Project title: “eyeSpot” – leaf specific herbicide applicator for weed control in field vegetables

Project number: CP 134

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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

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GROWER SUMMARY

Headline

- Experiments on precision manual applications of herbicide droplets to leaves of the natural weed infestation in field trials with cabbages and leeks. Dose – response relationships to droplet applications were compared for glyphosate and glufosinate-ammonium in a glasshouse study. Performance characteristics of a protoype herbicide droplet applicator were determined.
- Weed control in cabbages by glyphosate droplets reduced herbicide active applications by up to 98% compared to pendimethalin pre-emergence spray. In leeks, applications of herbicide actives were reduced by 74% compared to pendimethalin pre-emergence spray or 50% compared to post-emergence bromoxynil. These herbicide reductions were achieved in both crops *without affecting marketable crop yield or quality* compared to the weed-free, hand-weeded control.
- The field experiments with leeks and cabbages in 2017 included droplet applications of glufosinate-ammonium. Adequate weed control was achieved and although it was less effective than glyphosate. So, if approval for use of glyphosate were to be withdrawn, an alternative product has been identified and tested.
- Performance of the prototype applicator was optimised at 20 psi. Deviations due to wind during operation of the applicator were consistent and so could be compensated for in real applications.
- For commercial field vegetable crops, sequential treatments with droplets should take account of the crop's 'critical weed-free period' so that late germinating weeds, with potential to affect crop yield, are controlled. This approach will also mitigate 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 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.

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 plant-specific, with no direct herbicide applications to the crop or the soil. The concept is to apply single droplets of a non-selective, systemic herbicide to the individual leaves of unwanted plants (i.e. weeds). The approach is the state of the art 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 reduce 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. Even were the labour available and willing to hand-weed crops, the process is unlikely to be cost-effective and the task is dull, difficult, dirty and perhaps even dangerous (the four “Ds” of robotics).

The proposed system also offers advantages over mechanical intra- and inter-row tillage systems. Energy and fuel use will be much lower and the absence of soil disturbance means fewer weed seeds will be stimulated to germinate.

The project therefore explores the possibility of achieving leaf-specific weed control using an autonomous platform. If successful, the project will demonstrate a pre-commercial system as an alternative to other systems that 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. A detailed comparison of the directed spraying option with eyeSpot is available on request, but perhaps the essence of the difference is that the former targets large individual weeds such as potato volunteers, whereas eyeSpot will target weed seedlings of field vegetable crops soon after they emerge.

Summary

Precision targeting of glyphosate droplets to leaves of weeds is a leading edge procedure. The droplets are very small (1-2 microlitres) – so that one teaspoonful (5 ml) is enough to treat 2500-5000 individual weeds if one droplet is put on each weed. A distinctive fature is that discrete droplets are emitted. The droplets are much larger than those used when spraying so that there is no risk of spray drift but there is still the potential for spattering on impact and some shattering of droplets on ejection from an applicator and the droplets are likely to be deflected by wind. So in 2017/18, trials were carried out in the USA to assess the impact of applicator pressure and distance from target on spattering. The effect of wind on deflection of droplets was also investigated in a multifactorial experiment comprising windspeed and direction, applicator pressure and distance from target as factors. Provided windspeed and direction are known, deflection could be modelled and compensated for. Applicator pressure of 20 psi avoided all spattering in our tests.

To avoid risks of resistance and to provide an alternative, we have also tested glufosinate ammonium this year. This active ingredient has limited systemic action and so is less suitable than glyphosate for droplet application, but it appeared to achieve reasonable efficacy. Trials in 2018 will explore combining both actives with 2,4-D.

Doses applied in every case are linked approximately to the ground cover of the weeds. There is a potential issue as regards approval, for although the amount of product applied to each square metre of field will always be less than the permitted dose, the same would not be true for every square millimetre. There are of course one million mm^2 in each square metre and the current approvals rules do not take account of more focussed targeting.

In this year’s field trials with cabbages and leeks, respectively, herbicide inputs per unit land area with droplets reduced herbicide inputs by 98% and 74% compared to

a pendimethalin pre-emergence spray. Efficacy of weed control and crop yields were not significantly lower than the hand-weeded (“weed-free”) control.

Financial Benefits

Detailed analysis is planned for 2018/19 when all experimental results are available.

Action Points

As the research is not intended to produce a commercial product, no immediate action is needed. Growers are, however, encouraged to indicate their willingness to adopt an autonomous system.

SCIENCE SECTION

Introduction

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

Activities in 2017/18 comprised four main areas:

1. Images in natural weed infestations in leeks and cabbages 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 if the system is commercialised.
2. Prototype applicator evaluated to determine optimisation criteria (pressure, distance from target) and the impact of wind and wind direction on targeting.
3. Field trials to prove concept of droplet application system in real crops. Activities in 2017 tested the hypotheses that
    a. droplet applications 'satisfactorily' controlled the natural weed infestation in field crops of cabbages and leeks
    b. droplet applications were at least as effective as pre-emergence or post-emergence herbicides in controlling the natural weed infestation
    c. use of droplet applications for weed control did not incur any significant yield penalty compared to the weed-free control
    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
4. Glasshouse dose-response trials with glufosinate-ammonium and glyphosate continued in order to expand the range of weed species tested.
5. Glasshouse dose-response trials in Kentucky USA together with laboratory testing of herbicide application system (work carried out by UoR personnel but facilities funded and provided as an in-kind contribution by Concurrent Solutions llc).

Materials and methods

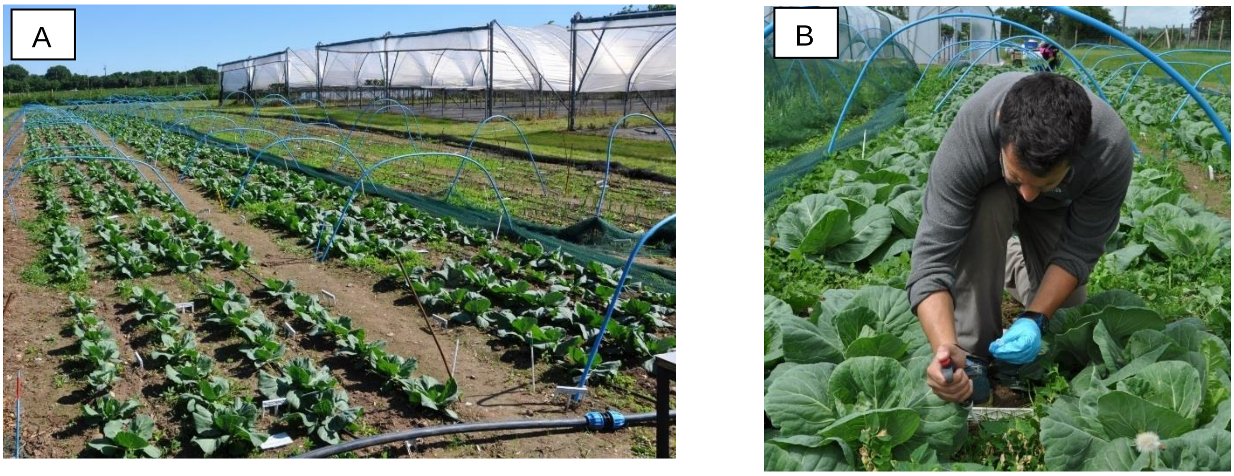


Figure 1 (A) Cabbage and leek experiments at Sonning Farm in 2017. Hoops supported netting for bird protection. The pipe in the foreground fed the drip irrigation system.

(B) Manual herbicide droplet application.

Image capture

The camera was attached to a small-plot sprayer boom on a tractor-mounted sprayer. Images were captured approximately weekly in both leeks and cabbages at Reading. The camera was orientated to capture images as would be carried out using an autonomous platform.

Plant material & Experimental design

Cabbage (cv. Surprise F1) and leek (cv. Krypton F1) seedlings were obtained from Westhorpe nursery (Westhorpe Plants Ltd, Boston, UK) on 20/04/2017 courtesy of Phil Lilley of Hammond Produce. They were transplanted to the field at the Crops Research Unit, Sonning Farm on 27/04/2017 at the 3 to 4 leaf stage. The soil was loamy sand with 87.1% sand, 6.4% clay, 5.3% silt and 12.9% stone content.

Experimental design and plot layouts are shown in Appendix 1. Cabbage / leek seedlings were respectively planted with 50 / 40 cm row spacing and 30 / 20 cm between plants within the rows with distances of 60 / 80 cm between the plots. Plots (width x length) were 2.5 m x 2.1 m (cabbages) and 2.0 m x 1.6 m (leeks). Both crops had four single rows with 28 plants per plot. (Appendix 1). A randomised block experimental design was used with four and three blocks for cabbages and leeks, respectively. Fertilizer application was carried out one week after transplanting using sulfur (SO_3) and nitrogen (N) at the rates of 50 kg ha^{-1} and 100 kg ha^{-1} respectively. Plants were individually irrigated daily for one hour using an automated drip irrigation system. The site of the field trial contained a natural infestation of *Senecio* *vulgaris* (Common groundsel), *Matricaria recutita* (Scented mayweed), *Chenopodium album* (Fat-hen), *Poa annua* (Annual meadow-grass), *Polygonum persicaria* (Redshank), *Capsella bursa-pastoris* (Shepherd's purse), *Achillea millefolium* (Common yarrow), *Geranium molle* (Dove's-foot Cranesbill), *Polygonum arenastrum* (Small leaved knotweed), *Taraxacum officinale* (common dandelion) and *Trifolium dubium* (little hop clover).

Various chemical treatments were tested together with four controls. In addition to weedy and weed-free plots which remained untreated and hand-weeded, respectively, throughout the trial, whole plot pre- and post-emergence herbicide controls simulated conventional practice (Table 1).

Table 1 Chemical weed control treatments in Summer 2017 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 (weeks)**
	CABBAGES	
Droplet x1 gly	36 µg of glyphosate/seedling	3
Droplet x2 gly	36 µg of glyphosate/seedling	3 and 5
Droplet x3 gly	36 µg of glyphosate/seedling	3, 5 and 6
Droplet x3 gly (adj)	9 µg of glyphosate per leaf	3, 5 and 6
Droplet x3 glu	60 µg of glufosinate-ammonium/seedling	3, 5 and 6
Droplet x3 glu (adj)	7.5 µg of glufosinate-ammonium per leaf	3, 5 and 6
Post-emergence	Sultan® 50 SC (500 g L^{-1} metazachlor) 1.5 L ha^{-1}	4
Pre-emergence	Stomp Aqua® (455 g L^{-1} pendimethalin) 2.9 L ha^{-1}	Three days before planting
	LEEKS	
Droplet x5 gly	36 µg of glyphosate/weed	2, 6, 8, 10, 12,
Droplet x10 gly	36 µg of glyphosate/weed	2, 4, 5, 6, 7, 8 9, 10, 11, 12
Droplet x10 gly (adj)	9 µg of glyphosate per leaf	2, 4, 5, 6, 7, 8, 9, 10, 11, 12
Droplet x10 glu	60 µg of glufosinate/weed	2, 4, 5, 6, 7, 8, 9, 10, 11, 12
Droplet x10 glu (adj)	7.5 µg of glufosinate-ammonium per leaf	2, 4, 5, 6, 7, 8, 9, 10, 11, 12
Post-emergence	Buctril (225 g L^{-1} bromoxynil) 1.5 L ha^{-1}	4 and 7 (Before BBCH 19)
Pre-emergence	Stomp Aqua® (455 g L^{-1} pendimethalin) 2.9 L ha^{-1}	Three days before planting

Herbicide application details

Pre-emergence herbicide (Stomp Aqua®, 455 g L^{-1} pendimethalin) and the post-emergence (Sultan® 50 SC for cabbages and Buctril for leeks) were applied with an electric knapsack sprayer (CP 15 Electric, Cooper-Pegler, Villefranche-sur-Saone, France). This sprayer was calibrated to deliver 1.310 L sec^{-1} using a spray volume of 200 L ha^{-1}.

All droplets had a volume of 2 µl and they were applied manually using a pipette (ErgoOne® Single-Channel, volume range from 0.1 to 2.5 µl, Starlab Ltd, Milton Keynes, UK). The droplet treatment areas in the centre of each plot (width x length) were 1.0 m x 1.2 m (cabbages) and 0.8 m x 0.8 m (leeks) (Appendix 1). For treatments with a single droplet per weed, 36 µg of glyphosate and 60 µg of glufosinate-ammonium were applied per seedling. For the glyphosate treatment 5% solution of Roundup® Biactive GL was used and for the glufosinate-ammonium 20% solution of Harvest® (150 g L^{-1}, SL, Bayer CropScience Ltd) and one droplet 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 adjusted (adj) treatments, droplets containing 9 µg of glyphosate and 7.5 µg of glufosinate-ammonium were applied to all leaves larger than 1 cm^2 in area (Figure 2). For adjusted treatments, solutions contained 1.25% Roundup® Biactive GL or 2.5% Harvest®.

Figure 2 Photo of an area inside a plot where droplet (adj) treatment was applied. Weed leaves tagged with a red circle (●) received 9 µg of glyphosate or 7.5 µg of glufosinate-ammonium. The scale is in cm.

Numbers of droplets applied per plot were counted so that an accurate estimate of herbicide application per unit area could be obtained and amounts of herbicide relative to conventional treatments could be assessed.

Assessments

Crops and weeds were harvested from the harvest area of the plots (Appendix 1). After cutting and weighing the above ground biomass, cabbages and leeks were trimmed (leeks trimmed to a length of 34 cm) and weighed (fresh weight) as for commercial sale. Leek stalk and cabbage head diameters were measured. Leeks were also classified into three categories according to their diameter (<25 mm, 25-35 mm and >35 mm) and were also weighed in different size categories: The dry biomass of the weeds was estimated after oven-drying fresh material for 48h at 80°C.

Statistical analysis

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

Results

Field experiments: Herbicide droplet application

From a practical perspective and because neither chemical has residual action, several applications were needed to ensure satisfactory control – three in cabbages and ten in leeks. Optimal glyphosate droplet treatments reduced the weed dry biomass in cabbages by 92-93% (*Figure 3*A – droplet x3), and by 97-99% in leeks (*Figure 3*B – droplet x10). For glufosinate ammonium, efficacy was significantly lower in leeks (79%) when only one droplet was applied per plant compared to treating each leaf in the adjusted (adj) treatment. Pre- and post-emergence treatments failed to control weeds satisfactorily in leeks achieving only 42 and 9% weed control, respectively (*Figure 3*B) whereas the pre-emergence herbicide gave satisfactory weed control (88%) in cabbages.

Hand-weeded control plots achieved marketable (trimmed) yields of 93.5 and 42.2 t ha^{-1} of cabbages and leeks, respectively (Figure 4). Yields in the optimal (adj) droplet treatments were not significantly lower than in these ‘weed-free’ crops and, in cabbages, all weed control treatments yielded similarly (Figure 4). By contrast, in leeks, yields were only maintained in the adjusted glyphosate droplet treatment; all other treatments significantly depressed crop yields: yield losses in conventional herbicide treatments were about 50% and even the glufosinate ammonium droplet treatment yielded 35% less (Figure 4).

Optimal droplet treatments achieved these high yields and weed control efficacies while reducing herbicide active use relative to the pendamethalin pre-emergence control by 98 and 74% in cabbages and leeks, respectively (***Table 2***).

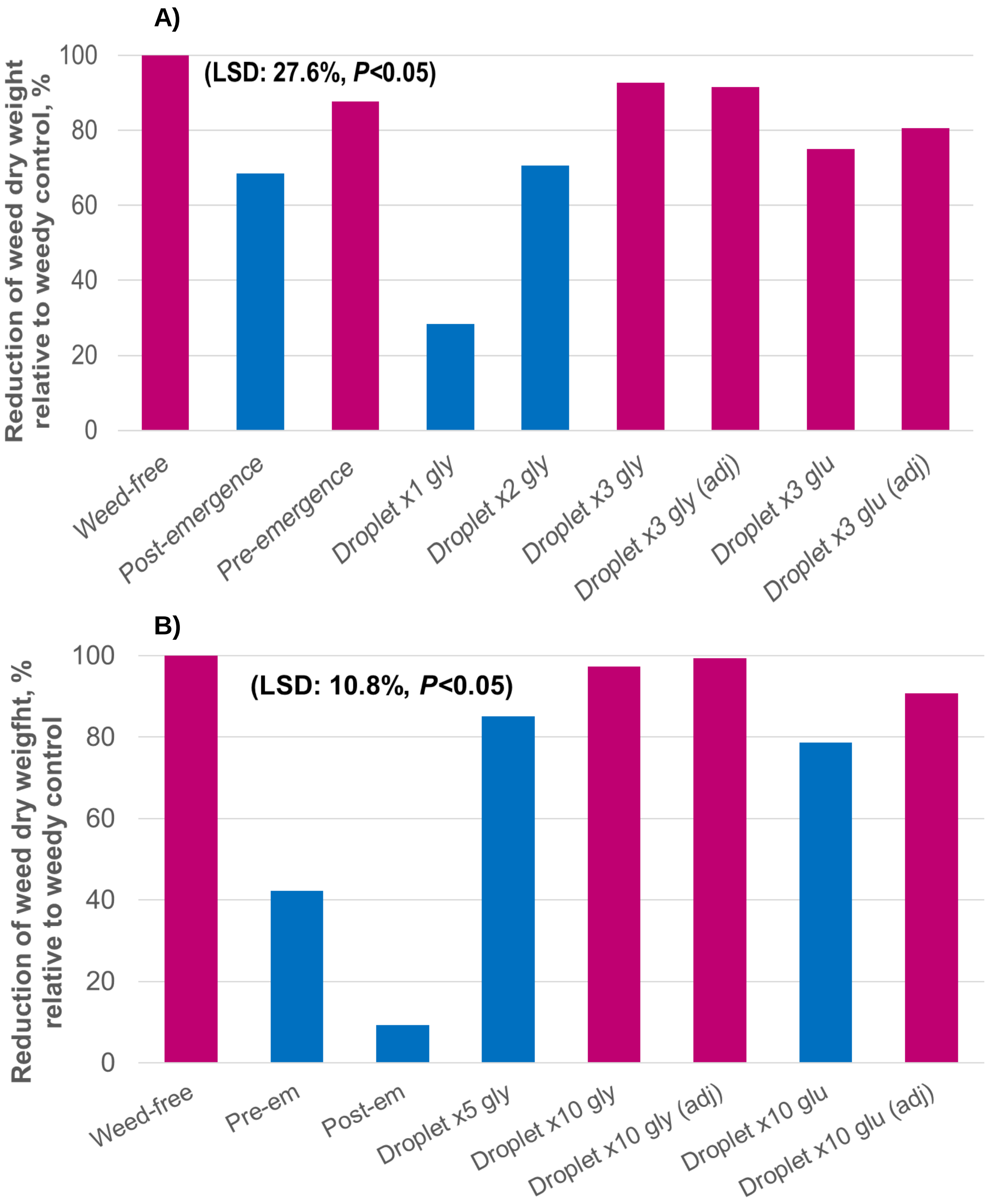


Figure 3 Reductions of weed dry biomass relative to the weedy plots achieved by various weed control methods in (A) cabbages and (B) leeks including hand-weeding, conventional pre- and post-emergence sprays, and various droplet treatments with glyphosate (gly) and glufosinate ammonium (glu). The dry weights of weeds in the weedy controls were (A) 393 g m^{-2} and (B) 537 g m^{-2}. See Table 1 for herbicide treatments. The number of droplet applications is indicated (x1 etc.). One droplet was applied per plant except in adjusted (adj) treatments when a droplet was applied to each leaf (**Figure 2**). Blue bars: significantly poorer weed control than hand-weeded (P < 0.05).

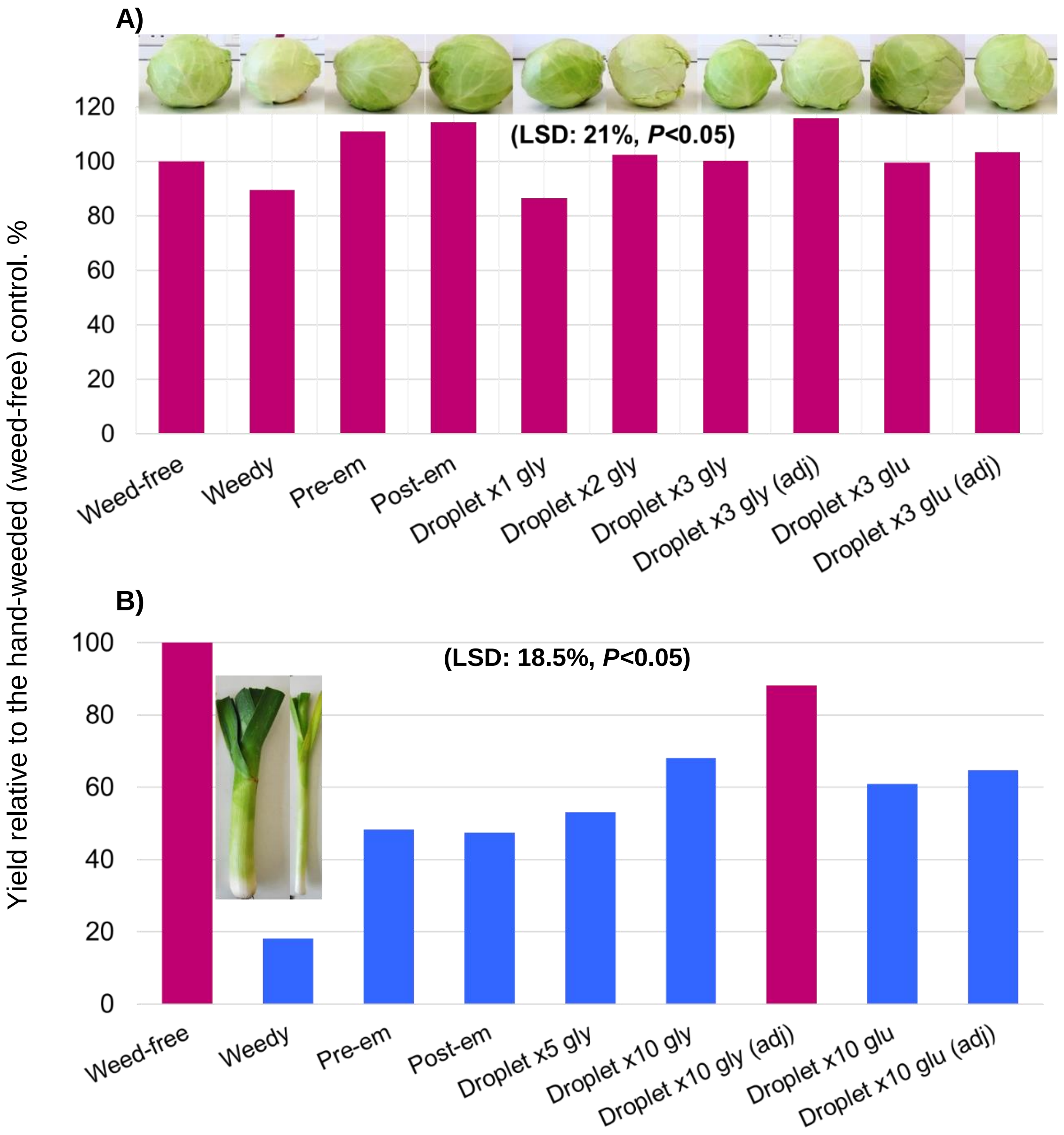


Figure 4 Marketable (trimmed) yields of (A) cabbages and (B) leeks expressed relative to the weed-free yield. Marketable yields in the weed-free controls were (A) 93.5 t ha^{-1} and (B) 42,2 t ha^{-1}. See Table 1 for herbicide treatments and **Figure** 3 for more details. Blue bars: significantly lower yield than hand-weeded (*P* < 0.05). The cabbages in (A) are typical trimmed examples for each treatment. The two leeks in (B) are from weed-free (left) and weedy (right) controls.

Table 2 Average amounts of herbicide applied (g of a.i. ha^{-1}) for the weed control treatments and reductions relative to the pre-emergence and post-emergence spray treatments. Label recommendations for glyphosate (gly) spray range from 540 to 1800 g ha^{-1} and for glufosinate-ammonium (glu) range from 450 to 750 g ha^{-1} with a maximum of 1500 g ha^{-1} per year if two treatments are applied. One droplet was applied per weed except In adjusted (adj) treatments, where a droplet was applied to each leaf of ≥1 cm^2 in area.

Treatments	**Average amount of herbicide applied, g ha^{-1}**	**Reduction relative to Pre-emergence, %**	**Reduction relative to Post-emergence, %**
	CABBAGES		**Metazachlor (750 g ha^{-1})**
Droplet x 1 gly	16.4	98.8	97.8
Droplet x 2 gly	41.0	96.9	94.5
Droplet x 3 gly	55.2	95.8	92.6
Droplet x 3 gly (adj)	28.1	97.9	96.3
Droplet x 3 glu	104.6	92.1	86.1
Droplet x 3 glu (adj)	40.2	97.0	94.6
	LEEKS		**Bromoxynil (675 g ha^{-1})**
Droplet x 5 gly	699.9	47.0	-3.7
Droplet x10 gly	930.1	29.5	-37.8
Droplet x10 gly (adj)	340.3	74.2	49.6
Droplet x10 glu	2120.5	-60.7	-214.2
Droplet x10 glu (adj)	646.1	51.0	4.3

From a regulatory perspective, the amounts of glyphosate applied in the cabbage crop over the whole growing season (28.1 g ha^{-1} in the optimal 'Droplet x 3 gly (adj)' treatment (Table 2) were 95-98% lower than would be used in a single glyphosate application at the recommended rate of 540 to 1800 g ha^{-1}. For glufosinate-ammonium, 40.2 g ha^{-1} was applied in adjusted treatment (Table 2), again, much lower than the label recommendation of 450 to 750 g ha^{-1} with a maximum of 1500 g ha^{-1} per year if two treatments are applied. In leeks, many more droplet treatments were applied, but amounts were still less than label recommendations for both chemicals (Table 2).

Dose-response trials with glufosinate-ammonium and glyphosate

Introduction

To minimise risks of herbicide resistance and of loss of approval by regulators, it is important that the system is not dependent on a single active ingredient. Technical prerequisites for leaf-specific weed control are that the active ingredient must be a non-selective (broad-spectrum) herbicide and it must be systemic. Dose-response relationships were reported in previous Annual Reports, but additional research was carried out to assess more precise dosing according to weed size. In July 2017, two new dose-response trials were established in glasshouse conditions, using mean weed ground cover to determine the recommended dose rate for both glufosinate-ammonium and glyphosate in Benton Kentucky (USA).

Seeds of *Amaranthus cruentus* L. (red amaranth) were obtained from the Two Willies Nursery (Lucedale, Mississippi, USA) and planted in Moisture Control Potting Mix from Miracle-Gro (The Scotts Company llc, Marysville, Ohio, USA). The trays used in this study 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 emergence, they were thinned to one seeding per cell. Seedlings were transplanted to 9 cm diameter pots at the 2-leaf stage. Trials comprised randomised complete blocks with seven replicates of 13 treatments for glyphosate and six replicates of 14 treatments for glufosinate-ammonium. The herbicides used were USA formulations of glyphosate (Envy™ Six Max, 540 g L^{-1}, Innvictis Crop Care, LLC™) and glufosinate-ammonium (Liberty® 280 SL, 280 g L^{-1}, SL, Bayer CropScience LP). When glufosinate-ammonium was applied the adjuvant Verimax Ams Dry (Ammonium sulfate, polyacrylamide, dimethylpolysiloxane, 100%, Innvictis Crop Care, LLC™) was used at 1% concentration for every solution of the herbicide.

In order to estimate the volume (µl), amount (µg) and number of herbicide droplets needed to apply the recommended rate of the herbicides to seedlings, individual images of the seedlings were taken using a Nikon D90 Digital SLR Camera with an 18-105 mm VR Lens Kit, mounted on a tripod (ManFrotto Compact Action). 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. Distilled water was used to prepare all the solutions and in order to achieve the label recommendation for glyphosate (631.8 g ha^{-1}) and glufosinate-ammonium (630 g ha^{-1}), 20% concentrations were prepared for both herbicides. Details of the droplet application can be found in Table 3. Fresh and dry weights of the weed seedlings were estimated two weeks after droplet application.

Table 3 Details of droplet application for the dose-response trials with *Amaranthus cruentus* seedlings when glyphosate (Envy™ Six Max, 540 g L^{-1}) and glufosinate-ammonium (Liberty® 280 SL, 280 g L^{-1}) were used. Calculations were based on the mean ground cover of the seedlings which was estimated at 51.04 cm^2.

*the droplets were applied on the same leaf and on the same spot

Herbicides	Glyphosate	Glufosinate-ammonium
Amount of active ingredient for 1x application (µg)	322.5	321.6
Volume (µl) of droplets	2.98	2.87
Number of droplets	1	2*
Concentration of herbicide for 1x (%)	20	20

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:

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

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.

Results

Both herbicides controlled *A. cruentus* with droplets, but the glyphosate was much more effective than glufosinate ammonium (Figure 5, Figure 6). While the ED50 was well below the recommended application rates, the ED90 for the latter was over three times the recommended compared to about one tenth of the recommended for glyphosate (Table 4)

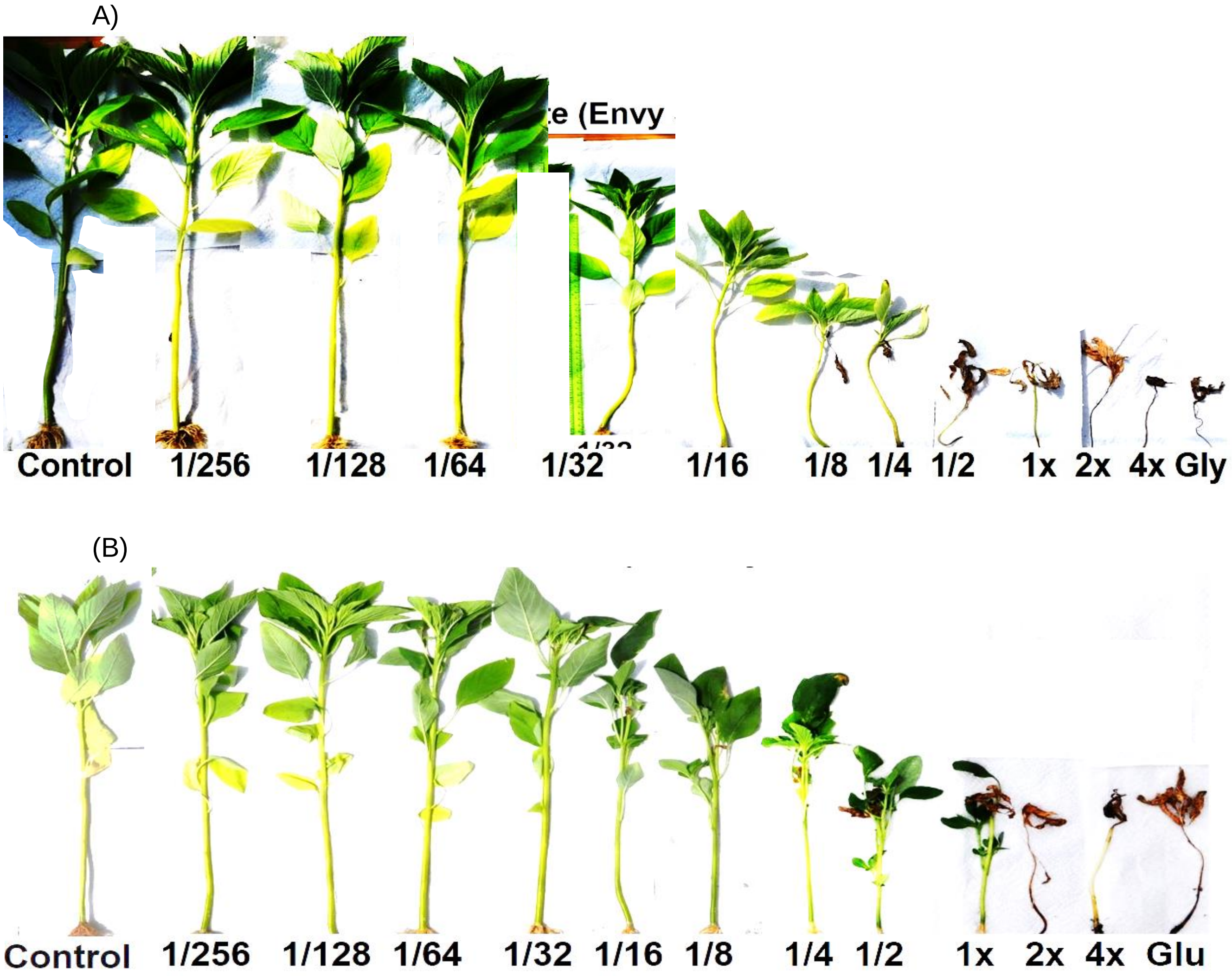


Figure 5 *Amaranthus cruentus* seedlings two weeks after application of droplets containing different concentrations of (A) glyphosate (Roundup Envy™ Six Max, 540 g L^{-1}) and (B) glufosinate-ammonium (Liberty®, 280 g L^{-1}). Droplet concentrations are expressed relative to the recommended rates per seedling (1x), which were (A) 322.5 µg and (B) 321.6 µg. Control treatments were treated with water, and undiluted product (Gly for glyphosate and Glu for glufosinate-ammonium). Seedlings were treated at the 6-leaf stage. The dose-response relationship for this experiment is in Figure 6.

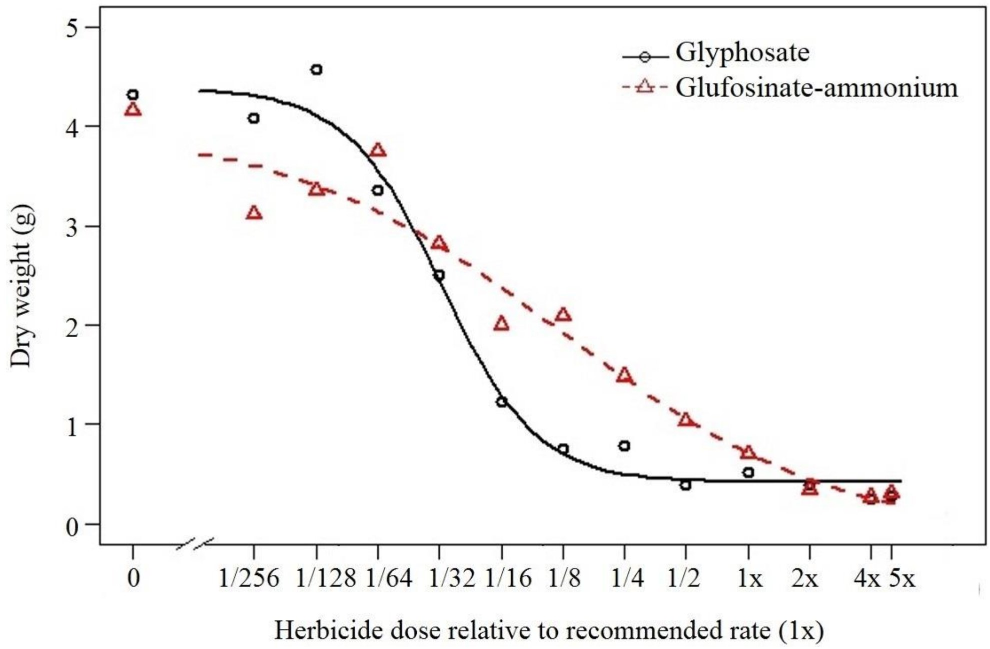


Figure 6 Dry weight of *Amaranthus cruentus* seedlings two weeks after herbicide droplet application, as a function of the dose expressed relative to the recommended rate per seedling (1x) of glyphosate and glufosinate-ammonium. Parameter estimates of fitted lines are in **Table 4**.

Table 4 Parameter estimates (±SE) of the *A. crunetus* dose-response regression curves (Eqn 1, Figure ***6***), 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 glyphosate and glufosinate ammonium. Dry weights were determined 2 weeks after applying the droplets.

Herbicides	**b**	**c (g)**	**d (g)**	**ED$_{50}$ (µg)**	**ED$_{90}$ (µg)**	**1x (µg)**
Glyphosate	1.87 (0.34) ***	0.44 (0.10) ***	4.38 (0.16) ***	10.3 (1.20) ***	33.5 (8)	322.5
Glufosinate-ammonium	0.64 (0.15) ***	-0.14 (0.39)	4.01 (0.24) ***	39.3 (14.8) **	1197 (1249)	321.6

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

Activities in the USA

Two experiments were carried out using the applicator which was mounted on a gantry system (Figure 7), in laboratory conditions. For both experiments distilled water was used which was coloured with blue food dye.

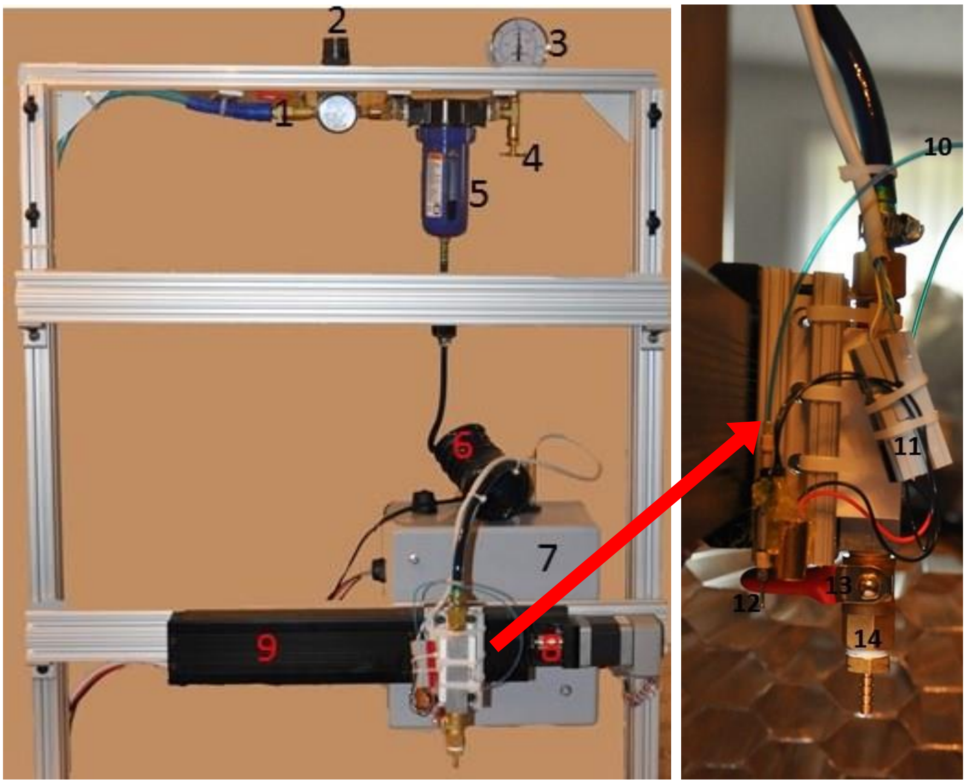


Figure 7 Applicator mounted on a gantry system comprised of the following parts: 1) air pressure shut off valve, 2) pressure regulator, 3) pressure gauge, 4) pressure bleed off valve, 5) liquid reservoir, 6) expandable tubing, 7) controller box, 8) motor, 9) linear actuator, 10) ejector tubing, 11) manifold, 12) **ejector nozzle/applicator**, 13) drain valve and 14) liquid drain.

Calibration test

In order to estimate the time needed for the applicator to dispense a single droplet of 1 μl under different pressures, a calibration test was carried out. The pressures tested were 10, 20, 30 and 40 psi and the times that the ejector nozzle/applicator would operate ranged from 1 to 10 ms. Treatments were replicated four times. A micro-tube was place underneath the ejector nozzle and one thousand droplets of distilled water were dispensed at different pressures for different periods (Figure 8). The weight of the micro-tube was recorded before and after droplets were applied.

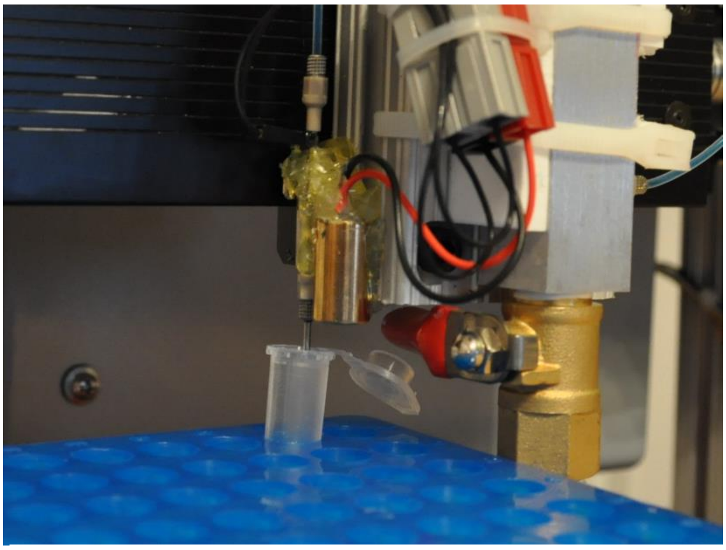


Figure 8 Calibration procedure in which a micro-tube was placed underneath the ejector.

Results from the calibration test indicated that when the applicator operated at 10 psi, it took 6 milliseconds to dispense one droplet of 1 µl, compared to 4, 3 and 2.5 ms at 20, 30 and 40 psi, respectively (Figure 9)

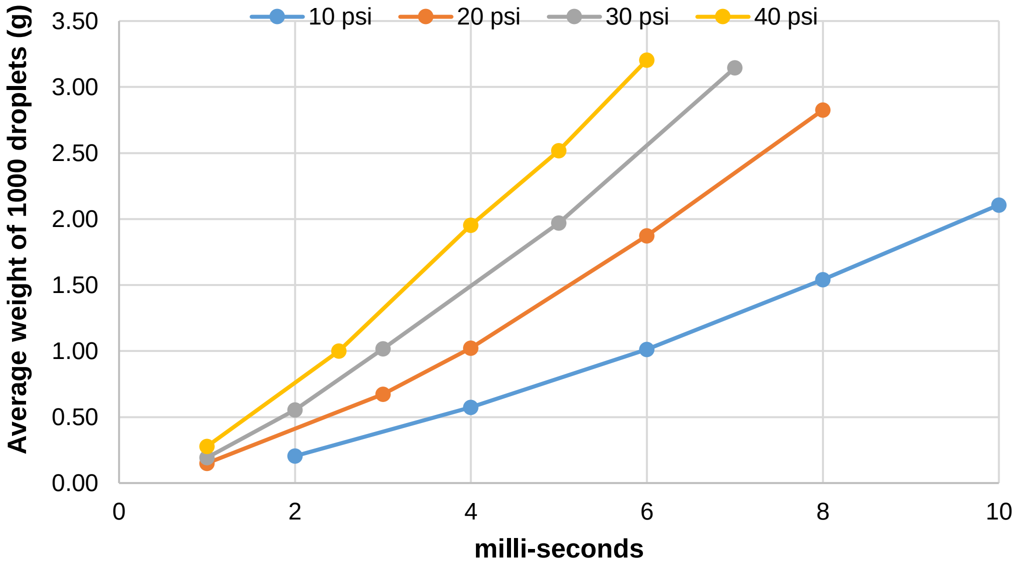


Figure 9 Weights of water dispensed by ejector operating at different pressures over different periods.

Targeting accuracy

The effects of ejector operating pressure, wind and distance of the ejector outlet from the target was assessed to quantify the targeting accuracy of the prototype applicator. Tests were performed both when the applicator was stationary and when it was moving. The target was a horizontally-displayed sheet of uncoated paper (A4 size), which was attached to a wooden board beneath the applicator gantry. A factorial trial comprised three distances from the target

(15, 30 and 50 cm), four application pressures (10, 20, 30 and 40 psi), a wind speed of 10 km/h blowing in three different directions when the applicator was moving (Figure 10) or four directions when it was stationary.

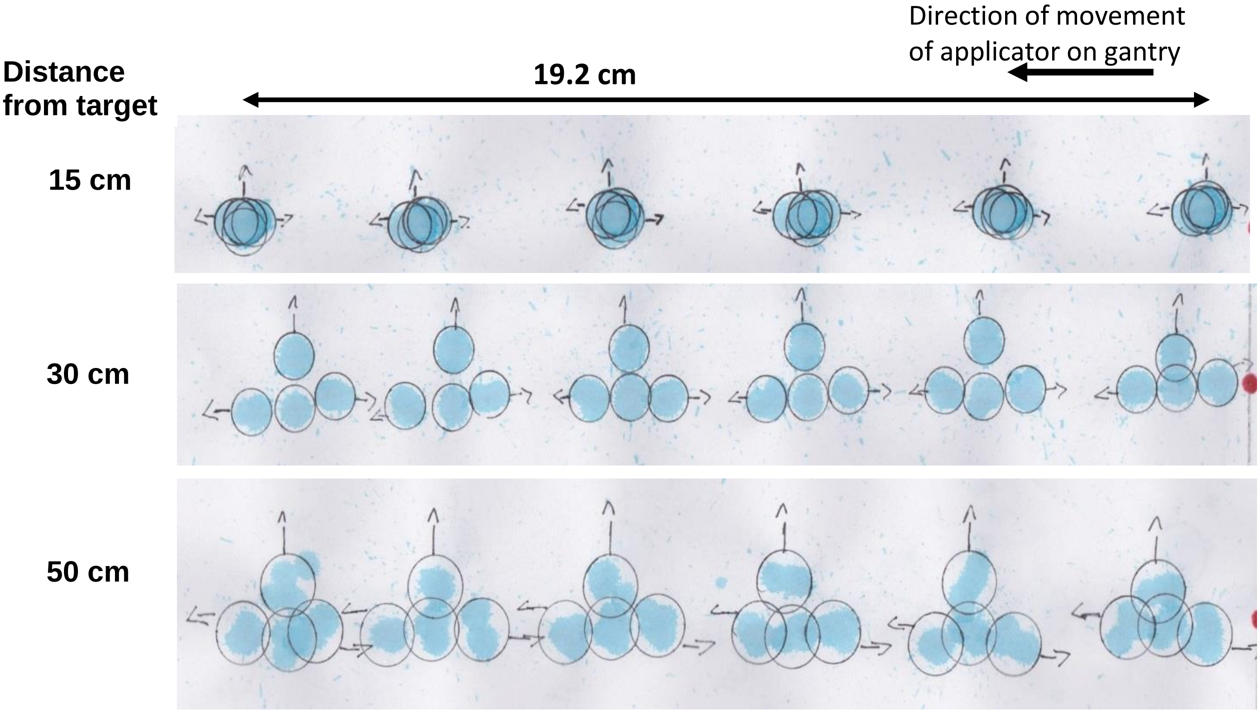


Figure 10 An example of droplet patterns from the moving applicator with the ejector operating at 20 psi and dispensing droplets from distances of 15, 30 and 50 cm above the target. The gantry traversed the area five times and circles were drawn by hand to enclose the positions where the five independently-applied droplets hit the paper target. Droplets marked with a circle in the middle indicate that they were applied with no wind. Circles with arrows mark an area where droplets were applied when wind was blowing at 10 km/h in the direction of the arrow. Apparent spattering on impact with the target is because the five droplets were applied over a short period of time and the surface was still wet at the time of application. No spattering occurred at 20 psi with the first droplet.

A digital caliper was used to measure the displacement of the droplets due to wind and results were expressed as displacement relative to that without wind. GenStat (16th Version) was used for data analysis and SigmaPlot (12th Version) was used to produce 3D graphs (*Figure 11*).

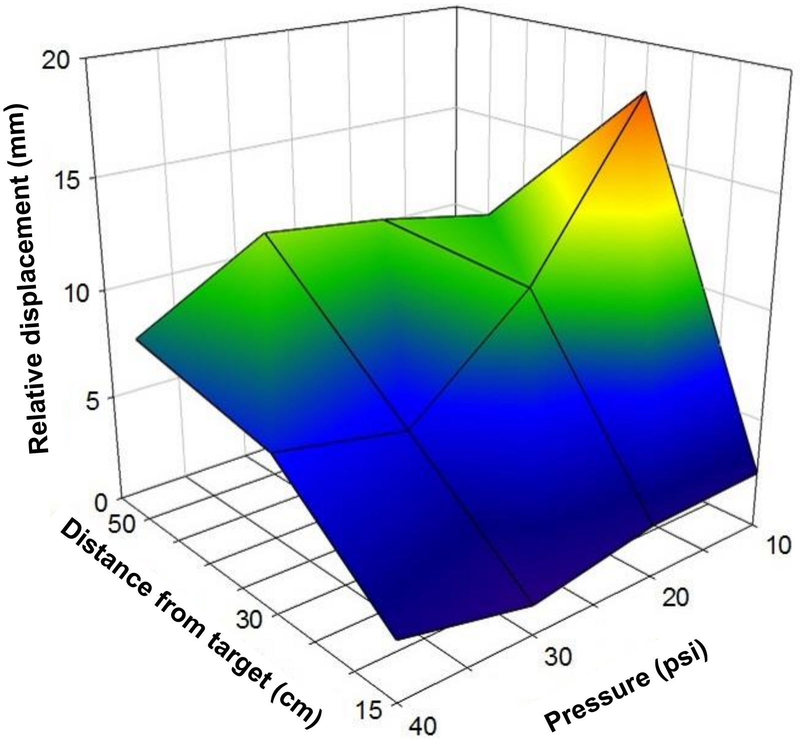


Figure 11 Relative displacement of droplets due to a cross-wind of 10 km/h during droplet application at different ejector pressures and for different distances of the ejector from the target.

- Targetting was slightly less precise for applications from 50 cm above the target – circles enclosing the droplets were slightly larger for this distance compared to those from 15 and 30 cm (Figure 10).
- Repeated applications consistently hit the same target area (Figure 10).
- Wind had little effect with a 15 cm separation (circles overlap, Figure 10), but the 10 km/h wind caused a 5-10 mm displacement for 30 and 50 cm distances (Figure 11).

Discussion

It is clear from the field trials, that the weed control efficacy of droplet applications shown in glasshouse studies were transferable to field trials and the results of 2016 field trials have been shown to be repeatable in 2017 and have been extended to include leeks.

We accepted our hypothesis that multiple treatments with a herbicide lacking residual activity like glyphosate would be necessary and multiple treatments are 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 more than one droplet treatment to ensure late emerging weeds are controlled. Although this approach is therefore valid for leaf-specific weed control, there is a strong case to start weed control as soon as seedlings are detectable and can be targeted with good accuracy. Small weeds are more susceptible to herbicides and will need less herbicide for effective control. Moreover, growers may prefer to avoid the risk of weeds becoming uncontrollable. The latter is much more likely if glufosinate ammonium becomes the herbicide of choice since this is not translocated easily and so the larger the weed, the lower its expected efficacy using droplets.

Glasshouse and field trials in 2017 confirmed that that droplet applications of glufosinate ammonium could be a valuable alternative to glyphosate for leaf-specific weed control. Both herbicides were applied without any evidence of crop damage, although this must clearly be retested with an autonomous system.

Precision targeting of glyphosate droplets to leaves of weeds is a leading edge procedure. The droplets are very small (1-2 microlitres) – so that one teaspoonful (5 ml) is enough to treat 2500-5000 individual weeds if one droplet is put on each weed. The use of large droplets avoids the risk of spray drift but there is still the potential for spattering on impact and some shattering of droplets on ejection from an applicator if applicator pressure is too high. The droplets are likely to be deflected by wind which must be accounted for in targeting. So in 2017/18, trials were carried out in the USA to assess the impact of applicator pressure and distance from target on spattering.

The effect of wind on deflection of droplets was also investigated in a multifactorial experiment comprising windspeed and direction, applicator pressure and distance from target as factors. Provided windspeed and direction are known, deflection could be modelled and compensated for.

To avoid risks of resistance and to provide an alternative, we have also tested glufosinate ammonium in the field. This active ingredient has limited systemic action and so is less suitable than glyphosate for droplet application, but it appeared to achieve reasonable efficacy. Trials in 2018 will explore combining both actives with 2,4-D.

Doses applied in every case are linked approximately to the ground cover of the weeds. There is a potential issue as regards approval, for although the amount of product applied to each square metre of field will always be less than the permitted dose, the same would not be true for every square millimetre. There are of course one million mm^2 in each square metre and the current approvals rules do not take account of more focussed targeting.

In this year's field trials with cabbages and leeks, respectively, Efficacy of weed control and crop yields were not significantly lower than the hand-weeded ("weed-free") control.

Conclusions

1. Leaf-specific droplet applications of herbicides provided very effective weed control in field-grown cabbages and leeks.
2. Glufosinate ammonium was effective for weed control in cabbages, but some yield loss occurred in leeks.
3. Droplet applications reduced amounts of herbicide applied to field grown cabbages by 97-98% compared to a pendimethalin pre-emergence spray for both herbicides. For leeks, reductions were 74% for glyphosate and 51% for glufosinate-ammonium.
4. Tests with a prototype applicator demonstrated consistent targeting without droplet shattering on ejection and without spattering on impact with an ejector pressure of 20 psi.

Knowledge and Technology Transfer

Students at Reading are exposed to leaf-specific weed control during lectures on weed technology and carry out laboratory practicals similar to the experiments carried out in the project, in which they determine dose-response relationships using droplet applications to individual weeds.

Publication

Murdoch, A., Koukiasas, N., Pilgrim, R., Sanford, S., De La Warr, P., Price-Jones, F. (2017). Precision targeting of herbicide droplets potentially reduces herbicide inputs by at least 90%. Aspects of Applied Biology 135, Precision Systems in Agricultural and Horticultural Production, pp. 39-44. Wellesbourne, U.K.: Association of Applied Biologists

[Abstract in Appendix 2.]

Tables of presentations and a list of media reports follows:

Presentations

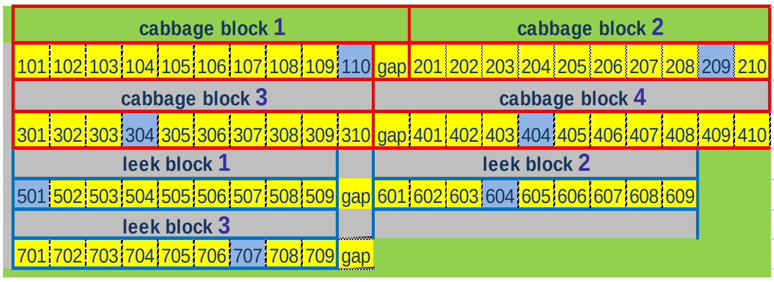
Event	**Date**	**Place**	**Topic**	**Type**	**Presenter**
AgriFood Charities Partnership Student Forum	6 April 2017	University of Hertfordshire	Targeted droplets reduced herbicide inputs in cabbages by at least 85%	Oral	Nikolaos Koukiasas
Automotive & Robotics in Agri food event. Innovate UK, Knowledge Transfer Network	4 July 2017	Kingsgate, Peterborough	Robotic weeding of cabbages offers potential reduction in herbicide inputs of at least 90%	Oral 'pitch'	Alistair Murdoch
Precision Systems in Agricultural and Horticultural Production. (AAB Conference)	27 October 2017	Pershore College, Worcestershire	Precision targeting of herbicide droplets potentially reduces herbicide inputs by at least 90%.	Oral + paper	Alistair Murdoch
British Onion Growers and British Leek Growers R&D Committee Meeting lunchtime seminar	31 October 2017	Cranfield University	CP134 EyeSpot – leaf specific herbicide applicator for weed control in field vegetables	Oral	Alistair Murdoch
Crop Production Group Student Symposium	2 Nov. 2017	University of Reading	Weed control in cabbages using droplets of glyphosate	Oral + Poster	Nikolaos Koukiasas
BCPC Weeds Review 2017	9 Nov. 2017	Rothamsted Research	Targeted droplets reduced herbicide inputs in cabbages by at least 85%	Oral + question panel	Alistair Murdoch
2017 AHDB Crops PhD Studentship Conference	6-7 Nov. 2017	Stratford Manor Hotel, Warwick	Targeted droplets reduced herbicide inputs in cabbages by at least 85%	Poster	Nikolaos Koukiasas
Presentation to AgriFood Charities Partnership	6 March. 2018	University of Reading	Precision agriculture research at Reading including eyeSpot	Oral	Alistair Murdoch

References

Bond, W. and Grundy, A.C. (2001). Non-chemical weed management in organic farming systems. Weed Research, 41:383-405.
Nieto H J, Brondo M A, Gonzalez J T. 1968. Critical periods of the crop growth cycle for competition from weeds. Pest Articles and News Summaries (C) 14:159–166.
Weaver, S.E., 1984. Critical period of weed competition in three vegetable crops in relation to management practices. Weed Research 24:317-325.

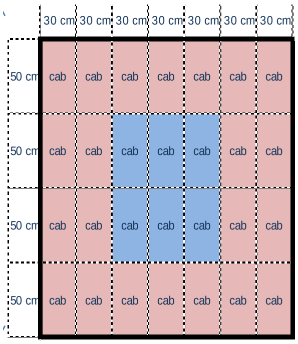
Appendix 1 Experimental design for field trials, summer 2017.

Two field experiments were planted in 2017 at Sonning Farm, one with cabbages and one with leeks. Both experiments comprised randomised complete blocks with ten treatments per block for the cabbages and nine for leeks. 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. Note that layouts are not drawn to scale. Pre-emergence plots (numbers 110, 209, 304, 404, 501, 604 and 707) are highlighted. Gaps between blocks were 60 cm (cabbages) and 100 cm (leeks)

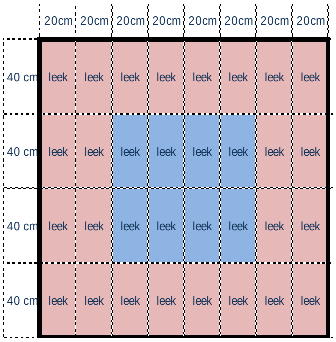


cabbage block 1											cabbage block 2									
101	102	103	104	105	106	107	108	109	110	gap	201	202	203	204	205	206	207	208	209	210
cabbage block 3										cabbage block 4										
301	302	303	304	305	306	307	308	309	310	gap	401	402	403	404	405	406	407	408	409	410
leek block 1										leek block 2										
501	502	503	504	505	506	507	508	509	gap	601	602	603	604	605	606	607	608	609		
leek block 3																				
701	702	703	704	705	706	707	708	709	gap											

Individual plot layouts are shown below and contained 28 cabbages (2.1 x 2.0 m) or 32 leeks (1.6 x 2.0 m). Treated areas for plots receiving droplet applications and final harvest areas in all plots were 1.0 x 0.9 m (six cabbages) or 0.8 x 0.8 m (eight leeks) and are shaded blue, other plants being guard rows. Individual plants are indicated: cab: cabbage)



	30 cm	30 cm	30 cm	30 cm	30 cm	30 cm	30 cm
50 cm	cab	cab	cab	cab	cab	cab	cab
50 cm	cab	cab	cab	cab	cab	cab	cab
50 cm	cab	cab	cab	cab	cab	cab	cab
50 cm	cab	cab	cab	cab	cab	cab	cab



	20cm	20cm	20cm	20cm	20cm	20cm	20cm	20cm
40 cm	leek	leek	leek	leek	leek	leek	leek	leek
40 cm	leek	leek	leek	leek	leek	leek	leek	leek
40 cm	leek	leek	leek	leek	leek	leek	leek	leek
40 cm	leek	leek	leek	leek	leek	leek	leek	leek

Appendix 2 Abstract of paper presented AAB conference, 27/10/2018

Murdoch, A., Koukiasas, N., Pilgrim, R., Sanford, S., De La Warr, P., Price-Jones, F. (2017). Precision targeting of herbicide droplets potentially reduces herbicide inputs by at least 90%. Aspects of Applied Biology 135, Precision Systems in Agricultural and Horticultural Production, pp. 39-44. Wellesbourne, U.K.: Association of Applied Biologists

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

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Summary

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

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

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

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

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