrations of glyphosate relative to the recommended dose (1x). Treatments were applied sequentially with a 2-week interval. Control treatments were treated with one droplet of 1% adjuvant. Plants were treated at the 8-10-leaf stage

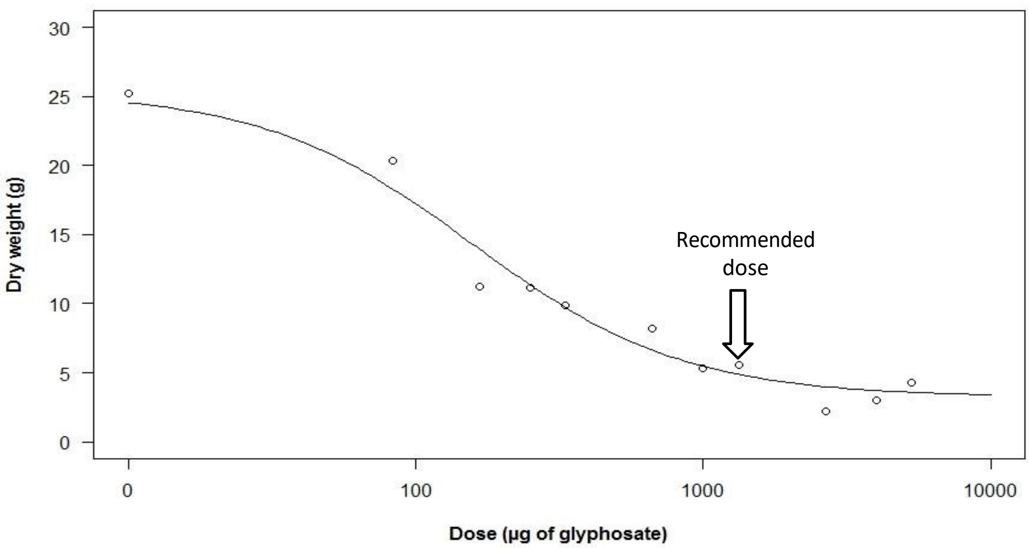


Figure 23. *Brassica oleracea var. sabauda* dry weight as a function of the dose of glyphosate (µg) applied per seedling for the sequential trial. Parameters of the fitted line are presented in Appendix 14.

5. Summarizing 1st year results

This study is a part of a project developing a system for herbicide droplet applications to individual leaves of weeds in field vegetables. The herbicide ejector will point and shoot high velocity droplets with a volume of approximately 1 µl using a non-selective herbicide. This is why for the purpose of this study the broad-spectrum and systemic herbicide glyphosate was used. Research was carried under glasshouse conditions at the University of Reading, in order to quantify the volume and number of herbicide droplets needed to control some of the most common weeds found in UK vegetable crops. Furthermore, treatments of glyphosate were applied to cabbage seedlings in order to record the susceptibility of the crop to herbicide droplet application. Most of the weeds were treated at the 2 to 4-leaf stage (BBCH:12-14) which is the most susceptible stage when foliar-applied herbicides are used (Streibig, 2010). However, *U. urens* seedlings were also treated in a later stage (BBCH:16-18).

According to the ED values a 50% reduction in the biomass of the weed seedlings at the BBCH growth stage 12-14 can be achieved with doses from 2.2 to 6 µg of glyphosate. However, from a farmer's point of view a 90% weed control is considered a reasonable level which for the annual weeds can be achieved with doses from 10 to 32 µg of glyphosate. These results are consistent with a field test when a drop-on-demand system was used which applied 22.6 µg of glyphosate per plant using 2.5 µl droplets, it achieved 82% control of *Solanum nigrum* (Lund *et al*., 2006; Urdal *et al*., 2014). However, if this amount of glyphosate is accidentally misplaced on a vegetable crop seedling it could cause up to 50% biomass reduction. Furthermore, in the case of a perennial or a more mature annual weed, doses with 460 µg are effective (Table 4). These results suggest that sequential application of herbicide is required or applications with higher doses in order to achieve a 90% weed control.

Table 4. Recommended dose rates and estimated effective doses to reduce biomass of the weed and crop species tested by 50% (ED50) and 90% (ED90) in µg of glyphosate per seedling (± se).

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
Urtica urens	137.2	460 ± 389	46.5 ± 17.0
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

It has also become evident that when the recommended dose rate is applied using droplets with a constant concentration of herbicide (25%), the mature and well developed leaves need to be targeted in order for the herbicide to be translocated and control the weed. Otherwise if younger and newly developed leaves are treated this could burn the leaves without controlling the weed. The danger of overdosing clearly needs to be taken into account.

Also when cabbage seedlings were treated as weeds in a sequential droplet application of different concentrations of glyphosate, differences were found when at the end of the trial same amounts of herbicide were applied at different times. However, these differences were not statistically significant.

From the results obtained to date, it is clear that weeds can be controlled leaf-specifically using droplet application using a systemic and broad-spectrum herbicide like glyphosate. However, a good targeting system is essential in order to avoid accidental crop contamination and treat the weeds at the area where the absorption and translocation of the herbicide can be achieved.

7.Doctoral training courses and summer school

As part of the University of Reading Researcher Development Programme (RRDP) five courses were attended in the first year of my PhD studies as required. Three further courses will be attended in the 2nd year and two in the 3rd. Courses attended so far are as follows:

1. How to write a paper, 2015/05/13
2. Managing your research project, 2015/05/14
3. Doctoral Research Conference, 2015/06/18
4. Preparing posters - one day session, 2015/11/02
5. How to summarise your research in 3 minutes, 2015/12/03
6. Ensuring confirmation of registration 2016/04/27 (Booked, not yet attended)

In addition, after attending a 3-day training course at the Berkshire Agricultural College I was able to obtain the certificate of competence in “Safe Use of Pesticide Knapsack Sprayer” (PA1/PA6 qualification).

In order to acquire more understanding of the principles behind the image analysis systems, I also attended the “Summer School on Image Analysis for Plant Phenotyping” at The University of Nottingham, Jubilee Campus from 7 to 10 July 2015.

8.Publications in Conferences

The results I obtained from the summer 2015 trials were presented in a poster entitled “Leaf-specific weed control in vegetable crops” at the BCPC Weeds Review 2015 which took place on 12 November 2015 at Rothamsted Research, Harpenden. In addition, I presented a similar poster together with a 5-minute oral presentation at the International Advances in Pesticide Application conference (13 - 15 January 2016, Barcelona, Spain). For the latter, a written paper was submitted under the title “Dose-response relationship of droplet applications of the leaf-specific weed control in vegetable crops” which was published at the conference’s proceedings (Aspects of Applied Biology, 132, pp. 343-348).

9.Provisional Thesis Chapter Outline

Chapter 1. Introduction

Chapter 2. Literature Review

Chapter 3. General Materials and Methods

Chapter 4. Dose-response relationships of leaf-specific droplet applications of herbicides for controlling common weeds of vegetable crops in the UK.

Chapter 5. Efficacy of weed control in vegetable using a herbicide applicator mounted on an autonomous platform under field and glasshouse conditions

Chapter 6. Economic analysis of use of leaf-specific herbicide applicator in row vegetable crops

Chapter 7. Conclusions

References

BASI, S., HUNSCHE, M., DAMEROW, L., LAMMERS, P. S. & NOGA, G. 2012. Evaluation of a pneumatic drop-on-demand generator for application of agrochemical solutions. Crop Protection, 40, 121-125.

BISSOLI, R., PATTEE, E., VIANELLO, G. & ANTISARI, L. 2008. Water Framework Directive 2000/60/EC. La qualità delle acque di superficie., 9-12.

BLACKMORE, S. 2013. In: Notes of a meeting, 16 July 2013, Digital Farming: The Next Agricultural Revolution. 2-3.

CHAN CUPUL, W., HEREDIA ABARCA, G., RODRÍGUEZ VÁZQUEZ, R., SALMONES, D., GAITÁN HERNÁNDEZ, R. & ALARCÓN GUTIÉRREZ, E. 2014. Response of ligninolytic macrofungi to the herbicide atrazine: dose-response bioassays. Revista argentina de microbiología, 46, 348-357.

CHRISTENSEN, S. 1994. Crop weed competition and herbicide performance in cereal species and varieties. Weed Research, 34, 29-36.

CHRISTENSEN, S., SØGAARD, H. T., KUDSK, P., NØRREMARK, M., LUND, I., NADIMI, E. S. & JØRGENSEN, R. 2009. Site-specific weed control technologies. Weed Research, 49, 233-241.

CIBA-GEIGY 1975. The scoring system proposed by EWRC. Field Trial Manual

CLARKE, J., WYNN, S., TWINING, S., BERRY, P., COOK, S., ELLIS, S. & GLADDERS, P. 2009. Pesticide availability for cereals and oilseeds following revision of Directive 91/414/ECC; effects of losses and new research priorities. HGCA Research Review.

FAO 2009. The lurking menace of weeds. Rome. Available at: http://www.fao.org/news/story/en/item/29402/icode/

FENNIMORE, S. A., HANSON, B. D., SOSNOSKIE, L. M., SAMTANI, J. B., DATTA, A., KNEZEVIC, S. Z. & SIEMENS, M. C. 2014. Field Applications of Automated Weed Control: Western Hemisphere. 151-169.

GERHARDS, R. & OEBEL, H. 2006. Practical experiences with a system for site-specific weed control in arable crops using real-time image analysis and GPS-controlled patch spraying. Weed Research, 46, 185-193.

GRAGLIA, E. Importance of herbicide concentration, number of droplets and droplet size on growth of Solanum nigrum L, using droplet application of glyphosate. XIIeme colloque international sur la biologie des mauvaises herbes, 2004 Dijon, France

HARKER, K. N. & O'DONOVAN, J. T. 2013. Recent weed control, weed management, and integrated weed management. Weed Technology, 27, 1-11.

HESS, M., BARRALIS, G., BLEIHOLDER, H., BUHR, L., EGGERS, T., HACK, H. & STAUSS, R. 1997. Use of the extended BBCH scale—general for the descriptions of the growth stages of mono; and dicotyledonous weed species. Weed Research, 37, 433-441.

JABRAN, K., MAHAJAN, G., SARDANA, V. & CHAUHAN, B. S. 2015. Allelopathy for weed control in agricultural systems. Crop Protection, 72, 57-65.

KLOSE, R., RUCKELSHAUSEN, A., THIEL, M. & MARQUERING, J. Weedy–a sensor fusion based autonomous field robot for selective weed control. 66th International Conference

Agricultural Engineering/AgEng, Stuttgart-Hohenheim, VDI-Verlag, Conference Proceedings, 2008. 167-172.

KNEZEVIC, S. Z., SIKKEMA, P. H., TARDIF, F., HAMILL, A. S., CHANDLER, K. & SWANTON, C. J. 1998. Biologically effective dose and selectivity of RPA 201772 for pre-emergence weed control in corn (Zea mays). Weed technology, 670-676.

LEE, W. S., SLAUGHTER, D. & GILES, D. 1999. Robotic weed control system for tomatoes. Precision Agriculture, 1, 95-113.

LUND, I., SØGAARD, H. & GRAGLIA, E. Micro-spraying with one drop per weed plant. Third Danish Plant Production Congress, Denmark, 10-11 January, 2006, 2006. Danish Institute of Agricultural Sciences, 451-452.

MEIER, U., BLEIHOLDER, H., BUHR, L., FELLER, C., HACK, H., HEß, M., LANCASHIRE, P. D., SCHNOCK, U., STAUß, R. & VAN DEN BOOM, T. 2009. The BBCH system to coding the phenological growth stages of plants–history and publications. Journal für Kulturpflanzen, 61, 41-52.

MIDTIBY, H. S., MATHIASSEN, S. K., ANDERSSON, K. J. & JØRGENSEN, R. N. 2011. Performance evaluation of a crop/weed discriminating microsprayer. Computers and electronics in agriculture, 77, 35-40.

MILLER, P., TILLETT, N., HAGUE, T. & LANE, A. 2011. The development and field evaluation of a system for the spot treatment of volunteer potatoes in vegetable crops. Aspects of Applied Biology, 113-120.

MONSANTO 2011. Roundup® Biactive® herbicide label. Monsanto UK Limited.

MURDOCH, A. J., LA WARR, D., NICHOLAS, P. & PILGRIM, R. A. 2010. Proof of concept of automated mapping of weeds in arable fields.

OERKE, E.-C. 2006. Crop losses to pests. The Journal of Agricultural Science, 144, 31-43.

PACANOSKI, Z. 2015. Herbicides and Adjuvants.

PARLIAMENT, E. 2009. Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides. Off. J. Eur. Union, 309, 71-86.

POWER, E. F., KELLY, D. L., STOUT, J. C. & GHERSA, C. 2013. The impacts of traditional and novel herbicide application methods on target plants, non-target plants and production in intensive grasslands. Weed Research, 53, 131-139.

REGULATION, E. 2009. No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. EU, Brussels.

RITZ, C. 2010. Toward a unified approach to dose–response modeling in ecotoxicology. Environmental Toxicology and Chemistry, 29, 220-229.

RUCKELSHAUSEN, A., BIBER, P., DORNA, M., GREMMES, H., KLOSE, R., LINZ, A., RAHE, F., RESCH, R., THIEL, M. & TRAUTZ, D. 2009. BoniRob–an autonomous field robot platform for individual plant phenotyping. Precision agriculture, 9, 1.

SINGH, M., BHULLAR, M. S. & CHAUHAN, B. S. 2014. The critical period for weed control in dry-seeded rice. Crop Protection, 66, 80-85.

SLAUGHTER, D. C., GILES, D. K. & DOWNEY, D. 2008. Autonomous robotic weed control systems: A review. Computers and Electronics in Agriculture, 61, 63-78.

SØGAARD, H. T. & LUND, I. 2007. Application Accuracy of a Machine Vision-controlled Robotic Micro-dosing System. Biosystems Engineering, 96, 315-322.

STREIBIG, J. C. 2010. Assessment of herbicide effects.

URDAL, F., UTSTUMO, T., VATNE, J. K., ELLINGSEN, S. A. A. & GRAVDAHL, J. T. Design and control of precision drop-on-demand herbicide application in agricultural robotics. Control Automation Robotics & Vision (ICARCV), 2014 13th International Conference on, 2014. IEEE, 1689-1694.

WOZNICA, Z. 2015. RE: AS 500 SL Adjuvant. Type to MURDOCH, A.

YOUNG, S. L. & GILES, D. K. 2014. Targeted and Microdose Chemical Applications. 139-147.

YOUNG, S. L., MEYER, G. E. & WOLDT, W. E. 2014. Future Directions for Automated Weed Management in Precision Agriculture. 249-259.

WOZNICA Z, IDZIAK R, WANIOREK W & SIP M. 2015. An optimized glyphosate formulation. Proceedings 17th European Weed Research Society Symposium, Montpellier, France, 55-56.

Appendices

Appendix 1. Protocol followed for preparing dilution series of glyphosate using two-fold serial dilution. Dilution series started from the highest concentration (4x) until the desired glyphosate concentrations have been prepared.

1. Beakers and micro-tubes were labelled properly.
2. Store 1ml of Roundup in a micro-tube.
3. Store 5 ml of deionized water to a beaker.
4. After diluting glyphosate shake ten times each micro-tube to ensure hominization of the solution.
5. <u>4-times the recommended dose (4/1)</u>: Add 400 µl of Roundup to a micro-tube with 600 µl of deionized water. Then take 250 µl and store in another micro-tube with 2.5µl of adjuvant. The rest of the solution will be used for further dilution.
6. <u>2-times the recommended dose (2/1):</u> Take 500 µl out of the 4/1 solution to 500 µl of deionized water in a micro-tube. Then remove 250 µl and store it in another micro-tube with 2.5 µl of adjuvant for trial application. The rest solution will be used for further dilution.
7. <u>Recommended dose (1/1):</u> This is for preparing a 10% Roundup dilution. Take 500 µl out of the 2/1 solution and dilute with 500 µl of deionized water in a micro-tube. Then 250 µl and store it in another micro-tube with 2.5 µl of adjuvant for trial application.
8. <u>Half of recommended dose (1/2):</u> Take 500µl out of the 1/1 solution to a micro-tube and add dilute with 500 µl of deionized water. Take 250 µl and store it in the micro tube with 2.5µl of adjuvant for trial application. The rest was utilized for further dilution.
9. Repeat step (8) until the desired series of concentrations have been produced.
10. Adjuvant Control: Take 250 µl of deionized water and store it in the micro tube with 2.5µl of adjuvant for trial application. Put a proper label on the micro-tube.
11. Pure Product Control: Take 250 µl pure MRB from the beaker and store it in the micro tube with 2.5µl of adjuvant for trial application. Put a proper label on the micro-tube.
12. Water Control: Keep 250 µl of deionized water in a micro-tube.

Appendix 2. Parameters estimates (±se), T-values and P-values of the dose-response model and ED90 value for the fresh weight data of the *Chenopodium album* drc trial.

Parameters	**Estimate**	**T-value**	***P*-value**
b	1.000 ± 0.236	4.237	<0.0001
c	0.037 ± 0.010	3.655	0.0003
d	0.158 ± 0.006	25.300	<0.0001
e (ED50) (µg)	3.545 ± 1.014	3.493	0.0005
ED90 (µg)	31.875 ± 18.559	NA	NA

Residual standard error: 0.061 on 425 degrees of freedom
NA: not applicable

Appendix 3. Parameters estimates (±se), T-values and P-values of the dose-response model and ED90 value for the fresh weight data of the *Rumex crispus* drc trial.

Parameters	Estimate	T-value	*P*-value
b	0.535 ± 0.210	2.544	0.0115
c	0.123 ± 0.016	7.682	<0.0001
d	0.203 ± 0.011	18.336	<0.0001
e (ED50) (µg)	5.307 ± 4.853	1.094	0.2751
ED90 (µg)	321.60 ± 639.26	NA	NA

Residual standard error: 0.064 on 271 degrees of freedom
NA: not applicable

Appendix 4. Parameters estimates (±se), T-values and P-values of the dose-response model and ED90 value for the dry weight data of the *Galium aparine* drc trial.

Parameters	Estimate	T-value	*P*-value
b	1.871 ± 0.800	2.337	0.0207
c	0.009 ± 0.002	3.663	0.0003
d	0.032 ± 0.001	24.002	<0.0001
e (ED50) (µg)	5.953 ± 1.544	3.855	0.0002
ED90 (µg)	19.26 ± 11.78	NA	NA

Residual standard error: 0.011 on 161 degrees of freedom
NA: not applicable

Appendix 5. Parameters estimates (±se), T-values and P-values of the dose-response model and ED90 value for the dry weight data of the *Matricaria recutita* drc trial.

Parameters	Estimate	T-value	*P*-value
b	1.439 ± 0.450	3.194	0.0016
c	0.008 ± 0.004	1.835	0.0680
d	0.063 ± 0.004	16.642	<0.0001
e (ED50) (µg)	2.218 ± 0.648	3.424	0.0007
ED90 (µg)	10.215 ± 6.562	NA	NA

Residual standard error: 0.026 on 197 degrees of freedom
NA: not applicable

Appendix 6. Parameters estimates (±se), T-values and P-values of the dose-response model and ED90 value for the dry weight data of the *Urtica urens* drc trial.

Parameters	Estimate	T-value	*P*-value
b	0.959 ± 0.277	3.457	0.0007
c	0.076 ± 0.011	6.709	<0.0001
d	0.177 ± 0.007	23.739	<0.0001
e (ED50) (μg)	46.534 ± 17.013	2.735	0.0068
ED90 (μg)	460.03 ± 389.19	NA	NA

Residual standard error: 0.038 on 191 degrees of freedom
NA: not applicable

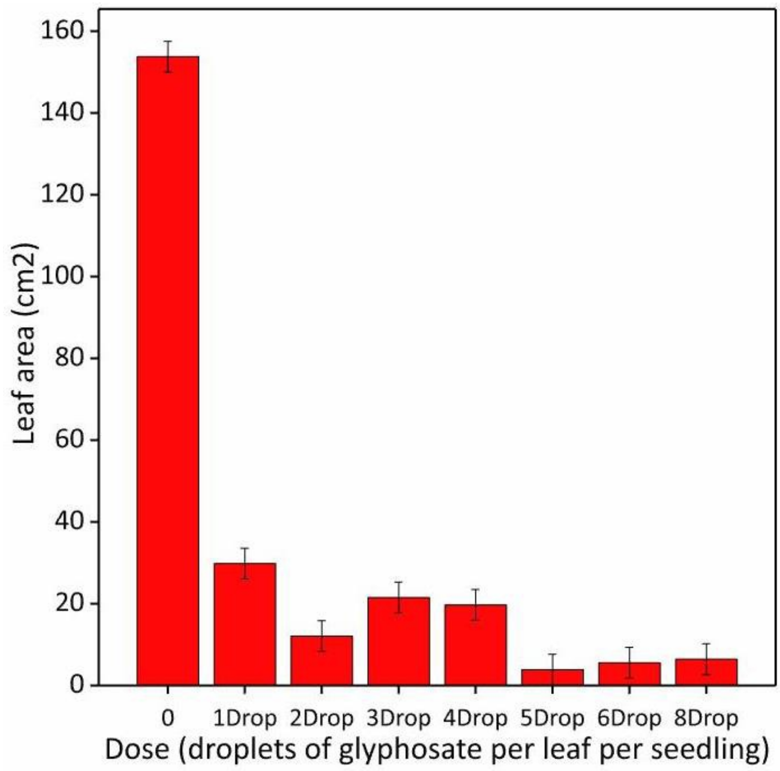
Appendix 7. Anova table after one-way analysis of variance of the dry weight of the *U. urens* seedlings and the doses of glyphosate applied.

Source of variation	Degrees of freedom	Sum of squares	Mean square	Variance ratio	*P*-value
Dose	7	4.627649	0.661093	91.83	<0.001
Residual	143	1.029436	0.007199		
Total	150	5.657085			

Appendix 8. Anova table after one-way analysis of variance of the leaf area of the *U. urens* seedlings and the doses of glyphosate applied.

Source of variation	Degrees of freedom	Sum of squares	Mean square	Variance ratio	*P*-value
Dose	7	335124.4	47874.9	177.67	<0.001
Residual	144	38801.8	269.5		
Total	151	373926.1			

Appendix 9. Means and standard errors of leaf area (cm^2) data of *U. urens* seedlings 3 weeks after application of different number of droplets containing 25% concentration of Roundup Biactive.



Appendix 10. Parameters estimates (±se), T-values and P-values of the dose-response model and ED90 value for the dry weight data of *Brassica oleracea var. sabauda* drc trial.

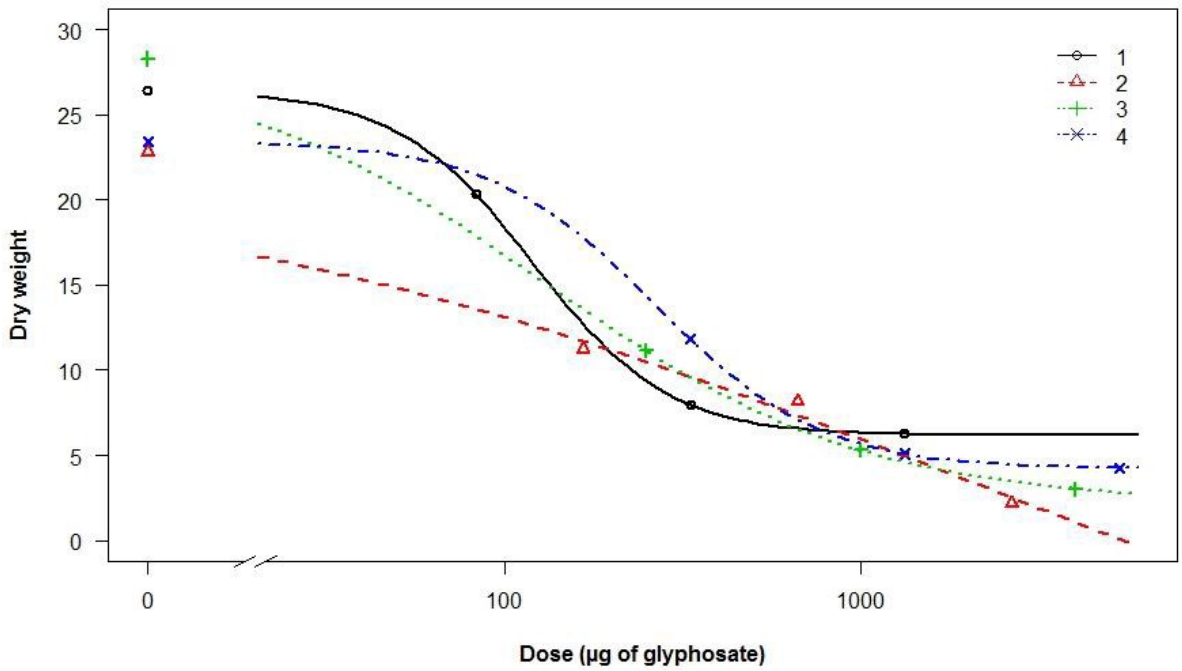
Parameters	**Estimate**	**T-value**	***P*-value**
b	0.970 ± 0.166	5.831	<0.0001
c	0.168 ± 0.031	5.455	<0.0001
d	0.665 ± 0.018	37.066	<0.0001
e (ED50) (µg)	35.918 ± 7.987	4.498	<0.0001
ED90 (µg)	345.83 ± 171.37	NA	NA
ED10 (µg)	3.730 ± 1.469	NA	NA

Residual standard error: 0.160 on 386 degrees of freedom
NA: not applicable

Appendix 11. Parameters estimates (±se), T-values and P-values of the dose-response model and ED90 value for the dry weight data for each of the times of droplet application of *Brassica oleracea var. sabauda* sequential trial.

Parameters	Times of application	Estimate	T-value	*P*-value
b	1	2.281 ± 2.784	0.819	0.425
c	1	6.171 ± 5.130	1.203	0.247
d	1	26.422 ± 4.156	6.358	<0.0001
e (ED50) (µg)	1	120.14 ± 81.65	1.471	0.1618
ED90 (µg)	1	314.78 ± 527.91	NA	NA
b	2	0.311 ± 0.220	1.417	0.1757
c	2	-28.384 ± 73.966	-0.383	0.7062
d	2	22.794 ± 2.248	10.140	<0.0001
e (ED50) (µg)	2	10184.10 ± 85735.64	0.119	0.9069
ED90 (µg)	2	11755,139 ± 153374919	NA	NA
b	3	0.960 ± 1.019	0.942	0.360
c	3	2.078 ± 4.396	0.472	0.643
d	3	28.296 ± 1.852	15.277	<0.0001
e (ED50) (µg)	3	128.622 ± 73.521	1.750	0.0994
ED90 (µg)	3	1267.9 ± 2697.8	NA	NA
b	4	1.887 ± 2.740	0.689	0.5008
c	4	4.198 ± 2.463	1.704	0.1077
d	4	23.430 ± 2.077	11.283	<0.0001
e (ED50) (µg)	4	265.96 ± 98.33	2.704	0.0156
ED90 (µg)	4	851.53 ± 1270.06	NA	NA

Appendix 12. *Brassica oleracea var. sabauda* dry weight as a function of the dose of glyphosate (µg) applied per seedling, after 1, 2, 3 and 4 times of droplets application. Parameters estimates are presented in Appendix 11.



Appendix 13. Anova table after two-way analysis of variance between doses of glyphosate applied and times of application in response of the dry weight data of the *Brassica oleracea var. sabauda* seedlings.

Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean square**	**Variance ratio**	***P*-value**
dose ignoring application	10	5739.47	573.95	15.63	< 0.001
dose eliminating application	10	5538.53	553.85	15.08	< 0.001
application ignoring dose	3	277.89	92.63	2.52	0.066
application eliminating dose	3	76.95	25.65	0.7	0.557
dose.application	2	62.18	31.09	0.85	**0.434**
Residual	63	2313.78	36.73		
Total	78	8192.38	105.03		

Appendix 14. Parameters estimates (±se), T-values and P-values of the dose-response model and ED90 value for the dry weight data of *Brassica oleracea var. sabauda* sequential trial.

Parameters	**Estimate**	**T-value**	***P*-value**
b	1.191 ± 0.497	2.398	0.0190
c	3.254 ± 2.059	1.580	0.1183
d	25.327 ± 1.293	19.583	<0.0001
e (ED50) (µg)	158.03 ± 43.82	3.606	0.0006
ED90 (µg)	999.19 ± 860.63	NA	NA

Residual standard error: 5.8287 on 75 degrees of freedom
NA: not applicable