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Research Article

Chlorophyll fluorescence technique to determine the effects of herbicides on *Arundo donax* L.

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Abstract

This paper reports the use of chlorophyll fluorescence analysis (CFA) to determine the effects of herbicides on photosynthetic activity in *Arundo donax* L. (giant reed). A field experiment was conducted with five systemic herbicides. Glyphosate 36% was applied in each of the following ways; 1) on adult mass of giant reed plants (3–4 metres in height), 2) on sprouts after initial cutting (80 cm in height) and glyphosate 54% was applied by injection into the stem with full-strength product. The other herbicides were applied on giant reed after the initial sprouting following cutting, on-label application rate. The CFA showed that glyphosate 36% applied on adult mass of plants at 10 L a.i. ha^{-1} had a significant effect (70% reduction) on photosynthetic activity 60 days after treatment (DAT). Glyphosate 36% at 4 L a.i ha^{-1} applied on sprouts resulted in 50% reduction and glyphosate 54% at 4 L a.i ha^{-1} applied by injection into the giant reed stems reduced their photosynthetic activity by 60%. Profoxydim 20% at 0.75 L ha^{-1} caused a 50% reduction at 60 DAT, cyhalofop-butyl 20% and penoxsulam 2.04% reduced 12 and 15% respectively, and azimsulfuron had no significant effect on photosynthetic activity of giant reed. Visual evaluations of giant reed presence showed similar results at 168 DAT. These results show that CFA could be used to measure the response of invasive plants to herbicides, and that glyphosate 36%, and possibly profoxydim 20%, might be used as an integrated control of giant reed.

Key words: giant reed; glyphosate; invasive weed; riparian environment; integrated control

Introduction

Arundo donax L. (giant reed) is a highly invasive, perennial weed of riparian zones in Spain. It is native to eastern and southern Asia, but is now widely distributed in the Mediterranean region where it has altered wildlife habitats and displaced native species (Sanz Elorza et al. 2004). Giant reed propagates vegetatively through rhizome elongation and stem pieces (Decruyenaere and Holt 2001). It transpires more water per unit area than native vegetation and increases the risk of fire in invaded areas. Giant reed alters the physical characteristics of ecosystems by providing a habitat unsuitable for native wildlife (Iverson 1994; Bell 1997).

Nowadays, giant reed is often controlled mechanically by stem cutting or through excavation of the entire plant (Bell 1997). However, these measures may be ineffective and insufficient, due to its high rate of reproduction. Presently, there are limited chemical control options available for giant reed and the information on proper timing of herbicide application to maximize control of giant reed is scarce. In this sense, Bell (2011) has observed that glyphosate and imazapyr at low rates are more effective when applied in fall. Giant reed has also shown tolerance to several herbicides (Odero and Gilbert 2012). In addition, control with herbicides may be restricted because giant reed invades very herbicide-sensitive habitats such as riparian areas. Glyphosate has been the most widely used nonselective systemic herbicide for control of invasive nonnative plants in Mediterranean rivers over the last four decades. It is also an integral component of programs which aim to restore native vegetation (Finn and Minnesang

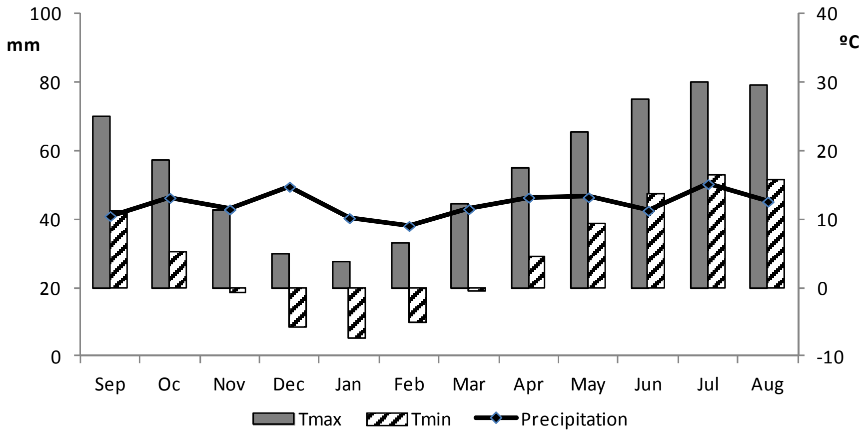


Figure 1. The climate conditions recorded in experimental site (left axis - precipitation, mm, right axis - temperature average monthly).

1990; Bell 1997; Jackson 1994). Although data on rate and efficacy of glyphosate on giant reed is limited, some authors have carried out the evaluation of glyphosate on giant reed management (Lowrey and Watson 2004). Bell (1997) reported that 2 to 5 % glyphosate solution was effective in controlling giant reed when applied to cut stems, and Jackson (1994) recommended a glyphosate solution of 1.5% for the control of the giant reed. More recently, a number of authors have suggested different formulations of glyphosate and other active ingredients for giant reed management (Odero and Gilbert 2012; Spencer et al. 2008) although the literature contains little more information in this respect.

Field and greenhouse herbicide efficacy evaluations can take several weeks and require suitable infrastructure, meaning that simpler methods or techniques that are faster than conventional methods are needed for herbicide efficacy evaluation. Chlorophyll fluorescence analysis (CFA) offers a fast, noninvasive and reliable method for assessment of photosynthetic activity in plants. The CFA technique has been used for early detection of physiological herbicide effects on plants (Spencer et al. 2009; Puértolas et al. 2010). Currently, there is no data on evaluation of giant reed response to herbicides using CFA. Utilization of CFA can bridge the knowledge gap on the physiological response of giant reed to herbicides. Therefore, the objectives of this study were to 1) compare initial giant reed response to glyphosate applied over-the-top of plants, after initial cutting, and by stem injection using CFA, and 2) evaluate the response of giant reed to four systemic herbicides (profoxydim, azimsulfuron, cyhalofop-butyl, penoxsulam) commonly used for control of weeds adjacent to surface water, after initial cutting using CFA.

Material and methods

Field study

The field study was initiated in the spring of 2010 and continued through fall of 2011 in Harnina River Basin, Almendralejo (Badajoz, Spain). The experimental site is characterized by a Mediterranean climate. During the experimental year, the weather conditions were close to long-term average for the previous 10 years (Figure 1). The average long-term annual precipitation is 532 mm range from 440 to 601 mm. The minimum and maximum average annual temperatures ranging from 4.4ºC in January (from -7.2ºC to 16.4ºC) to 17.4ºC in July (from 3.8ºC to 29.9ºC). The experimental location was selected because it showed a homogeneous distribution and high abundance of well-established giant reed. The experiment was arranged as completely randomized design with three replications of seven treatments, with each of experimental plot measuring 3.3 m × 9 m. Treatments consisted of broadcast foliar application of five systemic herbicides commonly used against grasses and perennial weeds; the active ingredients (a.i.) were glyphosate 36% and 54%, azimsulfuron 50%, cyhalofop-butyl 20%, penoxsulam 2.04%, and profoxydim 20%.

Glyphosate was chosen because it is the standard herbicide used by many land managers for giant

Table 1. Analysis of variance results (p-value in italics) for herbicide treatments. Mean values by relative quantum yield of fluorescence (QY) of giant reed sprouts, expressed as a percentage of the non-treated controls, at 30 and 60 days after treatment.

	QY (%) 30 DAT	**QY (%) 60 DAT**
Herbicide Treatment	*0,0001* ***	*0,0001* ***
Glyphosate on mass	50,64 c	31,81 e
Glyphosate by injection	-	39,39 de
Glyphosate on sprout	50,00 c	49,45 d
Profoxydim on sprout	46,75 c	48,48 d
Penoxsulam on sprout	85,71 b	85,49 c
Cyhalofop-butyl on sprout	85,71 b	88,31 bc
Azimsulfuron on sprout	94,80 a	96,53 ab
Nontreated	100,00a	100,00 a
R^2	98,84 %	92,74 %

Different letters in each column indicate a difference between treatments according to Tukey´s HSD Test based on linear-mixed effects model, at the 0.05*, 0.01** and 0.001*** significance level.

reed management. Herbicide treatments were applied in all experimental plots, on-label application rate (OLA), the first week of October 2010, immediately after flowering. When herbicide treatments were applied on grown sprouts, the cutting was realized at last of summer and sprouts were treated five weeks later. Glyphosate 36% was applied at 10 L a.i. ha^{-1}over-the-top of established giant reed plants (3 to 4 m in height) and after initial sprouting following cutting (80 cm in height) at 4 L a.i. L^{-1}, glyphosate 54% was injected with full-strength herbicide into stems (6 mls of product were injected into the second Knot) of giant reed sprouts, after the initial cutting, at 4 L a.i. L^{-1}. Azimsulfuron 50% (OLA 50 g a.i. ha^{-1}), cyhalofop-butyl 20% (OLA 1.5 L a.i. ha^{-1}), penoxsulam 2.04% (OLA 2 L a.i. ha^{-1}), and profoxydim 20% (OLA 0.75 L a.i. ha-1) were applied on giant reed after the initial sprouting following cutting. A nonionic surfactant at 0.25% (v/v) was added to the spray mix as recommended by the herbicide labels. All herbicide treatments were applied using a CO_2-presurized knapsack sprayer calibrated to deliver 187 L ha^{-1} at 276 kPa. During herbicide application, there was complete coverage of giant reed foliage. The effect of the herbicides on giant reed was quantified using CFA by measuring the quantum yield of fluorescence (QY):

$$QY = (Fm-Fo) / Fm$$

Where QY is a measure of photosystem II efficiency, *Fm* is maximal fluorescence intensity, and *Fo* minimal fluorescence intensity of selected object.

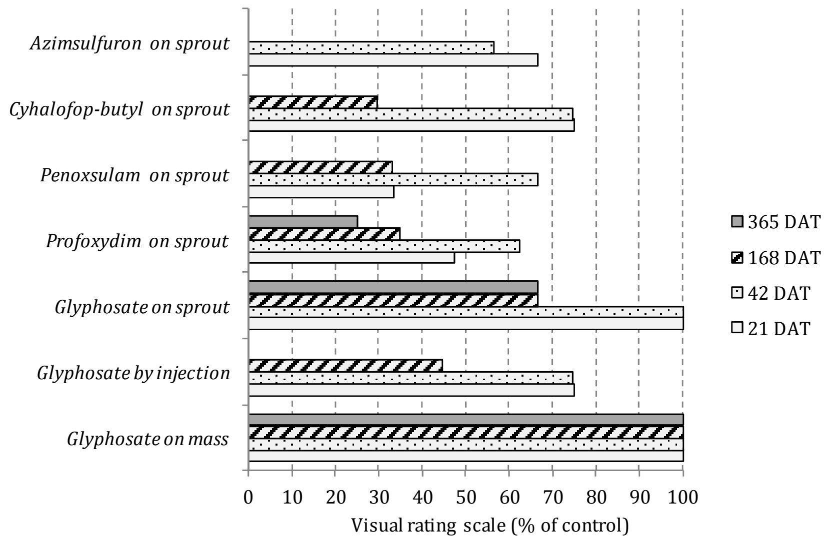
QY is a measure of “state of health of plants” and ranges from 0 to 1; healthy plants in the field show a maximum value of around 0.8, while a value below 0.7 indicates that a plant has suffered damage (Kourek et al. 2009). The QY was measured using a portable Pulse Amplitude Modulated fluorometre PAM 2000 (H. Walz, Effeltrich, Germany) at 30 and 60 days after treatment (DAT) to establish the response of giant reed to herbicides (expressed as a percentage of the non-treated control).

Visual evaluations utilized a qualitative rating scale from 0 to 100, where 0 equals no control and 100 is total control. The giant reed live stems or sprouts values were recorded from three randomly selected 0.25 m^2 quadrants within each plot. This scale was transformed to percentages by angular transformation (Little and Hills 1972). One day prior to treatment, visual evaluation values were recorded. At 21, 42, 168 and 365 DAT the visual evaluation was calculated by taking the difference between the initial situation prior to treatment and values taken on the subsequent evaluations.

Statistical analysis

The QY data were subjected to ANOVA and means separated using Turkey’s honestly significant difference multiple comparison test (5% level of significance) to evaluate herbicide effect on giant

Figure 2. Giant reed live stems or sprouts measured by visual ratings scale, and expressed as percentage (from 0%= no control to 100% = total control) at 21, 42, 168 and 365 DAT, means of three replications.



reed. Also, we estimated the relationship between QY data and visual estimations of giant reed sprouts by linear regression equation.

All statistical analysis was performed using Statgraphics Plus 5.0 software package (Statgraphics Plus for Windows 1998).

Results

The response to herbicides on giant reed sprout was examined using CFA techniques and showed a reduction in photosynthetic efficiency of plants treated with the herbicides (Table 1). All treatments with the exception of azimsulfuron significantly reduced QY compared with the non-treated control plants at 30 and 60 DAT (Table 1). At 30 DAT, glyphosate (10L a.i. ha^{-1}) applied over the top of established plants, glyphosate (4L a.i. ha^{-1}) and profoxydim both applied on sprouts, after initial cutting, had the highest effect on giant reed with QY value of 50% (equivalent to 50–54% reduction in photosynthetic efficiency). However, penoxsulam and chyhalofop-butyl reduced photosynthetic efficiency by 14% while azimsulfuron caused a 5% of reduction in photosynthetic efficiency at 30 DAT.

Similar results we obtained at 60 DAT, glyphosate (10L a.i. ha^{-1}) applied over-the –top of established plants had the greatest effect on giant reed with QY value of 31% (equivalent to 69% reduction in photosynthetic efficiency). Both glyphosate (4 L a.i. ha^{-1}) and profoxydim applied after initial stem-cutting reduced photosynthetic efficiency by 50% at 60 DAT. However, glyphosate (4 L a.i. ha^{-1}) injected into stems of established plants resulted in 40% reduction in photosynthetic efficiency at 60 DAT. In contrast, penoxsulam, cyhalofop-butyl, and azimsulfuron resulted in 15, 12, and 3% respective reduction in photosynthetic efficiency at 60 DAT.

There was a significant effect of the different herbicide treatments on giant reed visual injury estimation at 21 to 365 DAT. Glyphosate application over-the–top of established giant reed plants gave 100% control at all evaluation times (Figure 2). Glyphosate applied to regrown shoots also gave 100% control at 21 and 42 DAT, but control was only 65% by 168 and 365 DAT. Glyphosate injection controlled giant reed 75% at 21 and 42 DAT, but only 45% at 168 DAT, and injected plants had fully recovered by 365 DAT. Profoxydim applied to regrown shoots reduced giant reed presence by 45%, 65%, 35% and 25% at 21, 42, 168, and 365 DAT respectively. The other products applied to regrown shoots displayed mixed results. Giant reed control with azimsulfuron was 50% at 42 DAT, but no visible control was observed at 168 and 365 DAT. Chyhalofop-butyl controlled giant reed by 75% at 21 and 42 DAT, but was reduced to 30% at 168 DAT. Control with penoxsulam ranged from 35 to 70% at the first three evaluations; no control was visible by 365 DAT.

We found a significant relationship (R^2= 0,77) between the transformed QY values at 60 DAT and visual control of giant reed at 168 DAT (Figure 3). This indicates that CFA measured at 60 DAT can be used to predict the level of giant reed control expected at 168 DAT.

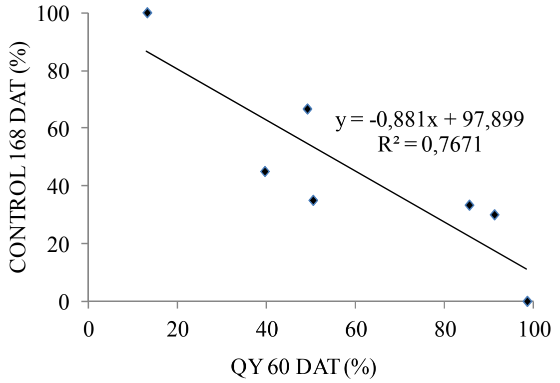


Figure 3. Regression equation of QY data at 60 DAT and the visual estimations of new giant reed sprout at 168 DAT, n=21.

Discussion

The CFA technique can provide a less laborious means of testing the effect of herbicides on giant reed and other invasive plants. CFA is a quick, easy, and reproducible method; the confirmation of its efficacy would provide a useful tool for land managers. In previous experiments carried out in glasshouses, we confirmed that CFA techniques could be used to easily monitor the effects of herbicide treatments on new giant reed sprouts following cutting (Santín-Montanyá et al. 2013).

Our results show that the effects of the different herbicides on QY at 60 DAT mirrored the effects visually observed on presence of new giant reed sprouts at 168 DAT. Meaning that efficacy of herbicide treatment can be predicted 108 days sooner and with the CFA technique in a more objective manner. However, it should be verified by further studies, that CFA techniques could be used to monitor the long term effects of herbicide treatments on giant reed and to confirm the ability of the giant reed to recover from injury.

The results also suggest that glyphosate applied over-the-top of established giant reed plants or to 80 cm shoots arising following earlier cutting both provide an excellent means of controlling this invasive plant species. The application of glyphosate by injection controlled the presence until 168 DAT, but eventually the plants recovered by 365 DAT. Profoxydim applied on sprouts of giant reed would also appear to hold promise. The effects of profoxydim on sprouts lasted until the end of the experiment (365 DAT). However, long-terms studies of the effects of these herbicides (glyphosate and profoxydim) on the control of this invasive species, and new studies to measure utility in combination with other products are now required.

Lack of response of giant reed to grass herbicides has been previously reported. Odero and Gilbert (2012) conducted a study about the response of giant reed to asulam and trifloxysulfuron. These herbicides are known to control rhizomatous perennial grass in sugarcane plantations, but neither provided complete control of giant reed, and their results showed that containment of aggressively spreading giant reed would not be possible with the currently available (authorized) herbicide control options in the United States. Bottoms et al. (2011) evaluated the activity of glyphosate, cyhalofop-butyl and penoxsulam on the growth and vegetative reproduction of creeping rivergrass (*Echinochloa polystachya* Kunth A. S. Hitchc) an invasive aquatic grass. It was found that herbicides cyhalofop, glyphosate and penoxsulam reduced the fresh weight 25–50% at 14 DAT and 63–80% at 28 DAT compared with the non-treated plants. This was partially in contrast to our results with giant reed, penoxsulam and cyhalofop-butyl did not seem to provide good control according to our QY and visual data.

Spencer et al. (2008 and 2009) reported the response of giant reed to different glyphosate concentrations, and found that the higher the concentration, the greater the control achieved, whereas experiments with imazapyr have produced inconsistent results. Later experiments showed both imazapyr and glyphosate treatments worked well applied alone, and the combined treatments also may provide control (Bell 2011). Mateos-Naranjo (2009) demonstrated that treatment with glyphosate could be an appropriate control method for *Spartina densiflora* L., because at high doses it has a negative effect on the photosynthetic apparatus and growth of this plant. These results are similar to those we obtained with glyphosate on new giant reed sprouts following cutting.

Cutting giant reed stems in combination with glyphosate application can be used to control and reduce the competitive ability of the invasive species, and it should be used together with species afforestation restoration programs to improve the success of invasive plant control. Given an infested area (with invasive plants), the first step to re-establish a native population would be to treat with herbicide in order to reduce the invasive plant biomass. The next step is to restore the area with native species.

Renz and DiTomaso (2006) found that one particular pretreatment (mowing and ploughing) enhanced efficacy of glyphosate on perennial pepperweed (*Lepidium latifolium* L.). Also a similar pretreatment with tillage improved the control of Canada thistle (*Cirsium arvense* L. Scop.) with glyphosate (Hunter 1996). In one long term study of *Alianthus altissima* L., under Mediterranean conditions (Constán-Nava et al. 2010), it was indicated that joint cut-stem and herbicide application is the only effective treatment to reduce this species in the long-term. Although, Miller and D´Auria (2011) concluded that in areas where tillage is not possible, glyphosate treatment could provide satisfactory control of the perennial weed wild chervil (*Anthriscus sylvestris* L.).

The high invasive potential of giant reed is based on its widespread distribution and inherent weedy characteristics, which greatly increases the likelihood of its post-treatment survival, and subsequent environmental damage (Barney and DiTomaso 2008).

Conclusions

Giant reed has been cultivated for thousands of years in Mediterranean areas; it was used to satisfy local necessities, such as training stakes, baskets and mats, walking sticks and fishing canes, and it was employed as bioenergy crop (Mariani et al. 2010). It became invasive in riparian areas and over-runs native plant and riverside habitats, and there is a need to increase the efficiency of current control techniques established to contain giant reed infestation in these areas (Bell 2011). Our results suggest that CFA techniques can be easily used to monitor the effects of combined different herbicide treatments over-the-top of established giant reed plants and after the initial sprouting following cutting in giant reed plants. This data would provide a guideline for field expectations when treating invaded areas.

In this study, at 60 DAT glyphosate 36% (10L a.i. ha^{-1}) applied over-the–top of the established giant reed plants had the highest effect; 69% reduction in photosynthetic efficiency. Both glyphosate 36% (4 L a.i. ha^{-1}) and profoxydim 20% (0.75 L a.i. ha^{-1}) applied after the initial sprouting following cutting reduced photosynthetic efficiency by 50% at 60 DAT.

The combined technique of herbicide treatment and stem-cutting should be included in giant reed management plans in our Mediterranean riverside habitats. The results of this study show great promise for this combined technique, however long term monitoring is necessary in order to assess its real potential, bearing in mind the main objective of any treatment plan, being to ensure native species colonization and ecosystem recovery.

The findings of this study should encourage further experimental analyses on response of herbicides and environmental conditions that may influence field efficacy. We also suggest further study of effects with respect to combined treatment of different herbicides, herbicide dosages and herbicide application timing, analyzing how treatment affects giant reed.

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