Project title:	"eyeSpot" – leaf specific herbicide applicator for weed control in field vegetables
Project number:	CP 134
Project leader:	Alistair Murdoch, University of Reading
Report:	Annual Report, 2017
Previous report:	Annual Report for 2016
Key staff:	Dr Alistair Murdoch (PI), Mr Nikolaus Koukiasis (PhD student), Mr Paul de la Warr (software engineer), Mr Liam Doherty, Mrs Caroline Hadley and Mr Richard Casebow (technical staff). Miss Fern Price (Undergraduate student intern) External advisors: Dr Robert Pilgrim, Mr Shane Sanford and Mr Steve Brueckner (Concurrent Solutions llc)
Location of project:	University of Reading: (a) Agriculture Building, Earley Gate, (b) Controlled Environment Laboratory, Whiteknights and (c) Crop Research Unit, Sonning Farm Linked activities (not funded by AHDB): Concurrent Solutions, Benton, Kentucky, USA
Industry Representative:	Mr Phil Lilley, Hammond Produce Ltd., New Farm, Redhill, Nottingham NG5 8PB
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Alistair John Murdoch

Associate Professor of Crop and Weed Science

University of Reading

Signature   Date   4 April 2017

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CONTENTS

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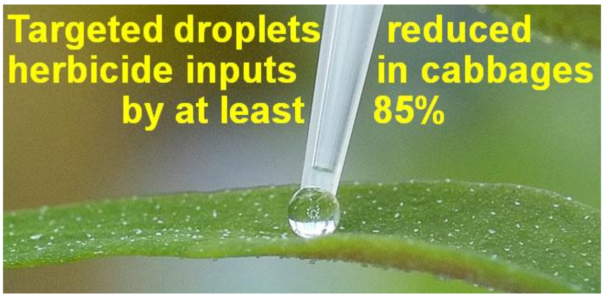
GROWER SUMMARY

Headline



- Precision targeting of glyphosate droplets to leaves of weeds in field trials with savoy cabbages was shown to reduce amounts of herbicide applied by 85% compared to a single inter-row spray and by 94% compared to a pendimethalin pre-emergence spray.
- Glasshouse trials showed efficacy of droplet applications of glufosinate-ammonium so that if approval for use of glyphosate were to be withdrawn, an alternative product is available.
- Use of alternative products is also essential to avoid the risk of herbicide resistance.
- Three sequential treatments with droplets achieved the maximum crop yield and weed suppression. This strategy is also mitigates risks of herbicide resistance, since weeds surviving an initial treatment, would be retreated on a subsequent visit.

Background

Weeds and their control play a vital role in maintaining vegetable yields and quality and herbicides are a highly efficient method of managing weeds. However, improper or inappropriate use of herbicides may have adverse effects on human health and the environment. Even though their use is subject to stringent regulation in the UK, the EC Regulation No. 1107/2009, the Water Framework Directive (2000/60/EC) and the Sustainable Use Directive (2009/128/EC) are leading to the loss of herbicide actives and make it more difficult for new compounds to gain approval. This predicament is worse for field vegetable growers because of their reliance on a limited and old range of herbicides which require a lot of funding and effort in order to keep them in the market.

This project represents a paradigm shift to post-emergence weed control in field vegetables. Some use of chemicals is retained, but it explores an engineering solution rather than chemistry and genetics (e.g. herbicide-tolerant crops). Moreover, the concept is no direct application of herbicides to the soil, none to the crop, simply leaf-specific droplet

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applications of a non-selective, systemic herbicide to the leaves of unwanted plants (i.e. weeds). It is the ultimate in precision agriculture. Overall objectives are 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 run-off to the soil, crop and non-target organisms; and
- provide an engineering alternative to herbicide tolerant crops (whether by conventional plant breeding or by genetic modification).

Plant specific weeding by hand is what growers have traditionally done. Individual plants are examined and if unwanted are hoed or removed. Such a task is dull, difficult, dirty and perhaps even dangerous and of course economically impossible on a large field scale. The project therefore explores the possibility of achieving leaf-specific weed control using an autonomous platform. If successful, this state of the art project will demonstrate a pre-commercial system as an alternative to other systems which approximate to plant specific weed control using directed sprays, lasers or electrocution. The former is currently available and the latter two are the subject of research. Each method has advantages and disadvantages, which are not discussed here, but a comparison of the directed spraying option with eyeSpot is available on request. The system here is designed to control all weeds in the field including young seedlings before they have had any yield or quality impact on the crop. The immediate application is to field vegetables after transplanting or drilling into bare soil.

Summary

Precision targeting of glyphosate droplets to leaves of weeds is a leading edge procedure. We have been applying droplets manually this year for proof of concept and for evaluation

of potential benefits while the automated droplet applicator is being developed. The droplets very small (1-2 microlitres) – so that one teaspoonful (5 ml) would be enough to treat 2500-5000 individual weeds if one droplet is put on each weed. Nevertheless, the droplets are much larger than those used when spraying so that there is no risk of either spray drift but nor are they large enough for spatter. In the experiments carried out in 2016/17, droplets were applied manually – mostly by Nikos Koukiasas, the PhD student on the project, and also by Fern Price-Jones an undergraduate summer intern funded by the University. In 2017, we hope to repeat and extend these trials in the UK and also carry out tests with an automated applicator in the USA.

The herbicide must be non-selective since the same product is applied to all weeds but no application is made to the crop. The chemical must also move from the point of application to other leaves and the roots. Glyphosate is therefore ideally suited to this application and we have used Roundup® Biactive GL (360 g/l, SL, Monsanto (UK) Ltd.) in this year's trials although we plan to use other formulations. To avoid risks of resistance and to provide an alternative, we have also successfully applied glufosinate ammonium (Harvest®, 150 g/L, SL, Bayer CropScience Ltd.) in glasshouse trials over the past year.

The dose applied is approximately based on the area of ground covered by an individual weed. In this way we can calculate how much herbicide would have been applied to the same ground area if one assumes that the amount, which would have been applied by conventional spraying, were sprayed uniformly. It is therefore possible to estimate exactly how much product is in the equivalent of the "recommended" dose for an individual weed plant. We have constructed dose-response curves on this basis.

In this year's (2016) field trials with savoy cabbages, we have shown that we can reduce herbicide inputs by 94% compared to a pendimethalin pre-emergence spray (Stomp Aqua®, 455 g/l pendimethalin, CS, BASF plc). Three sequential treatments, with droplets 3, 5 and 7 weeks after transplanting the seedlings, achieved the highest crop yield among weed control treatments, and weed suppression and was actually superior to the pendimethalin treatment. Note that the triple treatment also mitigates risks of herbicide resistance, since weeds surviving or omitted in an initial treatment, would be retreated on a subsequent visit.

- Glasshouse trials showed efficacy of droplet applications of glufosinate-ammonium so that if approval for use of glyphosate were to be withdrawn, an alternative product is available.
- Use of alternative products is also essential to avoid the risk of herbicide reistance.

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- Three sequential treatments with droplets achieved the maximum crop yield and weed suppression. This strategy is also mitigates risks of herbicide resistance, since weeds surviving an initial treatment, would be retreated on a subsequent visit.

Financial Benefits

Evaluation of the economics is planned for 2018

Action Points

No action needs to be taken by growers at this stage in the eyeSpot project.

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SCIENCE SECTION

Introduction

Background information to the project is provided in the Grower Summary.

Activities in 2016/17 comprised four main areas:

1. Image capture in natural weed infestations in leeks, savoy cabbages, red cabbage and Chinese leaf. Images were captured automatically using a customised camera and custom-built computer system (supplied by Concurrent Solutions llc). This system is a prototype of that we expect to use in the final autonomous platform (robot). Custom-written software, provided by Paul de la Warr, was used by Fern Price-Jones to tag individual weeds according to species in approx. 500 images.
2. Field trials to prove concept of droplet application system in real crops. Activities in 2015/16 were limited to the glasshouse and proved the concept in controlled environments. This year, the aim was to demonstrate efficacy of weed control in field vegetable crops and to test the hypotheses that
    a. droplet applications were effective in controlling the natural weed infestation in the field
    b. droplet applications were at least as effective as pre-emergence herbicide in controlling the natural weed infestation
    c. use of droplet applications for weed control did not cause significant yield penalty
    d. use of droplet applications for weed control would reduce herbicide use by at least 90% without yield penalty and would achieve at least 90% weed control
3. Glasshouse dose-response trials with glufosinate-ammonium as part of a strategy of exploring alternatives to glyphosate.
4. Installation of glasshouse in Kentucky for preliminary testing of herbicide application system (funded by Concurrent Solutions llc)

Materials and methods

Image capture

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Figure 1. EyeWeed camera system mounted on the boom of a sprayer, capturing images of weeds and red cabbage crop (left). Image of a weedy plot with red cabbages as it was captured using EyeWeed (right). The hoops were to support fleece.

The camera was attached to a small-plot sprayer boom on a tractor-mounted sprayer. Images were captured weekly starting three weeks after and finishing seven weeks after transplanting (Figure 1) to include the critical weed free periods for the crops. Camera was orientated to capture images as would be carried out using an autonomous platform.

Plant material & Experimental design

Savoy cabbage seeds (*Brassica oleracea* var. *capitata*), Famosa F1 variety, red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) Integro F1 variety and chinese cabbage (*Brassica rapa* subsp. *Pekinensis*) Manoko F1 variety were provided from Elsoms Seeds (Elsoms Seeds Ltd, Lincolnshire, United Kingdom) and were sown under glasshouse conditions on Seed & Modular compost (Clover Peat, Dungannon, N. Ireland). Six weeks after sowing, cabbage seedlings were transplanted to the field at the 3 to 4 leaf stage.

Experimental design and plot layouts are shown in Appendix 1. The following description relates only to the Savoy cabbage experiment as most leek plants were severely affected by wireworms and that the leek experiment was abandoned. Cabbage seedlings were planted with 50 cm row spacing and 30 cm between plants within the rows. A distance of 90 cm was left between the plots which remained unplanted and untreated throughout the trial. 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 irrigated daily for one hour using an automated drip irrigation system except on days when rainfall exceeded 0.2 mm, when it was turned off. The site of the field trial contained a natural infestation of *Chenopodium album*, *Senecio vulgaris*, *Matricaria recutita*, *Spergula arvensis*, and *Poa annua* weeds. *Equisetum* also affected the plots but was removed by hand as this is not a typical weed of field vegetables.

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The area planted with savoy cabbages only was used for the droplet application trial and the experimental design was randomized complete block with four replications of eight treatments (Table 1). Control treatments involved the use of weedy and weed-free plots which remained untreated and hand-weeded respectively throughout the trial. Plots were 2.5 m wide and 2.1 m long having four single rows of cabbages with 28 plants per plot (Appendix 1).

Table 1. Chemical weed control treatments in Summer 2016 field trial with cabbages and leeks. In addition, there were weedy and weed-free (hand-weeded) controls.

Treatments	Treatment description	Time of application relative to date of transplanting
Pre-em	2.9 l/ha, Stomp Aqua®, 455 g/l pendimethalin	One week before
Inter-row spray	1.5 l/ha, Roundup® Biactive, 360 g/l glyphosate between the rows	3 weeks after
Droplet x1	36 µg of glyphosate per weed	3 weeks after
Droplet x3	36 µg of glyphosate per weed	3, 5 and 7 weeks
Droplet x3 (adj)	9 or 18 µg of glyphosate per weed	3, 5 and 7 weeks
Inter-row +Droplet x1	1.5 l/ha, Roundup® Biactive between the rows + 36 µg of glyphosate per weed	Inter-row spray 3 weeks and single droplet 5 weeks after

Herbicide application details

Application of the pre-emergence (Pre-em) herbicide (Stomp Aqua®, 455 g/l pendimethalin, CS, BASF plc) and the inter-row glyphosate spray (Roundup® Biactive GL, 360 g/l, SL, Monsanto (UK) Ltd.) were carried out using an electric knapsack sprayer (CP 15 Electric, Cooper-Pegler, Villefranche-sur-Saone, France). This sprayer was calibrated to deliver 1.310 l/sec. For the inter-row application of glyphosate a spray shield (38 cm) was used to ensure that no herbicide was applied to the crop.

Application of glyphosate droplets was carried out manually, using a pipette with a volume range from 0.1 to 2.5 µl (ErgoOne® Single-Channel, Starlab Ltd, Milton Keynes, UK) in a 1 m wide and 1.5 m long treatment area in the centre of each plot (Appendix 1). In order to apply 36 µg of glyphosate per weed seedling (Droplet x1 and Droplet x3), 5% solution of

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Roundup® Biactive GL was used and one droplet of 2 µl volume was applied to one leaf of each weed or in the case of *Spergula arvensis* to the central meristem. No adjustment was made for weed size in these treatments. For the Droplet x3 (adj) treatment, however, droplets containing 9 or 18 µg of glyphosate per weed were applied if the ground covered by all the leaves of an individual seedling was visually estimated to be less or more than 1 cm^2, respectively (Figure 2). For this adjusted treatment, a 2.5% solution of Roundup® Biactive GL was used and one droplet of 1 µl volume was applied (9µg) or two droplets (18µg) were applied per weed.

Figure 2. Photo of an area inside a plot where droplet x3 (adj) treatment was applied. Weed seedlings tagged with a red circle (●) received 18 µg of glyphosate and the ones tagged with a black triangle (▲) received 9 µg of glyphosate. The scale is in cm.

Assessments

The assessments that were carried out included an initial weed count of the plots, followed by two more assessments from the first droplet application with a 3-week interval. In addition, phytotoxicity symptoms were recorded using the EWRC scoring system. Eight weeks after transplanting, an intermediate harvest of weeds was carried out from an area size of 0.11 m^2 (between the first two cabbage crops of the 2nd and 3rd row) within the treated area of the plot and their fresh and dry weights were recorded. Finally, 18 weeks

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after transplanting, trimmed heads of cabbages and the fresh and dry weights of weeds were harvested from the treated area of the plots (Figure 3; Appendix 1).

Statistical analysis

GenStat (16th Version) was used and one-way ANOVA was carried out to analyse weed biomass data and cabbage fresh shoot weights.

Results

Image capture

After images were captured they were analysed using School's image analysis software. Cabbages and weeds were tagged using a red circle for each leaf and a blue circle to mark the growing point of each plant (Figure 4).

Figure 3. Image captured using the camera system from a plot where Droplet x3 treatment was applied 4 weeks after transplanting. Tagging of the crop - Savoy cabbages (left) - and of *Chenopodium album* weed seedlings (right) is shown. This is an aid to weed discrimination software development and constructing an album of images for validation (image truthing). Leaves have been tagged using a red circle ( ) and central growing points with a blue one ( ). The tagging software allows species to be identified

Field experiments on precision weed control in cabbages and leeks

Crop yield in the Droplet x3 treatment was not significantly lower than that in hand-weeded control in contrast to the pre-emergence and inter-row spray treatments, which were (Figures 6). The Droplet x3 treatment reduced weed biomass by 92% (Figure 5) despite using 94% and 85% less herbicide than the pre-emergence and inter-row spray treatments, respectively (Table 2). A single droplet application (Droplet x1) was, however, insufficient to achieve a satisfactory level of control and produced the lowest yield (Figures 5-6).

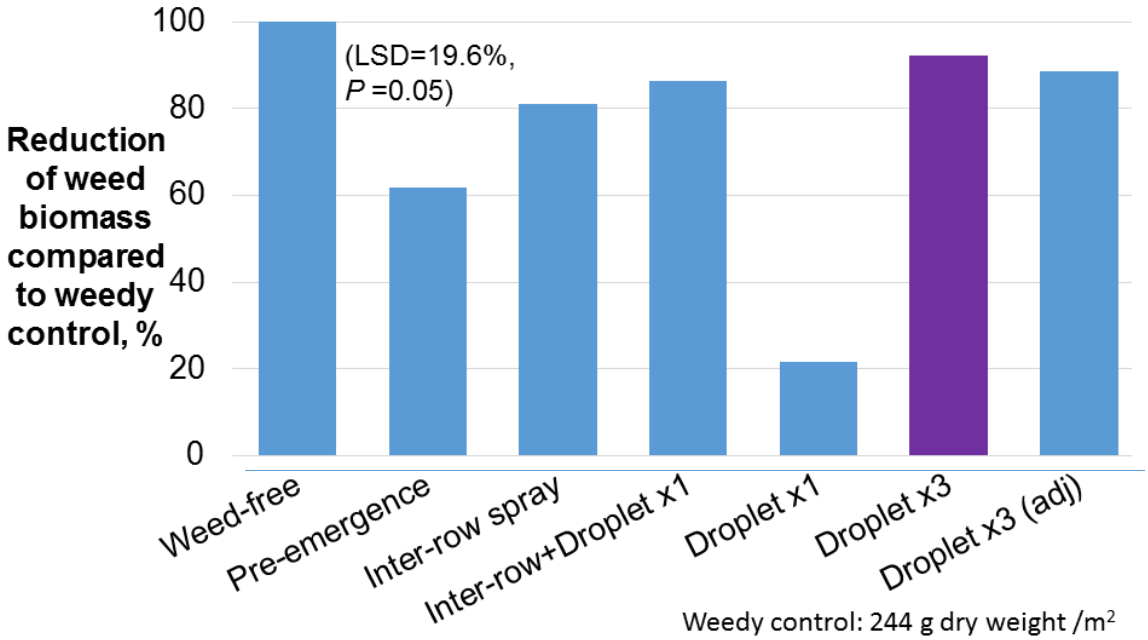

Figure 4. Reduction of weed dry biomass relative to the weedy plots.

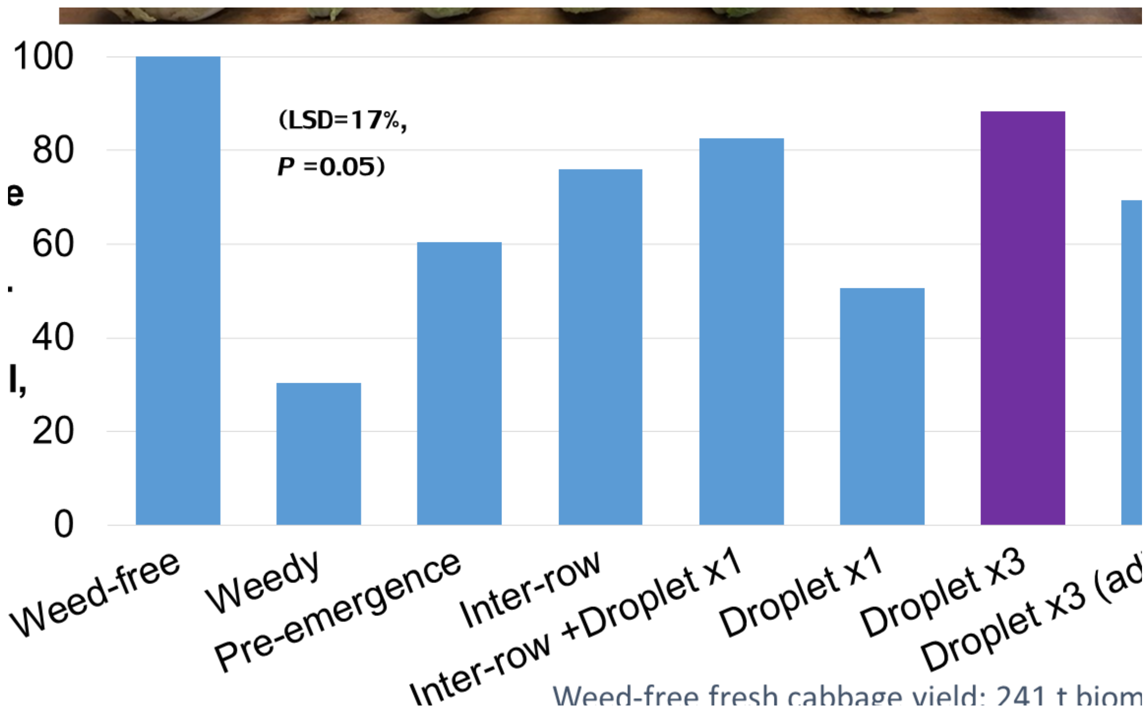

Figure 5. Cabbage biomass yield relative to the hand-weeded, weed-free plots.

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Table 2. Average amounts of herbicide applied (g of a.i./ha) for the weed control treatments and reductions relative to the pre-emergence and inter-row spray treatments.

Treatments	Average amount of herbicide applied, g a.i./ha	Reduction relative to pre-emergence, %	Reduction relative to inter-row spray, %
Droplet x1	53.9	95.9	90.0
Droplet x3	83.3	93.7	84.6
Droplet x3 (adj)	119	91.0	77.9
Inter-row spray	540	59.1	0.0
Inter-row + Droplet x1	562	57.4	-4.1
Pre-emergence	1320	0.0	-144

Glasshouse dose-response trials with glufosinate-ammonium and glyphosate

To minimise risks of herbicide resistance and also of loss of approval by regulators, It is important that the system is not dependent on a single active ingredient (ai). Technical prerequisites for an ai are that it be a non-selective (broad-spectrum) herbicide and it must be systemic. Dose-response relationships for glyphosate were reported in the Annual Report for 2015/16, but some additional research is needed to assess more precise dosing according to weed size. In August 2016, three new dose-response trials were established using mean weed ground cover to determine the recommended dose rate for both glufosinate ammonium and glyphosate.

Materials and Methods

Glasshouse trials were carried out during August 2016 with mean temperatures 26/17°C (day/night). Weed seeds were provided from Herbiseed Ltd. and were sown on the surface of J. Arthur Bower's multi-purpose compost in multi-cell plastic trays. The trays consisted of 84 cells with individual cell size being 35mm x 35mm and 45mm deep. Five to seven weed seeds were sown in each cell and after germination they were thinned to one seeding per cell. After germination, the seedlings were transplanted to individual pots (9 cm diameter) where they were treated. All trials were randomized complete blocks with 12 replicates.

In order to estimate the volume (μl), amount (μg) and number of herbicide droplets needed to apply the recommended rate of the herbicides (L/ha) individual images of the seedlings

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were taken using a Nikon D90 Digital SLR Camera with an 18-105 mm VR Lens Kit, mounted on a tripod (ManFrotto Compact Action). These images were then analysed using the WinDIAS Leaf Image Analysis System (Delta-T Devices Ltd, Cambridge, UK) and ground cover was estimated in cm^2 by the proportion of green pixels in an image of known area (Table 3). Means and standard deviations of weed ground cover were 9.03 (±3.63), 4.84 (±1.56) and 6.25 (±2.11) cm^2 for *Stellaria media*, *Chenopodium album* and *Urtica urens*, respectively. Droplets of glufosinate-ammonium (Harvest®, 150 g/L, SL, Bayer CropScience Ltd.) were applied to *U. urens* and *C. album* and droplets of glyphosate (Roundup® Biactive GL, 360 g/L, SL, Monsanto (UK) Ltd.) were applied to *S. media*.

Dose rates ranged from 1/128 to 6x of recommended for glufosinate-ammonium and from 1/256 to 4x for glyphosate. In the former, the recommended dose of glyphosate was also applied. There were three controls: water only, water with adjuvant (1%) and undiluted herbicide. Deionised water was used to prepare all the solutions and in order to achieve the label recommendation for glyphosate (540 g of a.i. ha^{-1}) and glufosinate-ammonium (450 g of a.i. ha^{-1}), A 10% concentration was prepared for both herbicides. 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.

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

Regression analysis

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. The four-parameter log-logistic model (Eqn 1) was fitted by non-linear regression:

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

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.

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Results

Figure 6. *C. album* seedlings two weeks after application of droplets containing different concentrations of glufosinate ammonium relative to the recommended dose (1x). Control treatments were treated with water, 1% adjuvant (Conadj) and undiluted herbicide (Glu). Seedlings were treated at the 6-leaf stage.

Figure 7. *Urtica urens* seedlings two weeks after application of droplets containing different concentrations of glufosinate ammonium relative to the recommended dose (1x). Control treatments were treated with water, 1% adjuvant (Conadj) and undiluted herbicide (Glu). Seedlings were treated at the 6-leaf stage. Scale is 30 cm.

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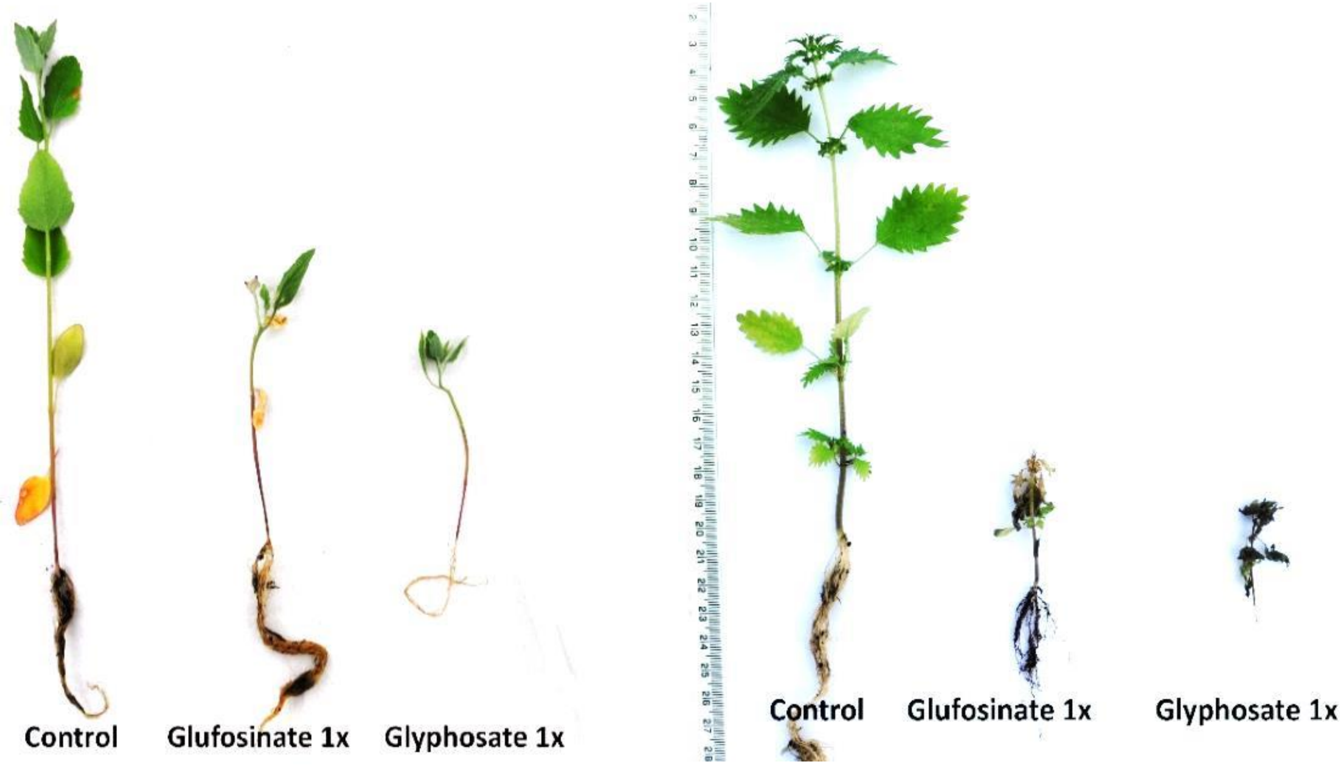

Figure 8. *C. album* (left) and *U. urens* (right) seedlings two weeks after application of droplets containing the recommended doses of glufosinate-ammonium and glyphosate. Controls were treated with purified water. Scale is 26 cm

Figure 9. *S. media* seedlings three weeks after application of droplets containing different concentrations of glyphosa relative to the recommended dose (1x). Control treatments were treated with purified water (Control) or with 1% adjuva (Conadj) or Roundup Biactive (Gly). Seedlings were treated at the 6 to 8-leaf stage. Scale is 28 cm.

Dose-response relationships are visualised in Figures 6, 7 and 9 and the statistically analysed curves shown in Figures 10-12 for *C. album*, *U. urens* and *S. media*, respectively.

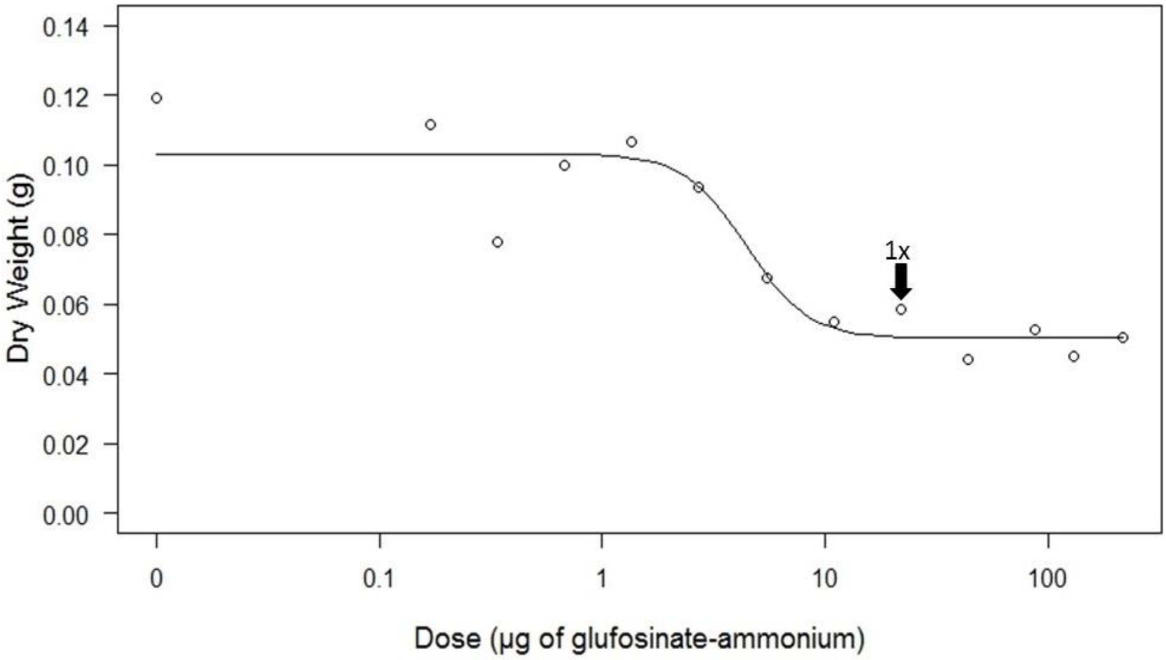

Figure 10. Dry weight of *Chenopodium album* seedlings two weeks after droplet application, as a function of the dose of glufosinate-ammonium applied per seedling. The recommended dose is shown (1x ↓). Parameter estimates of the fitted dose-response curve (Eqn 1) are in Table 4.

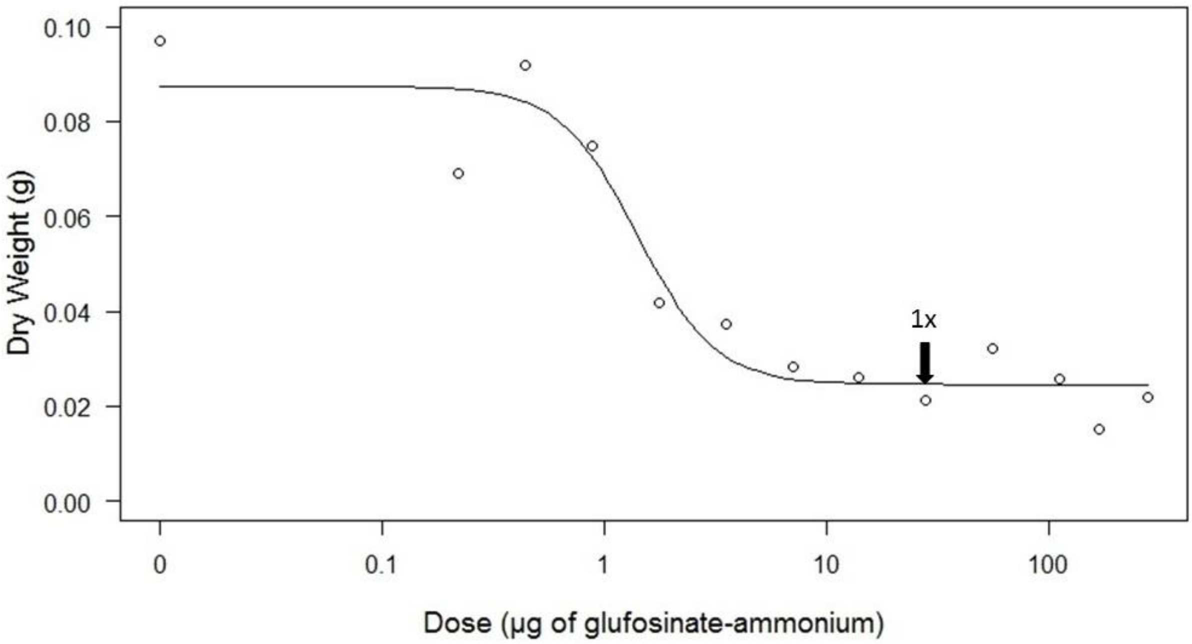

Figure 11. Dry weight of *Urtica urens* seedlings two weeks after droplet application, as a function of the dose of glufosinate-ammonium applied per seedling. The recommended dose is shown (1x ↓). Parameter estimates of the fitted dose-response curve (Eqn 1) are in Table 4.

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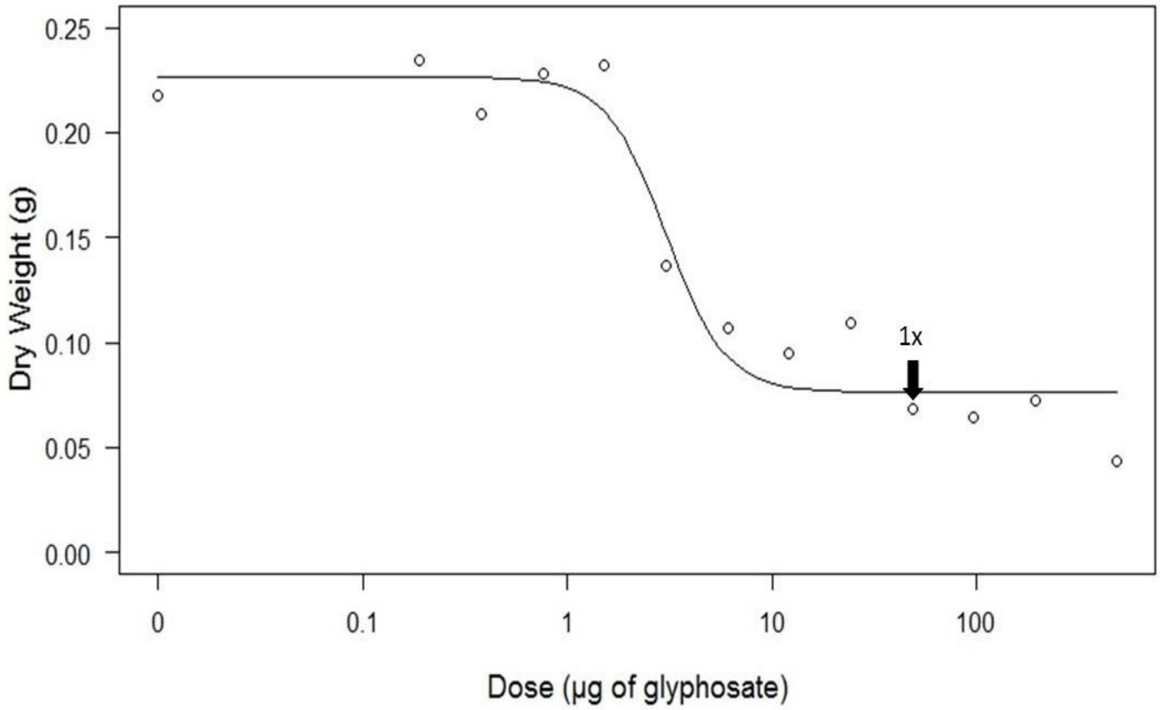

Figure 12. Dry weight of *Stellaria media* seedlings, 20 days after droplet application as a function of the dose of glyphosate applied per seedling. The recommended dose is shown (1x ↓). Parameter estimates of the fitted dose-response curve (Eqn 1) are in Table 4.

Table 3. Parameter estimates (±SE) of the seedling dose-response regression curves (Eqn 1; Figures 10-12), doses of a.i. per seedling estimated to reduce weed dry weight by 50 and 90% (ED_{50} and ED_{90}) and recommended seedling doses of a.i. (1x) for glufosinate ammonium (*Urtica urens* and *Chenopodium album*) and glyphosate (*Stellaria media*). Dry weights were determined 20 days after applying the droplets.

Weed species	**b**	**c (g)**	**d (g)**	**ED_{50} (µg)**	**ED_{90} (µg)**	**1x (µg)**
Chenopodium album	3.1 (2.6)	0.05 (0.005) ***	0.10 (0.005) ***	4.43 (1.2) **	8.99 (6.1)	21.8
Urtica urens	2.6 (1.9)	0.02 (0.004) ***	0.09 (0.006) ***	1.4 (0.3) ***	3.42 (2.4)	28.1
Stellaria media	3 (3.9)	0.08 (0.02) ***	0.23 (0.01) ***	3.04 (1.1) **	6.3 (7.8)	48.8

$P < 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

The ED_{90} values showed that applying 9 µg of glufosinate-ammonium to *C. album* and *U. urens* seedlings at the 6-8 leaf stage, effectively controlled these weeds even though the recommended treatments based on the mean ground cover of the individual plants were more than twice this amount (Table 4, Figures 6-7, 10-11). For *S. media*, droplets

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containing 6.3 μg of glyphosate controlled the weeds satisfactorily even though this was approx. 1/8th of the recommended dose (Figures 9, 12). When comparing efficacy of the two products at their recommended dose rates, glyphosate appears to have been more effective than glufosinate-ammonium (Figure 8).

Activities in the USA

As agreed with AHDB, instead of installing a glasshouse test rig at Reading, it would be installed at Benton near Murray in West Kentucky. The main activities here have been to create the system and results are expected to be available during summer 2017, when Nikos Koukiasas will make a visit there. A targeting test rig using lasers has been installed and is currently being tested for correct operation. A further rig with droplet applicator is available.

Discussion

It is clear from the field trials, that the weed control efficacy of droplet applications shown in glasshouse studies and reported in the 2015/16 Annual Report, was transferable to field trials. The 2016 field trials were limited to Savoy cabbages due to an infestation of wireworms which affected almost all plots in the leek trial. We intend to repeat both trials in 2017/18 in a different field and we are also obtaining nursery-grown leek plants which may reduce problems.

We accepted our hypothesis that multiple treatments with a herbicide lacking residual activity like glyphosate would be necessary. This inference was reinforced because a single glyphosate treatment gave a very poor weed control and lowest yield of all weed control treatments. Multiple treatments may be needed to keep the crop weed free during its critical period for weed control. The concept of critical period has two elements: (1) there is a time after planting when late emerging weeds will no longer be sufficiently competitive to reduce crop yield and (2) for early emerging weeds, there a period of time when they are too small to reduce crop development. The critical weed-free period is the interval between these two periods which ensures that early emerging weeds are removed before they cause damage and later emerging are not allowed to establish until the risk of their affecting the crop is eliminated (Nieto *et al.*, 1968). It is important to emphasise that this period varies with location, planting time, spacing within and between crop rows and cultivar. Onions and leeks are particularly vulnerable to competition (Hewson and Roberts, 1971) and are hypothesised to need several droplet treatments to ensure late emerging weeds are controlled. We were unable to test this hypothesis for leeks in 2016 but plan to in 2017. For cabbages, the literature suggests that a single weed control treatment may be sufficient. Weaver (1984) found a single inter-row spray treatment after 3-5 weeks was sufficient in

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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 period. UK organic farmers are 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. For droplets, therefore, it would be hypothesised that might appear that the triple treatment option was “overkill”. Nevertheless, we have to reject that hypothesis for droplets since the triple treatment was optimal achieving no significant reduction in crop yield or weed control while still reducing amount of herbicide applied by at least 85%, whereas the single droplet treatment amd single inter-row treatments gave poorer control (the single droplet being the worst). We plan to retest this hypothesis for both leeks and cabbages in the field in 2017.

Glasshouse trials showed for the first time to our knowledge that droplet applications of glufosinate ammonium could be a valuable alternative to glyphosate for leaf-specific weed control. This hypothesis will be tested in the field in 2017.

Results of the image capture have been passed over to our collaborators in the USA and will be used for software development. A particular challenge for herbicide targeting is that of species with needle-shaped leaves such as *Spergula arvensis*, of which there was a large number in the field experiment (e.g. there are several plants in Figure 2). Targeting in this case will be directed at the apical meristem but the target area available is not large (Figure 2). Provided accurate targeting is achieved, however, weed control efficacy was high in the field experiment.

Conclusions

1. Droplet applications with glyphosate reduced amounts of herbicide applied to field grown cabbages by 94% compared to a preemergence spray and by 85% compared to an inter-row spray with glyphosate.
2. The small reduction in cabbage yield due to weeds in the droplet treatment was not statistically significant and was less than that in the other treatments.
3. Three droplet applications at two weekly intervals achieved satisfactory weed control whereas a single droplet application did not.
4. Glufosinate-ammonium was identified as a potential alternative to glyphosate for droplet applications but this conclusion needs to be tested in the field.

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Knowledge and Technology Transfer

Students at Reading are exposed to the technology during lectures on weed technology and those doing an IPM module carry out a laboratory practical similar to the dose-response experiments carried out in the project, in which they determine dose-response relationships using droplet applications to individual weeds. Other students are involved in the project where feasible – specifically in 2016, Fern Price-Jones a second-year undergraduate, assisted with the field trials and did all of the image tagging as a university-funded six-week internship.

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Tables of presentations and a list of media reports follows:

Presentations

Event	Date	Place	Topic	Type	Presenter
SCI Young Researchers in Agri-Food 2016: Food Quality and Sustainability from Plough to Plate	12 May 2016	University of Reading	Dose response relationship of droplet applications for the leaf-specific weed control in vegetable crops	Oral + Poster	Nikolaos Koukiasas
SCI Young Researchers in Crop Sciences 2016	14 July 2016	Syngenta Jealott's Hill	Leaf-specific weed control on vegetable crops	Oral	Nikolaos Koukiasas
Crop Production Group Student Symposium	1 Nov. 2016	University of Reading	Weed control in cabbages using droplets of glyphosate	Oral + Poster	Nikolaos Koukiasas
BCPC Weeds Review 2016	10 Nov. 2016	Rotham-sted Research	Targeted droplets reduced herbicide inputs in cabbages by at least 85%	Oral + Poster	Nikolaos Koukiasas
2016 AHDB Crops PhD Studentship Conference	16-17 Nov. 2016	Stratford Manor Hotel, Warwick	Targeted droplets reduced herbicide inputs in cabbages by at least 85%	Poster	Nikolaos Koukiasas
Conference on Science and policy: nutrient management challenges for the next generation.	7 Feb. 2017	Massey University, Palmer-ston North, New Zealand	Robotic weeding of field vegetables offers potential reduction in herbicide inputs of at least 90%. Abstract in Appendix	Oral and paper	Alistair Murdoch

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Media reports

A range of media reports have been published following AHDB's press release on 21 November 2016. The PI has also been interviewed twice (once in the UK for *The Economist* and once in New Zealand for *Rural News [New Zealand]*). The university has also promoted the research. This following list is likely to be incomplete as we are not usually notified by the publishers. In addition to reports in *Horticulture Week*, *Fresh Produce Journal*, *Farmers' Weekly*, *Farmers' Guardian*, *Farming UK* and *Scottish Farmer* in the UK, media coverage has occurred in the USA (6), Canada (1), New Zealand (1), Switzerland (1) and the Netherlands (1).

1. 21 November 2016: AHDB Horticulture "The future of targeted weed control" https://horticulture.ahdb.org.uk/news-item/future-targeted-weed-control [Press release by AHDB Horticulture]
2. 21 November 2016: Horticulture Week "Trials show promise of automated topical herbicide application" http://www.hortweek.com/trials-show-promise-automated-topical-herbicide-application/edibles/article/1416273
3. 21 November 2016: Fresh Produce Journal "New weed control tech 'could slash herbicide use'" http://www.fruitnet.com/fpj/article/170647/new-weed-control-tech-could-slash-herbicide-use
4. 21 November 2016: Farmers' Weekly "Precision spraying could reduce herbicide use by 95%" http://www.fwi.co.uk/arable/precision-spraying-reduce-herbicide-use-95.htm
5. 23 November 2016: Farmers' Guardian Insight. "Targeted weed control system under development". https://www.fginsight.com/news/news/targeted-weed-control-system-under-development-16940
6. 29 November 2016: Farming UK "New research project aims to reduce herbicide inputs in weed control by 95 per cent" [Published online 21 Nov 2016] https://www.farminguk.com/news/New-research-project-aims-to-reduce-herbicide-inputs-in-weed-control-by-95-per-cent_44865.html
7. 16 December 2016, News Report, "The future of targeted weed control" in *Agroberichten Buitenland*, Washington DC, USA. http://www.agroberichtenbuitenland.nl/verenigde-staten/future-targeted-weed-control/
8. 4 January 2017.Article in the *Scottish Farmer* http://www.thescottishfarmer.co.uk/news/15001410.Weed_control_by_robot/
9. 10 January 2017, News Report, "Robots: The Future of Weed Control" in *AgWeb – the Farm Journal,* Philadelphia, Pennsylvania, USA. http://www.agweb.com/article/robots-the-future-of-weed-control-naa-ben-potter/
10. 11 January 2017. "Robots: The Future of Weed Control – Tech Check News www.techchecknews.com/world/robots-the-future-of-weed-control/

11. 13 January 2017. “Can robots destroy weeds better than a farmer driving a sprayer?” In HortiDaily. Tholen, The Netherlands
    http://www.hortidaily.com/article/31576/Can-robots-destroy-weeds-better-than-a-farmer-driving-a-sprayer
12. 13 Jan 2017 Robots: The Future of Weed Control | Weed Science Society of America
    wssa.net/2017/01/robots-the-future-of-weed-control/
13. 13 January 2017. “Researchers Developing Automated Spot Herbicide Ejector”
    i. In Growing Oregon.
       http://growingoregon.com/news/2017/01/researchers-developing-automated-spot-herbicide-ejector-2017-01-13/
    ii. Also in *Growing Georgia*
       http://growinggeorgia.com/news/2017/01/researchers-developing-automated-spot-herbicide-ejector-2017-01-13/
    iii. and in *Growing Kansas*
       http://growingkansas.com/news/2017/01/researchers-developing-automated-spot-herbicide-ejector-2017-01-13/
14. 18 January 2017. “Killing weeds is a job for robots” J-LYN Grains - Ontairo Farmer
    www.jlyngrains.com/ontairofarmer
15. 10 March 2017. “Reducing chemical inputs with robots” *Rural News* (New Zealand)
    http://www.ruralnewsgroup.co.nz/item/11578-reducing-chemical-inputs-with-robots
16. 12 March 2017. “Can robots help reduce chemical inputs” FruitWorldMedia
    [quoting #15]

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Hewson, R.T., and Roberts, H.A. (1971). The effect of weed removal at different times on the yield of bulb onions. Journal of Horticultural Science 46:471-483.

Nieto, J.H., Brando, M.A. and Gonzalez, J.T. (1968). Critical periods of the crop growth cycle for competition from weeds. PANS 14:159–166

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Weaver, S.E., 1984. Critical period of weed competition in three vegetable crops in relation to management practices. Weed Research 24:317-325.

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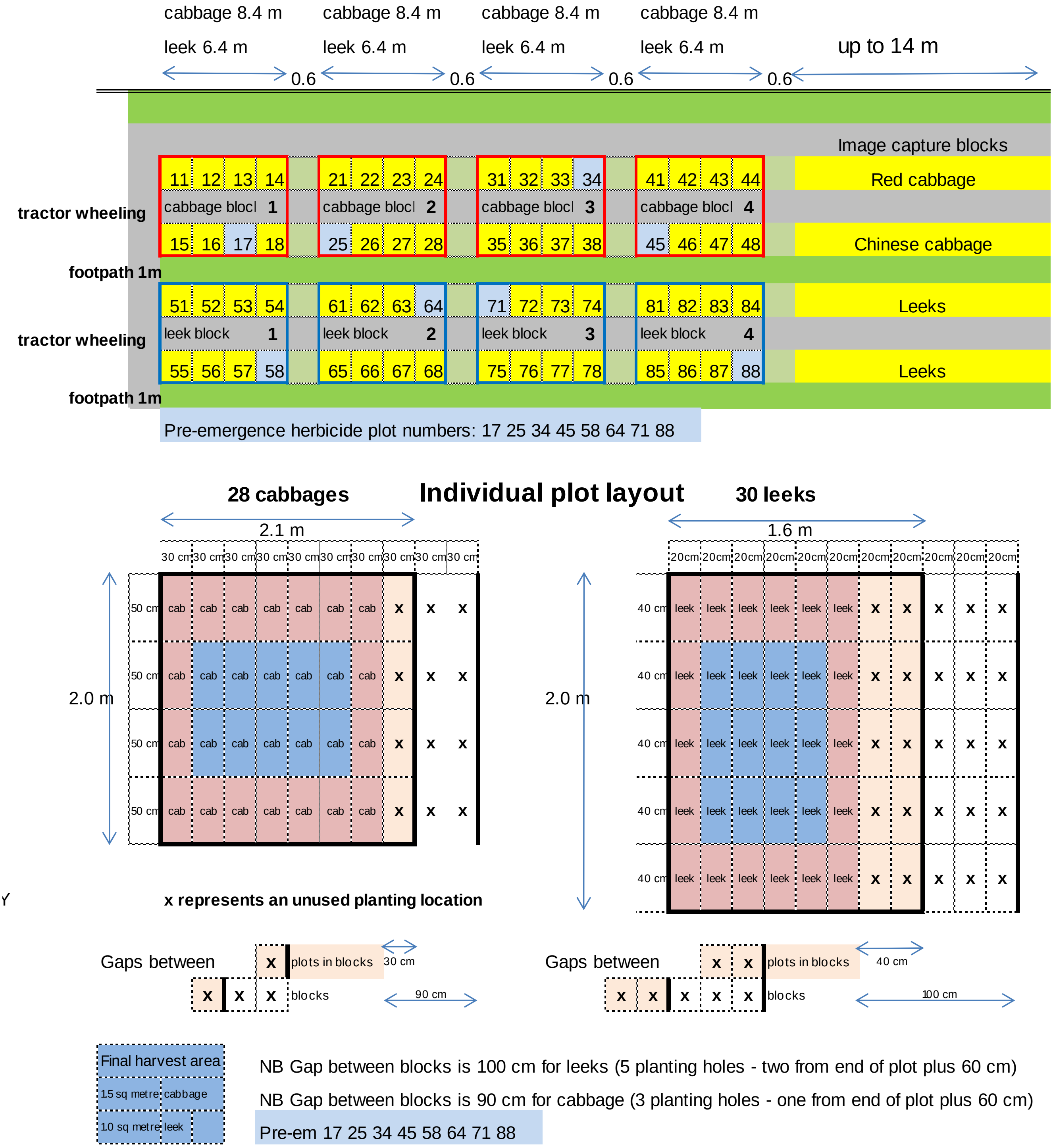
Appendix 1 Experimental design for field trials, summer 2016.

Two field experiments were planted in 2016 in Woodlands field, Sonning Farm, one with Savoy cabbages and one with leeks. The leek experiment was abandoned due to wireworm damage to a large number of the leeks. Both experiments comprised randomised complete blocks with the same eight treatments per block. Plot sizes, row and plant spacings, and final harvested areas were varied according to species as shown. Automated image capture was carried out in all plots using the tractor wheelings. To achieve a range of crop backgrounds, additional blocks for image capture were planted with red cabbage, Chinese cabbage and leeks. Note that the layouts are not drawn to scale.



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Appendix 2 Abstract of paper presented in New Zealand, 7/2/2017

Murdoch, A.J., Koukiasis, N., de la Warr, P.N., Pilgrim, R.A., Sanford, S., 2017. Robotic weeding of field vegetables offers potential reduction in herbicide inputs of at least 90%. In: *Science and policy: nutrient management challenges for the next generation*. (Eds L. D. Currie and M. J. Hedley). http://flrc.massey.ac.nz/publications.html. Occasional Report No. 30. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand. 1 page

Robotic weeding of field vegetables offers potential reduction in herbicide inputs of at least 90%

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Abstract

Weed control in field vegetables in the UK is increasingly challenging due to the loss of herbicide actives. Actives have been lost due to loss of approval by regulatory authorities and there is also little incentive to develop new selective herbicides for vegetables. Equally, policy makers and consumers demand fewer agro-chemical inputs. Selective herbicides are not, however, needed is removed if weed leaves are identified by image analysis and if droplets of herbicides are targeted to these leaves. No chemical is applied to the crop and none directly to the soil.

Research at Reading in conjunction with Concurrent Solutions llc in the USA, is developing a robotic weeder for field vegetables in the UK.

This paper describes

1) dose-response relationships for glyphosate (Roundup Biactive, 360 g/l glyphosate) applied to individual leaves of weeds, and
2) proof of concept field experiments with manually applied droplets to the naturally occurring weed population in a cabbage crop.

Efficacy of glyphosate droplet applications to control weeds in glasshouse and field and to prevent crop yield loss was assessed in comparison to weed-free (hand-weeded), and weedy controls. Reductions in herbicide were compared with use of the pre-emergence herbicide, pendamethalin (Stomp Aqua, 455 g/l pendimethalin at 2.9 l/ha before transplanting). For the field study, Savoy cabbages were transplanted at the 4-leaf stage in June 2016 using a randomized complete block design with 4 blocks.

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Droplet applications, 3, 5 and 7 weeks after transplanting gave most effective weed control, reducing weed biomass by 92% compared to the weedy control and giving a crop yield, which did not differ significantly from the weed-free control. At the same time, the amount of herbicide applied was 94% lower than the recommended rate for pendamethalin and 85% less than a band spraying (inter-row) glyphosate treatment. Pre-emergence and band spray treatments gave significantly lower yields than the weed-free.

Provided a systemic herbicide is used, droplets only need to be applied to one leaf but three treatments were essential to allow for differences in weed emergence times. The efficacy of droplet applications for controlling natural weed infestation in cabbages was demonstrated.

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