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Teaching/Education

Utilizing R Software Package for Dose-Response Studies: The Concept and Data Analysis

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Advances in statistical software allow statistical methods for nonlinear regression analysis of dose-response curves to be carried out conveniently by non-statisticians. One such statistical software is the program **R** with the *drc* extension package. The *drc* package can: (1) simultaneously fit multiple dose-response curves; (2) compare curve parameters for significant differences; (3) calculate any point along the curve at the response level of interest, commonly known as an effective dose (e.g., ED30, ED50, ED90), and determine its significance; and (4) generate graphs for publications or presentations. We believe that the *drc* package has advantages that include: the ability to relatively simply and quickly compare multiple curves and select ED-levels easily along the curve with relevant statistics; the package is free of charge and does not require licensing fees, and the size of the package is only 70 MB. Therefore, our objectives are to: (1) provide a review of a few common issues in dose-response-curve fitting, and (2) facilitate the use of up-to-date statistical techniques for analysis of dose-response curves with this software. The methods described can be utilized to evaluate chemical and non-chemical weed control options. Benefits to the practitioners and academics are also presented.
Key words: nonlinear regression, experimental design, weed dry matter.

The importance of dose was recognized more than 500 years ago. In the fifteenth century, Paracelsus (1494–1541), who practiced alchemy, suggested that the poison is in the dose ["*Alle Ding sind Gifft und nichts ohn Gifft. Allein die Dosis macht das ein Ding kein Gifft ist.*" (All things are poison and are not poison; only the dose makes a thing not a poison)]. In recent history, many researchers have examined various aspects of the dose-response in herbicide bioassays, and toxicological and ecotoxicological studies (Cedergreen et al. 2005; Hamill et al. 2000; Knezevic et al. 1998; Seefeldt et al. 1995; Sikkema et al. 1999; Streibig 1988).

In weed science research, the most common goal of a biological assay is to measure and compare the response of weeds and crops to physical, chemical, biological, or temporal stimuli. Often, summaries of biological assays require the use of nonlinear regression models with upper and lower limits, which provide information on the dose required to control the plant species of interest. The effective dose (ED), at a specific response level (e.g., ED50, ED90), can be determined through a variety of methods for data analyses. Some compare the effects of dose on plant response using multiple comparison techniques to separate treatment means (Biediger et al. 1992; Poston et al. 1992; Stamps 1992), whereas others utilize various nonlinear regression models (Knezevic et al. 1998; Seefeldt et al. 1995; Streibig, 1988). Berti et al. (1996) referred to the multiple comparison techniques and nonlinear regression models as the classical and functional approaches, respectively. Several authors have criticized the use of the classical (ANOVA) approach, suggesting that regression is a more appropriate method for analysis of structured data (e.g., herbicide doses in step-wise increments) (Knezevic et al. 1998, 2002; Seefeldt et al. 1995; Streibig et al. 1993). With advances in computer technology a variety of statistical programs have become available, and many have been utilized to fit nonlinear regressions and estimate their parameter values, including SAS[1] (Knezevic et al. 2002), Sigma Plot, S-PLUS®[2], Axum®[3] (Evans et al. 2003), and FIG-P®[4] (Knezevic et al. 2003a, 2003b).

Advances in statistical software allow both standard and more complex statistical methods for nonlinear regression analysis of dose-response curves to be carried out by non-statisticians. One such package, *drc* (dose-response curves) (Ritz and Streibig 2005), has been developed for the open-source language **R** and is available at no cost from the Internet.

R is a command-line driven software, and it is relatively user friendly. **R** can simultanously fit multiple dose-response curves, examine whether a chosen dose-response model is appropriate to describe the data, and calculate biologically relevant quantities such as a dose of interest (EDy). The user only needs to fit the regression model once and then all parameter combinations of choice can be compared for significance. The *drc* package contains programmed commands for dose-response analysis and enables **R** to graph the distribution of data and regression lines. Therefore, our goal is to: (1) provide a review of a few common issues in fitting dose-response curves, and (2) facilitate the use of up-to-date statistical techniques for analysis of dose-response curves with this software package.

The Dose-response Concept

Dose is a subject of research in many disciplines. In weed science, dose usually refers to the ED of an herbicide needed

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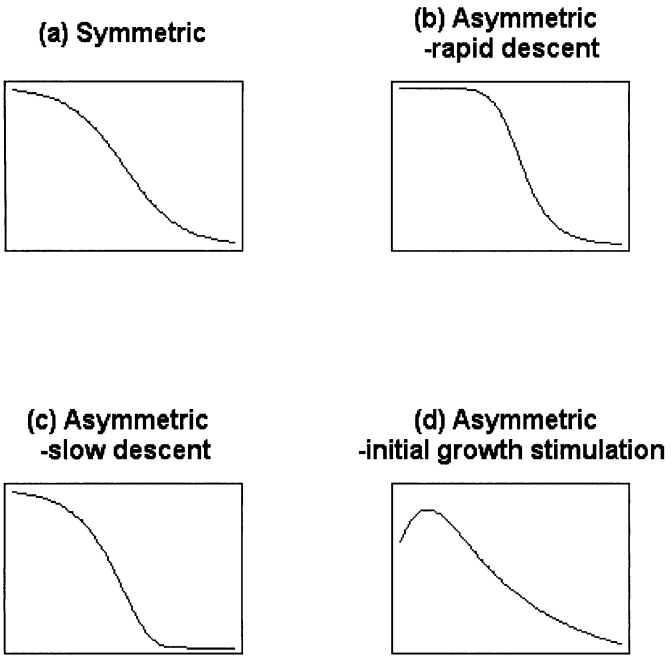

Figure 1. Symmetric and asymmetric dose-response curves: (a) Asymmetric dose-response curve (Equations 1 and 2); (b) An asymmetric curve where the descent from the upper limit is rapid, but the approach towards the lower limit is more slow (Equation 3); (c) An asymmetric curve where the descent from the upper limit is slow, but the approach towards the lower limit is more rapid (Equations 4 and 5); and (d) An asymmetric curve suitable for modelling initial growth stimulation such as hormesis (Equation 6).

to obtain a desirable effect on the plant species of interest (Streibig et al. 1993). Thus, the relationship between herbicide dose and plant response is of great interest for understanding herbicide efficacy. Furthermore, understanding of that relationship is critical for the proper design and interpretation of the dose-response studies (Streibig et al. 1993).

Dose-Response Curve. Typically, the shape of a dose-response curve is sigmoidal (Figure 1), with upper and lower limits, where the upper limit is defined by the response from nontreated plants (control), or from plants treated with a very low dose of herbicide, whereas the lower limit is determined by the response levels from a high dose of herbicide (Figure 1). The dose corresponding to the midpoint of plant growth response observed between the upper and lower limits is usually referred to as ED50 (Streibig 1988).

The most commonly used model for sigmoidal dose-response curves is the log-logistic model with three or four parameters (Figure 1a). The four-parameter log-logistic function is given by the equation 1:

$$Y = c + \{d - c / 1 + \exp[b(\log x - \log e)]\} \quad [1]$$

where e is the ED50. The upper limit is d and the lower limit is c. The parameter b denotes the relative slope around e. Interpretation of these parameters is discussed in detail by Streibig et al. (1993). The log-logistic function is symmetric around the parameter e, the inflection point. If c = 0, then the four-parameter model reduces to the three-parameter model (equation 2), with the lower limit being zero.

$$Y = d / 1 + \exp[b(\log x - \log e)] \quad [2]$$

Another commonly used model is the four-parameter Weilbull model given by equation 3:

$$Y = c + (d - c)\exp\{-\exp[b(\log x - e)] \quad [3]$$

where the parameters c and d are the lower and upper limits, as in the four-parameter log-logistic model (equation 1), and b is the relative slope around the inflection point e. The Weilbull model is not symmetric around any point (Figure 1b). If in the three-parameter Weilbull model (equation 4) the lower limit e = 0, then equation 3 becomes equation 4 (Figure 1c).

$$Y = d^{\#} \exp\{-\exp[b(\log x - e)]\} \quad [4]$$

In addition to the most commonly-used log-logistic models (equations 1 and 2), and the Weibull models (equations 3 and 4), there are several other dose-response models published in the literature. Brain and Cousens (1989) proposed using a model for studies of hormesis (Figure 1d). Hormesis (from the Greek for "setting in motion"), is a stimulation of plant response to a low dose of some herbicides, resulting in response values that are higher than in nontreated plants (Calabrese 2001; Calabrese and Baldwin 2001, 2003). Brain-Cousens model was obtained by modifying the four-parameter log-logistic model (equation 1) with the addition of the linear term f in the numerator.

$$Y = c + \{d + fx - c / 1 + \exp[b(\log x - \log e)]\} \quad [5]$$

Most of the models (equations 1 to 4) assume that the plant response (e.g., dry matter) is a decreasing function of a dose (Figure 1). However, the above models can be also utilized to describe plant response as an increasing function of a dose. For example, in visual ratings of weed control, the level of effects on weeds typically increases with an increase in dose. Therefore, in the descending curve, the value of ED10 represents an effective dose that provides 90% weed control, which is equivalent to the ED90 value in the ascending curve.

Typical Data Variables

Dose-response relationships can vary greatly, depending on the plant species, dose range and type of herbicide tested, and environmental conditions. Therefore, there is a minimum amount of data that should be collected in order to be able to fit dose-response curves. Variables commonly reported include plant fresh or dry weight (Streibig et al. 1993), relative biomass (Knezevic et al. 1998), and visual ratings of weed control (Knezevic et al. 2004). Additional variables to consider can include: leaf stages, height and developmental phases at regular time intervals (Hock et al. 2006), root length and weight measurements, various growth parameters (e.g., growth rates, harvest index, leaf area) (Evans et al. 2003), and environmental variables such as daily rainfall, average daily temperature (soil and air), soil moisture, and nutrient status (Evans et al. 2003). Knowledge of ancillary variables is critical

Table 1. Basics commands for fitting dose curves in order to determine the dose of interest.

Line	*R PROGRAM & OUTPUT*	*COMMENTS*
	Step 1: Reading a data file into R	
01	*library(drc)*	This function loads and activates the *drc* package in **R.**
02	*dataname<-read.csv ("c:\\dir\\subdir\\filename.csv"), skip=4)*	Assign a name to your data file (replace "*dataname*" with a name of your choice), and location (replace "*c:\\dir\\subdir\\filename.csv*" with the full path your data file). In the Anglo Saxon system, with (.) as decimal separator you should use read.csv () whereas elsewhere (,) is used as decimal point and then you use read.csv2(). To start reading data from a particular line in the data file, use *skip=4* code, which tells R to start reading data at the indicated line number (4).
03	*head(dataname)*	This line prints the first six lines of the data set, thus it is useful to double check if data have been read correctly. Example of first six lines are provided.

	g.ai.ha	TIMING	IMG.DM
1	0	1	128.0
2	0	1	179.0
3	0	1	102.6
4	225	1	113.4
5	225	1	110.6
6	225		101.0

Step 2: Fitting multiple dose-response curves

The *multdrc* function is the key function in *drc* for fitting dose-response curves.

04	*IMGDM<- multdrc(IMG.DM~g.ai.ha, TIMING, fct = l3(), data=dataname)*	*IMGDM* is a user-defined name which will contain the all information about the fitted model generated by the *multdrc* function. *IMG.DM* is the response variable (y-axis) and *g.ai.ha* is the dose (x-axis). *TIMING* is the classification variable for each curve; *fct=l3()* is the argument for the log-logistic curve with three parameters (equation 2). If the *fct* argument is not provided, by default a four-parameter log-logistic model is fitted to data; *data=dataname* identifies the name of data file. Note that *multdrc* does not produce any output. All the information on the model fit is stored in the object under the user defined name (e.g., IMGDM) to be read by further commands (e.g., lines 5–8).
05	*summary(IMGDM)*	The *summary* function provides a summary of the parameter estimates.
06	*anova(IMGDM)*	The *anova* function provides a lack-of-fit test, comparing your chosen dose-response model to the more general ANOVA model. In this particular instance it is a two-way ANOVA with TIMING and g.ai.ha as factors.
O7	*ED(IMGDM,c(90))*	The *ED* command provides ED values of your choice based on the fitted model. With the logistic model, ED50 value is in fact the parameter *e.* In weed control studies, the ED90 is of more interest.
08	*SI(IMGDM,c(90,90))*	*SI* is used to compare the relative differences of ED values among curves, i.e., the relative potencies among the herbicides. In this case we compared the relative potency at ED90 for the three curves.
09	*plot(IMGDM, conName="0", conLevel=100, xlim=c(0,10000), xlab="Glyphosate dose (g ai/ha)", ylab="Dry Matter(g/plant)", col = c(1,1,1), pch=c(1,2,3), legendText=c("1st timing", "2nd timing", "3rd timing"), main="Morningglory control as influenced by timing of application")*	To obtain visuals of the fitted curve the *plot* command is used. The argument *conName and conLevel* specify the label on the x axis for the control measurements and how the x axis is divided, respectively. *xlim* specifies the range on the x axis. The labels on the axes are provided using arguments *xlab* and *ylab*. The arguments *col* and *pch* determine the color and plot symbol used for each curve and the corresponding data points. Legend text and figure title are provided by arguments *legendText* and *main (Figure 2).* Visit http://www.R-Project.org, for additional information on how to do produce graphs within R environment.

Output from Step 2:

Model fitted: l3()

Parameter estimates:

	Estimate	Std. Error	*t*-value	P value
b:1	1.42243	0.25239	5.63575	4.373e-07
b:2	1.69003	0.28378	5.95546	1.264e-07
b:3	2.12887	0.42581	4.99964	4.863e-06
d:1	135.61721	10.43945	12.99084	8.838e-20
d:2	180.59099	9.84531	18.34284	2.940e-27
d:3	224.04626	8.64285	25.92273	1.288e-35
e:1	588.71401	112.96285	5.21157	2.203e-06
e:2	1,067.41642	132.90226	8.03159	3.142e-11
e:3	1,812.70392	122.84619	14.75588	2.045e-22

Estimated residual variance: 335.554

Output from *summary* command: parameter estimates in the three-parameter log-logistic model. The estimated b parameters: b:1, b:2, and b:3, of the three curves are the relative slope at e:1, e:2, and e:3, which are the ED50 for the three curves. d:1, d:2, and d:3 are the upper limits

Provided are also standard errors of the parameters and an approximate *t*-test with associated P value that is testing the hypothesis that the parameters are equal to 0

Table 1. Continued.

Line	*R PROGRAM & OUTPUT*					*COMMENTS*
Lack-of-fit test						Lack of fit test yields a p-value of 0.676 which is not significant at 5%, indicating that the nonlinear model provides acceptable description of data. Output from *ED*: The ED90 values for each of the three curves and the corresponding estimated standard errors.
Model	df	RSS[a]	df	*F* value	P value	
Two-way ANOVA	48	1360	1			
DRC model	63	21140	15	1.77	0.0676	
	Estimate		Std. Error			
1:90	2,759.0		636.62			
2:90	3,917.1		637.79			
3:90	5,088.2		904.25			
	Estimate		Std. Error	*t*-value	P value	Output from SI:
1/2:90/90	0.70436		0.19891	-1.48629	0.1422	The ED90 values were compared. ED90 values for curves 1 and 2 by analysing whether the ratio ED50(1)/ED50(2) is significantly different from 1.00 (P = 0.14); ED90 values for curves 1 and 3 are significantly different (P = 0.005); ED90 values for curves 2 and 3 are not significantly different (P = 0.22).
1/3:90/90	0.54224		0.15792	-2.89859	0.0052	
2/3:90/90	0.76984		0.18555	-1.24043	0.2194	

[a] Abbreviation: RSS, residual sum of squares.

for extrapolating the results to other conditions, and can help the user adjust the effective dose spatially and temporally (Knezevic et al. 2002).

Plant Biomass. Plant dry matter (DM) is one of the best biological indicators of plant growth, and has been suggested as the most objective measure for defining herbicide dose-response curves, and in determining effective dose for a specific weed species (Streibig et al. 1993). Some researchers have used plant DM as a measure for describing the concept of biologically effective dose. The biologically effective dose was defined as a dose that provides 90% reduction in weed dry matter (Dieleman et al. 1996; Knezevic et al. 1998). Knezevic et al. (1998) also suggested that the biologically effective dose can be utilized to ensure profit maximization and to reduce the amount of herbicide applied into the environment. Depending on the desired level of weed control, a herbicide dose could be selected to either control the weed, or reduce its growth, thereby offsetting its competitive ability with the crop (Dieleman et al. 1996).

Relative Biomass. Relative biomass reduction is expressed as the percentage of the nontreated control and is a common way of presenting data in weed science literature (Hamill et al. 2000; Knezevic et al. 1998; Sikkema et al. 1999). In this method, the plant DM data is expressed on a relative scale from 0 to 100%, as a percentage of the DM of nontreated plants.

There are several practical reasons why some scientists have used relative biomass data (Hamill et al. 2000; Knezevic et al. 1998; Sikkema et al. 1999). Relative biomass data provides a visual presentation of herbicide effects on plants that producers and practitioners can easily understand (Knezevic et al. 1998). The plant response data is presented at the y-axis utilizing a scale from 0 to100%, which is similar to the visual weed control rating scale commonly used in the weed science discipline.

We suggest to analyze raw data using actual DM, but to graph the results utilizing relative DM for the y-axis, as the x-axis (herbicide dose) is identical in both cases. One of the problems with using data expressed on a relative scale in the initial analysis is that the parameter estimates are not independent anymore because data have been scaled. Usually the resulting b and ED50 are the same in both cases, but the standard errors differ. The same is true for estimating other ED values, which makes statistical comparisons among relevant biological parameters difficult.

Percent Weed Control. Visual estimates of percent weed control is one of the most common ways of presenting data in the weed science literature. Weed control ratings are usually based on a scale from 0 to 100 (where 0 = no injury and 100 = plant death). The main advantage of visual ratings method is that the data collection process is easier than harvesting plant material for fresh and dry weights. A disadvantage of this method is that the visual ratings can differ among researchers (Knezevic et al. 1998), making comparisons of EDs difficult among the years and locations.

Timing of Data Collection. The timing of data collection typically depends on the hypothesis and objectives of the

experiment (Knezevic et al. 2002). For example, visual ratings of percent weed control in herbicide evaluation trials in a single growing season are routinely collected around 1, 2, 4, 8, and 12 wk after treatment (WAT), whereas in longer term studies, data are collected at 1, 2, or more years after treatments (Knezevic et al. 2004).

In dose-response studies, a curve provides a snapshot of the plant response to the herbicide at the time of evaluation. In reality, the development of herbicide symptoms and effects can depend on many factors, including the plant biology and life cycle, the herbicide mode and type of action, and environmental variables (Vencill 2002). For example, fast changes in plant growth immediately following spraying can be observed with contact-type herbicides (e.g., paraquat and diquat), whereas the effects from systemic herbicides (e.g., glyphosate) take longer to develop (Vencill 2002). The phenology of the plant is also crucial, because some species are more vulnerable at particular stages of development than others (Vencill 2002). The timing of data collection should capture those most obvious symptoms, and/or differences among the treatments. Otherwise, the data collection might be conducted on a sliding time scale (e.g., 2, 4, 6 WAT).

Number of Data Points Required. The shape of the dose-response curve is greatly influenced by the distribution of data it describes, thus the appropriate selection of herbicide doses is critical for dose-response analysis (Streibig et al. 1993). For example, a sufficiently high dose stops growth or might kill the plants, resulting in data grouping at the lower end of the curve, but sufficiently low doses have no effect on the plant resulting in data grouping around the upper end of the curve (Figure 1a). The line between the two ends of the curve is usually straight (Knezevic et al. 1998).

In theory, a four-parameter log-logistic curve (equation 1) could be described by four doses; one dose for each parameter. Based on our experience, we believe that it is better to reduce the number of replications from four to three and increase the number of doses. In essence, the larger number of data points will hopefully allow for possible shifts in the curve among the years and locations, and ultimately account for some of the variability in field experiments.

R Software and *DRC* Package

The **R** statistical software is available at http://www.R-project.org (R Development Core Team, 2006) from where it can be freely downloaded (binaries for Windows systems and source code for GNU/Linux and MacOs). The extension package *drc* is available at http://www.bioassay.dk (the homepage for the package), or from the central repository for extension packages for **R**, the Comprehensive **R** Archive Network. A particular useful editor for use with **R** is Tinn-R (http://www.sourceforge.net/projects/tinn-r). **R** with the *drc* package occupies about 70 Mb of a hard drive.

After installing **R**, the extension package *drc* must be also installed. The file drc_x-y.zip (x-y stands for version number, drc_0.9-9.zip) should be installed from within the **R** environment only, in order for the package to function properly. The zip file contains the current version; however, the *drc* package might be updated over time, and improved with additional features. The updates should not alter data analysis. It is useful to check for updates periodically because the guidelines on how to use them will be provided at http://www.bioassay.dk. Within the **R** program, click on "packages," select "install packages from local zip files," then find the file drc_x-y.zip, and **R** will unzip and install the package. **R** and *drc* are now ready for data input and analysis.

Data Organization and Input into R. Data can be transferred into **R** from a variety of spreadsheet programs. Smaller data sets can simply be copied and then pasted into **R** (Ritz and Streibig 2005). Larger data files can be read in a variety of ways; in this paper we use the comma separated files format ("filename.csv").

General Approach for the Suggested Statistical Analysis. Initial estimates of the parameters for the nonlinear regression model is typically the first step in nonlinear regression analysis. In most cases with *drc*, there is no need for initial estimates of the regression parameters as they are calculated from the data by the package (Ritz and Streibig 2005). With the drc package, the chosen regression model is only fit once and the desired parameters, such as effect dosages (ED10, ED90) and selectivity indices (SI) as relative potencies between dose-response curves are derived from the regression model utilizing the delta method (Van der Vaart 1998). This is in contrast to the common approach to reparameterize and refit the model in order to determine the initial parameter values (Schabenberger et al. 1999). Reparameterizing and refitting is relatively computer-intensive and requires some skill in manipulating mathematical expressions. The latter process is also vulnerable to lack of convergence of the nonlinear least squares algorithm, as the reparameterization could result in strongly correlated parameters, or poorly determined parameters, depending upon the distribution of the responses at the lower and upper limits.

Example 1: Fitting Single and Multiple Curves. ***Case study: Effect of Glyphosate Rate and Three Application Times on Control of Selected Weeds.*** In this experiment eight glyphosate doses were applied at each of the three growth stages of the weed, hereafter referred to as growth stages 1, 2, and 3. The eight doses included 0, 225, 449, 896, 1,346, 1,797, 2,330, and 2,888 g ai/ha, and were randomized within each of the three growth stages replicated three times. Specific details regarding the experimental site and results are provided by Knezevic and Klein (2005).

Basic commands for reading data into **R** and curve fitting are shown in Table 1. The code can be copied into **R** from various editors including the **R** script editor, and the saved file has suffix r. Selecting "file"/"open script" allows access and edit of the script of interest. There are several ways of executing scripts within the **R** environment. One way of using the **R** script editor is to execute each script line by placing the cursor on the script line of interest, then select the icon "run line or selection" or use Crtl R.

The first step in the procedure is to start the *drc* package, which is done using the *library* function (Table 1, Step 1, line 01). Line 02 assigns the name for your data file and location of the file. Line 03 prints the first 6 observations, providing

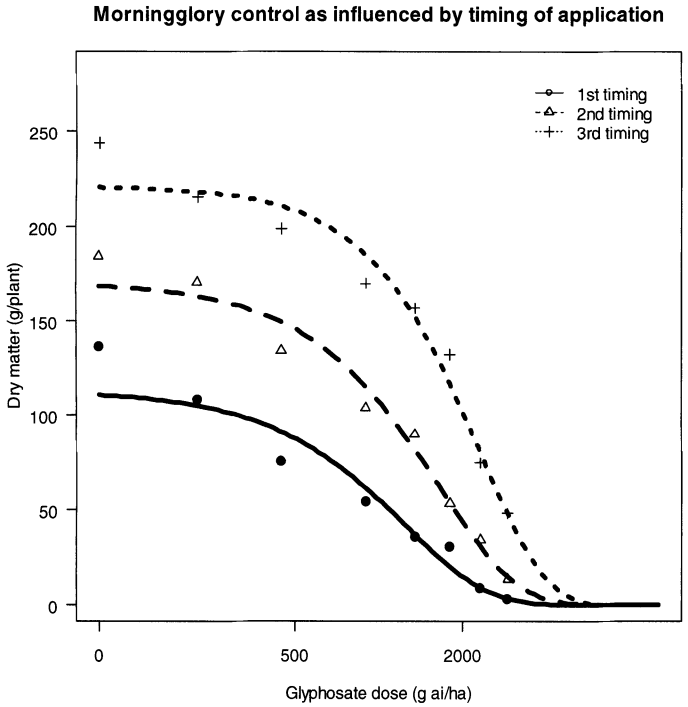

Figure 2. Output from the *plot* command (line 9, Table 1). The visual of the fitted dose-response curves, labels for x and y axis, line legends, and main title. Equation parameters are shown within the output section of Table 1.

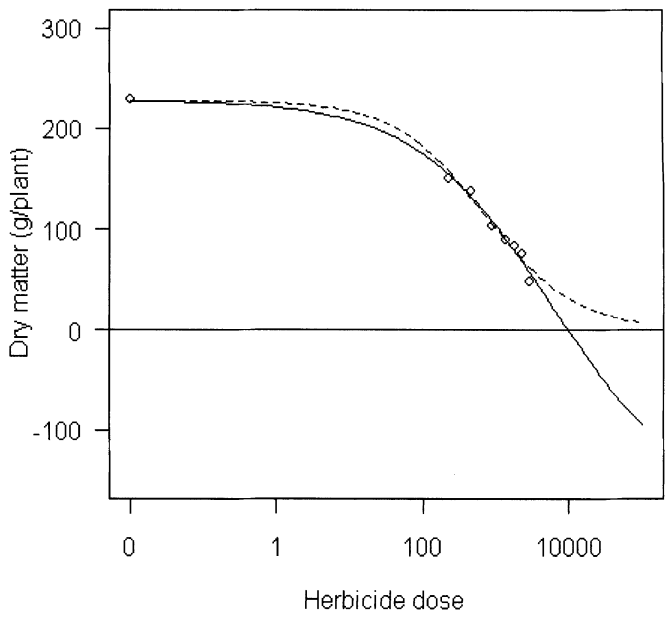

Figure 3. Comparison of the log-logistic model where the lower limits is estimated (solid line) and the log-logistic model where the lower limit is fixed at 0 (dashed line). Commands and equation parameters are shown in Table 2.

Table 2. Fitting curves to a data set lacking data points along the lower limit of the log-logistic curves, thus providing example for using the three-parameter over four-parameter log-logistic curve.

Line	*R PROGRAM & OUTPUT*	*COMMENTS*
	Comparison of two log-logistic models (equations 1 and 2)	
1	*IMG.equation1<- multdrc(IMG.DM~g.ai.ha,fct=l4(), data=dataname)*	Commands *multdrc* and *fct l4()* are used to fit a four-parameter log-logistic curve for ivyleaf morninglgory (*IMG.DM*) dry matter to glyphosate dose (*g.ai.ha*).
2	*plot(IMG.equation1, xlim=c(0,100000),* *ylim=c(-150,300),* *legend=TRUE,* *xlab="Dose",* *ylab="Dry Matter",* *data= IMG.equation1)*	The fitted model of data *IMG.equation1* is plotted. The ranges on the x and y axes are specified using the *xlim* and *ylim* argument: the x axis goes from 0 to 100,000 and the y axis from -150 to 300. Legend information is given (*legend=TRUE*) the names of the x and y labels are specified (*xlab* and *ylab*).
3	*IMG.equation2<- multdrc(IMG.DM~g.ai.ha,* *fct=l3())*	The command *multdrc* is used to fit a three-parameter log-logistic curve which is specified with the argument *fct=l3()*.
4	*plot(IMG.equation2, type="add",* *xlim=c(0,100000), lty=2)*	The fitted three-parameter log-logistic curves is added to the existing plot as dashed line using the argument *type="add"*. Line type 2 (dashed line) is specified for this fitted curve (*lty=2*).
	Outputs for four-parameter and three-parameter models:	
	Parameter estimates for l3:	Output for four-parameter model (equation 1). The estimated regression parameters are b, c, d, and e. Note the negative values for parameter c = -109.8 is huge compared to the upper limit d = 64 The line is shown in Figure 3 (solid line). This resulted in unrealistic ED values at any level, including ED90 of 95,166.5 g ai/ha, which is not appropriate.
	Estimate Std. Error *t*-value P value	
	b: 0.7 0.3 2.3 0.0231	
	c: -109.8 144.9 -0.7 0.4515	
	d: 64.3 10.4 6.1 6.141e-08	
	e: 5,994 1,115.8 0.5 0.5917	
	Estimate Std. Error	
	1:90 95,166.5 280,360	
	Parameter estimates:	Output for three-parameter model (equation 2). The estimated regression parameters with lower limit, (Figure 3, dashed line). The ED90 value of 2,472.9 g ai/ha suggests that the dose needed to control ivy leaf morningglory equals 2,472 g ai/ha, which is meaningful to practitioners.
	Estimate Std. Error *t*-value P value	
	b:1 5.3 1.0 5.3 1.466e-06	
	d:1 45.1 4.7 9.5 6.489e-14	
	e:1 1,640.5 144.1 11.3 3.185e-17	
	Estimate Std. Error	
	1:90 2,472.9 97.448	

Table 3. Fitting a hormesis model (Brain-Cousens model).

Line	*R PROGRAM & OUTPUT*	*COMMENTS*
	Analyzing data using a "hormesis model"	
1	*l3.model <-multdrc(SC.DM~g.ai.ha, fct=l3(), data=np05.mod)* *hormesis.model <-multdrc (SC.DM~ g.ai.ha, fct=bcl3(), data=np05.mod)*	The relation between dry matter (DM) of sweet clover (SC) and rates of glyphosate (g.ai.ha) is described using the three-parameter log logistic model (*fct=l3()*) and using the Brain-Cousens model (*fct=bcl3()*).
2	*anova(l3.model)* *summary(l3.model)*	The command *anova* is used to extract the lack-of-fit test for the fitted three-parameter log logistic model.
3	*anova(hormesis.model)*	Again, *anova* is used to obtain the lack-of-fit test, this time for the Brain-Cousens model.
4	summary(hormesis.model)	The *summary* of the fitted model, displaying parameter estimated with corresponding standard errors.
5	*ED(hormesis.model,c(10,30))*	The *ED* commands calculate values for ED10 and ED30, which are critical in ecotoxicology or crop tolerance-type studies.
6	*plot(l3.model, legend=TRUE, xlab="Dose", ylab="Dry Matter") plot(hormesis.model, type="add")*	The plot of the two fitted dose-response curves (overlayed) and the original observations.
	Outputs for three-parameter log logistic model and hormesis model:	
	Lack-of-fit test for l3	Output for three-parameter model (equation 2).
	Model df RSS df *F* value P value One-way ANOVA 16 327.65 DRC 21 739.08 5 4.0181 0.0149	There was a significant lack of fit test (P = 0.0149), suggesting that l3 model was not appropriate for data
	Parameter estimates:	The estimated regression parameters are b, d, and e. Parameter d is higher than in the Brain-Cousens model below. Parameter e, which corresponds to the ED50, is twice as large than for the Brain-Cousens model. The model fit is shown in Figure 4 (dashed line).
	Estimate Std. Error *t*-value P value b: 2.72 0.81 3.35 0.003 d: 26.59 2.22 11.92 8.166e-11 e: 989.93 144.02 6.87 8.569e-07	
	Lack-of-fit test for blc3	Output for Brain-Cousens model (equation 5).
	Model df RSS df *F* value P value One-way ANOVA 16 327.65 DRC 20 503.21 4 2.1433 0.1225	There was no significant lack of fit test (P = 0.12), suggesting model was appropriate for data.
	A 'Brain-Cousens' model was fitted.	Now parameter d is lower than in the three-parameter logistic model. Note that the parameter e in this case does not have the same meaning as in the logistic curve; it is not ED50. The line of the fit was shown in Figure 4 (solid line).
	Parameter estimates:	
	Estimate Std. Error *t*-value P value b: 2.68 0.47 5.69 1.409e-05 d: 20.61 2.82 7.28 4.808e-07 e: 553.85 14.98 4.81 0.0001 f: 0.05 0.02 2.26 0.0346	Note the values for ED10 = 733 and ED30 = 890 g ai/ha suggested the dose needed to provide sweet clover growth stimulation (hormesis) of 10% or 30%, respectively, which has practical value.
	Estimated residual variance: 25.16074	
	Estimate Std. Error 1:10 733.97 113.76 1:30 890.62 119.96	

a visual for checking if the data file has been correctly loaded into **R**.

The second step is the curve fitting procedure (line 04, Table 1) using the *multdrc* function. Line 05 summarizes parameter values for model fit of the three curves. The results from line 05 are shown in the output section (Table 1). Line 06 executes a lack-of-fit test. In this particular instance the test for lack of fit (Line 11) was not significant, which indicates that the data is well described by the selected model.

The ED command, line 07, calculates the user's choice of ED values. In this example, the ED50 (the parameter e:1, e:2, and e:3 in the output from *summary*) values were not considered because glyphosate was intended to control weeds, thus ED90 was of greater interest. In contrast, in some other types of studies (ecotoxicology, crop tolerance), the ED50, ED30, or ED10 might be of interest.

Line 08 provides estimated relative potencies between the curves (using the *SI* function), which compares ED values of interest among curves in order to detect significant differences among the curves. The line 09 illustrates the *plot* command for displaying the visual of fitted dose-response curves (Figure 2).

Example 2: Determining the ED90 Value with Data Sets that Lack Data Points Along the Lower Limit of the Curve. Often experimental data do not cover a sufficiently wide range of doses to provide information about the lower and/or upper limit of the curve (Figure 1). That is especially true for determining efficacy of new herbicides or determining the level of weed resistance. It can be highly misleading in those cases to fit a dose-response model where the parameters of the lower limits are also being estimated. For example, lack of data points around the lower limit of the curve can be the reason for having negative c values (the lower limit of the log-logistic curve with four parameters, equation 1).

In this example (Table 2), the four-parameter log-logistic model (equation 1) was fit to the data set with the function *fct*

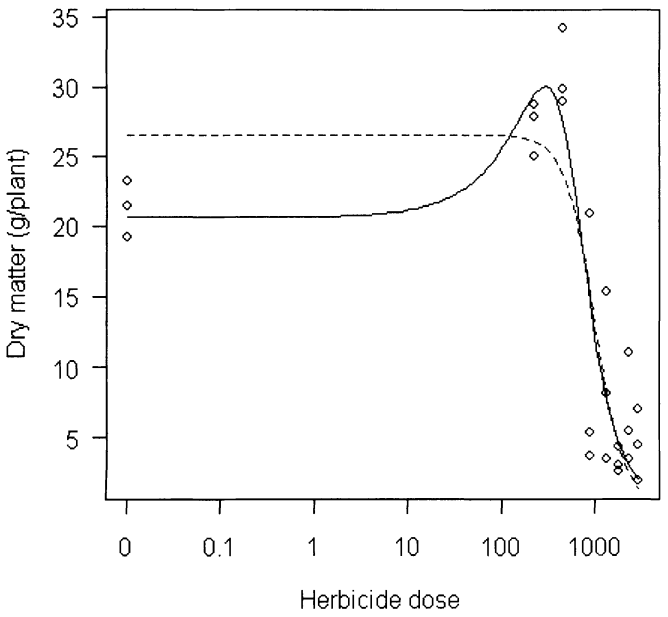

Figure 4. Fitted dose-response curves of Brain-Cousens model (solid line) and the three-parameter log-logistic model (dashed line) displayed together with the same dataset exhibiting initial growth stimulation. The log-logistic curve overestimates the upper limit. Commands and equation parameters are shown in Table 3.

l4 (line 01), whereas line 02 produced a plot of the fitted curve (Figure 3, solid line). Commands in line 4 plot the fitted curve together on the same figure resulting in the three-parameter model (dashed line) being more closely related to the data (Figure 3). Most importantly, the resulting ED90 value suggested that the dose needed to obtain 90% control of ivy leaf morningglory was 2,472 g ai/ha, which is biologically acceptable, and it is meaningful to practitioners. Therefore, we recommend to use the three-parameter log-logistic model (equation 2) and three-parameter Weilbull model (equation 4) because they have the advantage of leading to reasonable ED90 values for data sets that lack data points along the lower limit of the curve (Table 2). Similar response was observed in over 20 other data sets that exhibited lack of data along the lower limit of the curve (unpublished data) from a study in glyphosate-resistant soybeans (Knezevic and Klein 2005).

Example 3: Describing the Hormesis-Type Response. It is not unusual that an initial growth stimulation is observed in many plant species when exposed to a low rate of a herbicide, a phenomenon that is called hormesis (Cedergreen et al. 2005). Several models were published for describing the hormesis dose-response data (Cedergreen et al. 2005).

In this example (Table 3), we illustrated fitting the Brain-Cousens model (equation 5) utilizing function *bcl3* (line 1), to describe the relationship between glyphosate dose and DM of sweet clover (Knezevic, unpublished data). Other lines in that table are already described in Table 1.

The summary of pertinent statistics for the three-parameter log-logistic model (equation 2) and Brain-Cousens model (equation 5) is provided in output section (Table 3). There was the significant lack of fit (P = 0.0149), suggesting that three-parameter model was not appropriate for the data (Table 3). In addition, parameter d (upper limit of the curve) was significantly larger than in the Brain-Cousens model as shown in Figure 4 (dashed line). This can also imply that the effective doses associated with the upper limit of the curve (e.g., ED10, ED30, or ED50) can be overestimated. Similar issues were observed with parameter e (ED50), which was a two-fold larger value than in the more realistic Brain-Cousens model (Table 3).

The lack-of-fit test for Brain-Cousen's model was not significant (P = 0.12), suggesting that this model provided an acceptable fit to the hormesis data (Figure 4, solid line). The P value for testing the hormesis parameter f was 0.03 (Table 3), indicating that there was a significant effect of hormesis. Similarly, others have concluded that the Brain-Cousens model provided the best description of hormesis dose-response data when compared to several other models (Cedergreen et al. 2005).

Software Value to Users

A novel approach for determining the ED values of interest, and for fitting dose-response curves was illustrated using the package *drc* in the statistical program **R**. The additional value of this data analysis approach is that it can be easily adopted for other types of studies that are based on nonlinear regression modelling. Effects of herbicides and other control measures on crops and weeds, seed germination in response to time or chemical stimuli, and weed competition in crops (including plant growth curves), are just a few of the biological responses that can be described with nonlinear regression curves. Furthermore, procedures described in this paper can help in determining dose-response curves for weed control, not only with herbicides, but also with other weed control methods, including various no-chemical weed control options (e.g., flaming) (Ascard, 1994, 1995).

The concepts presented within this paper can provide general guidelines for conducting dose-response studies to practitioners and academics. Practitioners (e.g., university extension specialists, pesticide industry scientists, etc.) can use presented guidelines for fitting and comparing dose-response curves as part of their decision-making process for weed management. For example, knowing the effective dose (e.g., ED90) for a weed species of interests is critical in making plans for weed control options, which can result in less intensive management options and economic savings.

Academics and teachers can use the information presented here as a potential teaching tool for the dose-response concept in the classroom. Effective teaching can be achieved with addition of a simple greenhouse or growth chamber study during the class laboratory periods. For example, students can grow a few plant species, collect data, fit dose-response curves, and write a report describing their dose-response study. Such a laboratory exercise would also provide a practical and visual tool for comparing herbicide activity among plant species as part of an effective learning process.

We believe that **R**, with the add-on *drc* package, can be useful to many users because of the size of the package, ability

to relatively simply and quickly compare multiple curves, select ED-levels easily along the curve, and obtain relevant statistics. The software is free, has no licensing fees and the open source nature of the software can promote scientific collaboration.

Finally, the **R** and *drc* package can be easily accessed to provide benefits to many weed science colleagues worldwide. Many previously published procedures for data analysis (Knezevic et al. 2002; Seefeldt 1995) might not have been very useful to colleagues in parts of the world where commercial statistical packages are not readily available. **R** and *drc* can be downloaded at no cost anywhere with Internet access. The hope is also that the methods and software described in this manuscript can help achieve a small improvement in statistical usage as previously advocated by Cousens (1988, 1991).

Sources of Materials

[1] SAS®, Statistical Analysis Systems, Inc., Box 8000, Cary, NC 27511–8000.

[2] Sigma Plot, S-PLUS®, Analytical Software, Insightful Corp., Seattle, WA 98101.

[3] Axum®, Technical Graphics and Data Analysis, MathSoft Engineering and Education, Inc., 101 Main St. Cambridge, MA 02142–1521.

[4] FIG-P®, The scientific figure processor, Durham, NC.

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