A new method for assessing herbicide damage in weeds

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Summary Herbicide application is one of the most widely used weed management tools. Whilst herbicides are effective at killing plants [weeds], their application can lead to variable levels of control and uptake rates, as well as off-target damage, and as such assessments of their effectiveness are needed. The effectiveness of herbicide application is typically assessed using visual estimations of the level of damage present, either to individual leaves or whole plants, using a series of pre-determined categories (e.g. <25% leaf burn) over time. Such visual assessments lack accuracy and only provide information on the effectiveness of the herbicide applied. In many instances the weed is not killed by the herbicide application and thus information is also needed on how it responds to being damaged, but not killed. Here, we outline the use of several tools to increase rigor into assessments of herbicide effectiveness. First, the leaf area is determined using leaf area software to differentiate between healthy (green) and damaged (brown) areas of the leaf. Then each leaf is assessed to determine the changes occurring within the leaf to support the visual changes observed (i.e. damage). These tools assess the chlorophyll content, photochemical index, and stomatal conductance of the leaf. Results from a trial on four weed species showed that these tools provide greater accuracy for assessing herbicide damage, especially when combined, as well as how leaf function changes in relation to herbicide damage. Whilst the use of these tools may not be suitable for many land managers, the use of digital photos and leaf area software is very effective and should be considered as an alternative to visual assessments.

Keywords Herbicide effectiveness, leaf damage, new tools, leaf area software, leaf condition, leaf health.

INTRODUCTION

Previous attempts to examine the effects of herbicide spraying on weeds have generally relied on visual assessments made in the field by eye. As such, different observers may judge leaf condition/damage very differently, creating observer bias. To resolve this situation instrumentation can be used with a higher level of precision and thus provide a more objective assessment. For example, Dayan and Zaccaro (2012) recently suggested that chlorophyll content provides a good marker for examining herbicide mode of action. Other attributes that may influence herbicide effectiveness include the physiology of weeds (especially leaves), and as such physiological assessments of weeds have been increasingly undertaken (e.g. Pandey *et al*. 2003, Wooley *et al*. 2011).

An opportunity now exists to study herbicide effectiveness using methods from remote sensing to investigate the physiology of weeds. There is an extensive literature discussing how glyphosphate blocks the production of aromatic amino acids and thus affecting protein synthesis (Baylis 2000). This suggests that although a leaf may appear healthy, the herbicide may be affecting leaf function, as measured by stomatal conductance. Thus, a single measurement of leaf condition may not appropriately characterise leaf function, and several different measurements may be needed to properly assess the effectiveness of herbicide.

The assessment of plant condition and function of crops using remote sensing is well-established as a field of investigation. These studies have incorporated sensors mounted on satellites, airplanes, and digital cameras (Dash and Curran 2004, Gitelson *et al*. 2005, Hunt *et al*. 2011). Recent studies suggest that photos taken with digital cameras are able to capture basic aspects of leaf condition, including chlorophyll content (Hunt *et al*. 2011).

Here we applied a range of these methods to assess the effectiveness of herbicide on four different weed species to determine the application of such technologies to the field of weed management.

MATERIALS AND METHODS

Here we undertook a pilot study to assess leaf condition (and function) using four different tools and approaches, being: Leaf Chlorophyll (LC), Photochemical Reactive Index (PRI), Stomatal Conductance (SC), and the analysis of Digital Photos (DP) using leaf area software. LC and PRI are static measures of leaf condition related to the photosynthetic capacity of leaf tissue. SC is a dynamic measure of the transpiration flux of water vapour that occurs during photosynthesis. The use of DP represents an upgrade of previous visual approaches at examining leaf condition based on eyesight, with the further advantage that it can be analysed using a suite of available software products and the photos can be archived for future use or reference.

The four weed species examined were *Geranium molle* L. var *molle*, *Plantago lanceolata,* L., *Solanum nigrum* L., and *Taraxacum* sp. Juvenile plants of each species were collected in the field and transplanted into cylindrical (18 cm diameter, 1 L volume) pots and grown in the glasshouse for approximately 1 month before herbicide application. Fifteen plants of each species were transplanted into pots; for a total of 60 plants. Plants were watered every 2–3 days during the study.

Three herbicide treatments were applied to five plants of each species, being: unsprayed, sprayed (one application of Roundup® (glyphosphate) at 10 mL L^{-1}), and half-sprayed (i.e. the sprayed treatment applied to only one-half of the plant) to assess how plants respond to varying levels of damage.

Immediately prior to spraying, each plant was photographed using a Lumix FZ100 digital camera, with 10.5 megapixel resolution and square photo setting. The camera was positioned at a height of 1.5 m within a frame, ensuring that each photo was taken from the same perspective. All plants were also photographed under diffuse sunlight to minimise the effects of shading of leaves. The images were analysed using Windias 3.2 software (Dynamax®). First, we selected a subset of photos for each species to classify healthy and unhealthy leaves. Next, through a batch process, all photos for a species were analysed separately for each date (time since herbicide). Classifications were separated by species and date because each species responded differently to the herbicide and different observation dates had different sky [light] conditions.

Measurements of leaf condition were also taken at the same time from three representative, healthy (or most healthy) leaves on each of the 60 plants; taken from the upper leaf lamina, near the centre of the leaf. Leaf condition was assessed for (i) relative LC (using an Atleaf® Chlorophyll meter), with a scale of 0 to 100); (ii) PRI (using a PlantPen PRI 200® meter) which measures the difference in leaf reflectance between two narrow bands of light centred on 531 and 570 nm; and (iii) SC (using a Decagon SC-1 Leaf Porometer®), which measures transpirational flux (loss) of water vapour during photosynthesis. These measurements were repeated weekly for 14 days post-herbicide application.

Since a number of studies have shown significant effect of temperature and light levels on PRI (Gamon *et al*. 1997) and SC (Farquhar and Sharkey 1982), we minimised these effects by taking all measurements for each individual species within one hour, during the middle of the day. In addition, as the rate of water vapour loss during photosynthesis is also affected by light, temperature and humidity (vapour pressure deficit), we took all measurements within the same hour as PRI for each species.

RESULTS

Due to space limitations here, only the results for *Taraxacum* sp. are illustrated graphically (Figure 1). The mean relative LC content values remained unchanged during the study for unsprayed plants, however, there were significant differences in relative LC content values between the sprayed and unsprayed treatments from 1 week post-herbicide application onwards (Figure 1A). There were no significant differences between the sprayed and half-sprayed treatments until the last sampling date (i.e. 2 weeks post-herbicide application); when the fully sprayed plants had completely died off. Similar results were observed for the other three weed species. The PRI values show a similar trend to that observed for LC (Figure 1B). Similar results were observed for the other three weed species.

Both sprayed treatments had significantly lower SC values that unsprayed plants with the exception of an inconclusive result 2 weeks after herbicide application (Figure 1C). This result was similar for the other three species, where SC values were significantly lower for sprayed plants and that reductions in SC values occurred before changes were observed in LC and PRI.

Declines in leaf health, as determined using photographs, lagged behind the other three measures (Figure 1), with significant decreases observed 3 weeks after herbicide application. Similar results were observed for the other three weed species.

Due to space limitations here, only the results for *G. molle* var *molle* are illustrated graphically (Figure 2). Comparison between the results observed for each measure for each species showed that some values were heavily correlated. Leaf health was positively correlated with LC ($r^2 = 0.59$) and strongly correlated with PRI ($r^2 = 0.90$: Figure 2). These results were similar for the other species.

The results from comparing the half-sprayed with sprayed plants were largely inconclusive. There appeared to be no asymmetrical effect of herbicide damage based on visual inspection.

DISCUSSION

Results from this trial of assessing herbicide effectiveness for four weed species using a range of different tools has shown that greater accuracy can be obtained from using digital photos and leaf area analysis.

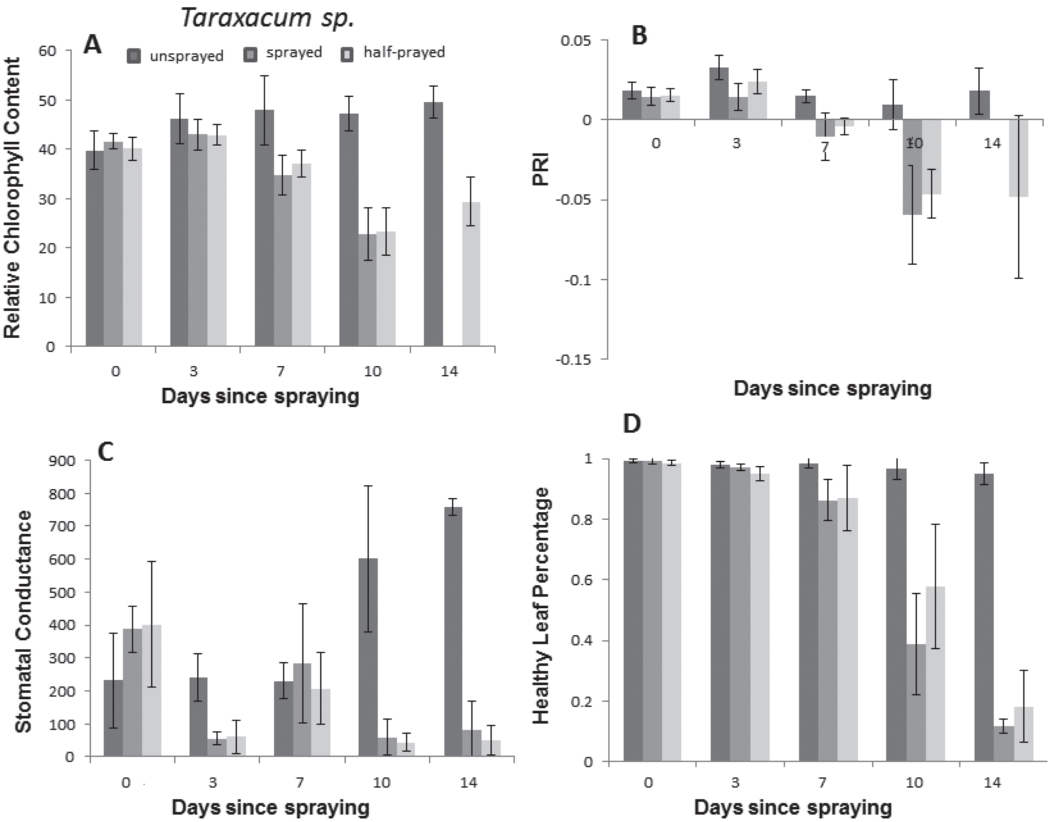

Figure 1. Changes in leaf condition following herbicide application, as measured through, (A) LC - relative chlorophyll content, (B) PRI - photochemical reactive index, (C) SC - stomatal conductance, and (D) leaf health obtained through digital photo analysis for *Taraxacum* sp. Error bars indicate ±2 standard errors.

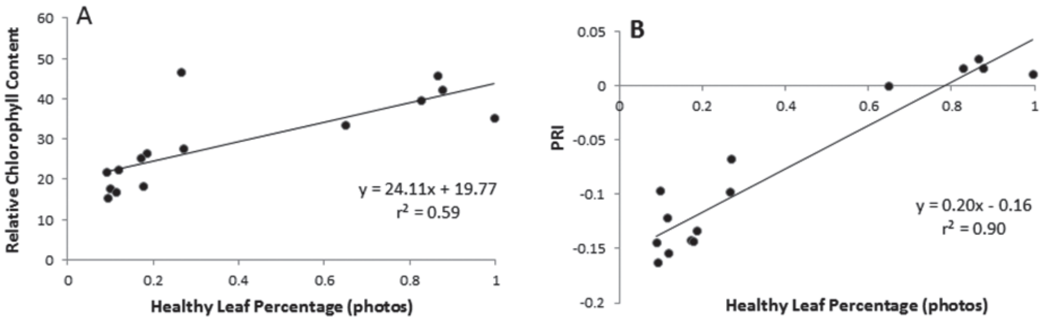

Figure 2. Relationship between percentage of healthy leaves obtained through digital photo analysis and (A) LC and (B) PRI measurements for *Geranium molle* var *molle*.

In addition, changes in the leaves, whilst correlated with the leaf damaged observed through photo analysis, occurred differently for each attribute measured, suggesting that some leaf functions were still occurring and that plant death occurs over a prolonged period of weeks.

Whilst the equipment required is expensive and the measurements are time consuming, the data collected provides clear insights into how leaves are affected by herbicide function as they die. Given that some herbicides (i.e. other than glyphosate) take significantly longer to result in weed mortality, data presented here illustrates that the plants may be functioning relatively well over much of this time period. How this affects later stages of flowering and seed production is unknown, but based on our results, it suggests that using herbicides with prolonged uptake rates during or even just prior to seeding, may not prevent seed production.

The results from comparing the half-sprayed with sprayed plants were largely inconclusive. The net effect of half-spraying appears to have been to dilute the treatment as observed in the later observation periods. The results from the half-spraying treatment also suggest that the four species readily translocated the herbicide throughout the entire plant, possibly through root uptake, which resulted in plant death.

For this study, similar results may be achieved using a different camera and freely available software, and thus such analysis should be used over visual assessments wherever possible.

Based on the recent findings of Dayan and Zaccaro (2012) and Hunt *et al*. (2011) and our finding in which information from multiple sets of analyses can provide a much greater understanding of leaf function, adoption of such techniques for assessing herbicide effectiveness in weeds is warranted.

REFERENCES

Baylis, A.D. (2000). Why glyphosphate is a global herbicide: strengths, weaknesses and prospects, *Pest Management Science* 56, 299-308.

Dash, J. and Curran, P.J. (2004). The MERIS terrestrial chlorophyll index, *International Journal of Remote Sensing* 25, 5403-13.

Dayan, F.E. and Zaccaro, M.L.D. (2012). Chlorophyll fluorescence as a marker for herbicide mechanisms of action, *Pesticide Biochemistry and Physiology* 102, 189-97.

Farquhar, G.D. and Sharkey, T.D. (1982). Stomatal conductance and photosynthesis. *Annual Reviews in Plant Physiology and Plant Molecular Biology* 22, 317-45.

Gamon, J.A., Serrano, L. and Surfus, J.S. (1997). The photochemical reflectance index: an optical indicator of photosynthetic radiation use efficiency across species, functional types, and nutrient levels. *Oecologia* 112, 492-501.

Gitelson, A.A., Vina, A., Ciganda, V., Rundquist, D.C. and Arkebauer, T.J. (2005). Remote estimation of canopy chlorophyll content in crops. *Geophysical Research Letters* 32, L08403.

Hunt, E.R., Daughtry, C.S., Long, D.S. and Eitel, J.U. (2011). Remote sensing of chlorophyll content at leaf and canopy scales using a visible band index. *Agronomy Journal* 103, 1090-9.

Pandey, D.K., Palni, L.M.S. and Joshi, S.C. (2003). Growth, reproduction, and photosynthesis of ragweed parthenium (*Parthenium hysterophorus*), *Weed Science* 51, 191-201.

Wooley, S.C., Smith, B., King, C., Seastedt, T.R. and Knochel, D.G. (2011). The lesser of two weevils: physiological responses of spotted knapweed (*Centaurea stoebe*) to above- and belowground herbivory by *Larinus minutus* and *Cyphocleonus achates*. *Biocontrol Science and Technology* 21, 153-70.