

Grower Summary

CP 134

"eyeSpot" – leaf specific herbicide applicator for weed control in field vegetables

Annual Report for 2015/6

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AHDB Horticulture, AHDB Stoneleigh Park Kenilworth Warwickshire CV8 2TL

Project title:	"eyeSpot" – leaf specific herbicide applicator for weed control in field vegetables			
Project number: CP 134	CP 134			
Project leader: Dr Alistair Murdoch	Dr Alistair Murdoch			
Report: Grower Summary for 2015/16	Grower Summary for 2015/16			
Key staff:	Dr Alistair Murdoch (PI), Mr Nikolaus Koukiasis (PhD student), Mr Paul de la Warr (software engineer), Mr T Yu (MSc student), Mr Liam Doherty, Mrs Caroline Hadley and Mr Richard Casebow (technical staff). External advisors: Dr Robert Pilgrim and Mr Shane Sanford (Concurrent Solutions IIc)			
Location of project:	University of Reading, UK			
Industry Representative:	Mr Phil Lilley, Hammond Produce Ltd			
Date project commenced:	1 April 2015			
Expected completion date:	30 September 2018			
Co-funders:	Douglas Bomford Trust, Edith Mary Gayton Trust Fund and the University of Reading			

# 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 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.

This project proposes 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 (i.e. GM). Moreover, the concept is no chemical to the soil, none on the crop, simply leaf-specific droplet 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 reduce run-off to the soil, crop and non-target organisms; and
- provide an engineering alternative to the biotechnological option of geneticallymodified herbicide tolerant crops.

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, the project will demonstrate a pre-commercial system as an alternative to other systems which approximate to plant specific weed control using directed sprays. Such systems are currently available but still cause some loss of crop plants and are most suited for controlling large weeds such as volunteer potatoes. The system here is focussed on all weeds in the field including young seedlings before they have had any yield or quality impact on the crop.

### Research at Reading in 2015/16

Dose-response relationships of glyphosate droplet applications have been studied and modelled for five weed species. Also herbicide droplets have also been applied to crop seedlings so as to record susceptibility of the vegetable to accidental droplet application. 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). Phytotoxicity symptoms (yellowing and stunting) were also recorded.

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. The cabbage seedlings, savoy variety, were supplied by Hammond Produce.

Roundup® Biactive® (Monsanto®, 360 g/l glyphosate) was used for droplet applications. To calculate the recommended rate of Roundup (1.5 l/ha) as µg of glyphosate per seedling, the ground cover of individual weed plants was assessed by image analysis of overhead photographs (Figure. 1).



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

The recommended application rate of Roundup Biactive was 1.5 l/ha for annual weeds, i.e. 540 g glyphosate /ha or 5.4  $\mu$ g glyphosate /cm<sup>2</sup>. According to the ground covered by the seedling, the recommended dose for an individual seedling was calculated as follows:

Glyphosate ( $\mu$ g/seedling) = ground cover of seedling (cm<sup>2</sup>) × 5.4

In order to improve the performance characteristics of glyphosate, the adjuvant AS 500 SL, Agromix was needed to achieve adequate wetting of waxy leaf surfaces using droplet application (Figure 2). The recommended concentration of the adjuvant for conventional spray applications is 1% v/v (Woznica Z., personal communication, July, 2015) and this was confirmed as appropriate for droplet applications also.



**Figure 2.** 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).

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. Dose response curves the biomass and leaf area data were fitted and ED50 and ED90 values were estimated from the fitted curves.

### **Results: Dose responses**

Examples of some dose response curves for three species are shown and others are summarised in Table 1.

## Chenopodium album

*C. album* seedlings were controlled leaf-specifically after applying droplets containing a series of glyphosate solutions (Figure 3). Although the recommended dose (based on initial seedling size) was estimated at 5.83  $\mu$ g per seedling, a 90% reduction in the fresh biomass was only achieved at 32  $\mu$ g of glyphosate per seedling (Figure 4).

### 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 5). The recommended dose was estimated at 8.44  $\mu$ g per plant and was able to reduce biomass of the seedlings by 43% (Figure 6).

#### Urtica Urens

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 8). Dry weight data were fitted to the dose-response curve with all of the parameters being statistically significant (Figure 8). A 50% reduction in the biomass was achieved with concentrations of glyphosate containing 3/8 of the recommended dose.

#### Matricaria recutita

The biomass of *M. recutita* seedlings was reduced after droplet application with different concentrations of glyphosate (Figure 9). Dry weight data showed a typical dose-response relationship as a function of the  $\mu$ g of glyphosate (Figure 10). The recommended dose of 16.7  $\mu$ g achieved almost 90% reduction of the dry weight of the seedlings.

### Summarizing 1<sup>st</sup> 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 droplets of a non-selective, translocatable herbicide. Although glyphosate is an ideal active ingredient, it is not the only option and indeed, alternatives must be used to avoid risk of herbicide resistance. The research quantified the volume and number of herbicide droplets needed to control some common weeds. Furthermore, treatments of glyphosate were applied to cabbage seedlings (detailed results are not shown here) 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 (Table 1). However, from a farmer's point of view a 90% weed control is considered a reasonable level which, for the annual weeds studied, was 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 1). These results suggest that sequential application of herbicide is required or applications with higher doses in order to achieve a 90% weed control.

**Table 1.** Recommended dose rates and estimated effective doses to reduce biomass of the weed and crop species tested by 50% (ED50) and 90% (ED90) in  $\mu$ g of glyphosate per seedling (± se).

Species		Recommended dose*	ED90 (µg)	ED50 (µg)
		(µg)		
Brassica olera sabauda	cea var.	112	346 ± 171	35.9 ± 8.0
Galium aparine		8.44	19.3 ± 11.8	5.95 ± 1.5
Matricaria recutita	1	16.9	10.2 ± 6.5	2.22 ± 0.7
Chenopodium alb	um	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

In other results not reported here, it was evident that when the recommended dose rate was 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 individual leaves clearly needs to be taken into account.

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.

Research in 2016/17 will continue dose response studies on other species and also using alternative chemicals. In addition, a pilot field trial will be carried out with cabbage and leeks. Efficacy of droplet applications in maintaining crop yield and quality will be assessed in comparison with both pre-emergence treatment, inter-row band-spraying and hand-weeded plots.

The field trials will also utilise the eyeWeed image capture system so that algorithm development can take place.

Clearly at this stage in the project, results are not directly applicable to growers' fields, however, the results obtained to date prove the concept that droplet applications can achieve satisfactory reductions in weed biomass in the absence of any spray drift or any application to soil or crop plants. The importance of this finding cannot be over-stressed and it is therefore particularly important that systemic, broad-spectrum active ingredients such as glyphosate remain available to farmers and growers.

#### **Publications in Conferences**

Some results obtained in 2015/16 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, a similar poster together with a 5-minute oral presentation was given at the International Advances in Pesticide Application conference (13 - 15 January 2016, Barcelona, Spain). A written paper under the title "Dose-response relationship of droplet applications of the leaf-specific weed control in vegetable crops" was published at the latter conference's proceedings (Aspects of Applied Biology, 132, pp. 343-348).



**Figure 3.** 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.



Dose (µg of glyphosate)

Figure 4. Fresh weight of Chenopodium album as a function of  $\mu g$  of glyphosate applied per seedling.



**Figure 5.** 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).



Figure 6. Dry weight of Galium aparine as a function of the dose of glyphosate (µg) applied per



**Figure 7.** 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).



Figure 8. Urtica urens dry weight as a function of the dose of glyphosate ( $\mu g)$  applied per seedling



**Figure 9.** 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).



Dose (µg of glyphosate)

Figure 10. Dry weight of M. recutita as a function of the dose of glyphosate ( $\mu$ g) applied per seedling.