Weed Science 2011 59:601–610

Nonlinear Regression Analysis of Herbicide Absorption Studies

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Although foliar herbicide absorption has been studied intensively, there is currently no standardized method for data analysis when evaluating herbicide absorption over time. Most peer-reviewed journals require the treatment structure of data be incorporated in the analysis; however, many herbicide absorption studies published in the past 5 yr do not account for the time structure of the experiment. Herbicide absorption studies have been presented in a variety of ways, making it difficult to compare results among studies. The objective of this article is to propose possible nonlinear models to analyze herbicide absorption data and to provide a stepwise framework so that researchers may standardize the analysis method in this important research area. Asymptotic regression and rectangular hyperbolic models with similar parameterizations are proposed, so that the maximum herbicide absorption and absorption rate may be adequately modeled and statistically compared among treatments. Adoption of these models for herbicide absorption analysis over time will provide a standardized method making comparison of results within and among studies more practical.

Key words: Asymptotic regression, exponential rise to maximum, rectangular hyperbola, nonlinear regression.

Foliar herbicide absorption has been intensively studied for many years. The weed science literature is filled with accounts of various combinations of herbicides and plants, often evaluating adjuvants or attempting to determine the mechanism of herbicide resistance or selectivity. As critical as this area of research is to our discipline, there is currently no standardized way of analyzing and presenting data sets that evaluate foliar herbicide absorption over time.

A recent, nonexhaustive literature search for the terms "herbicide" and "absorption" for the period 2006 to 2010 returned 27 peer-reviewed research articles where herbicide absorption was quantified over time (Table 1). Of this sample, 15 articles (56%) did not address the time structure of the analyzed data and did not model absorption over time using regression techniques. Instead, those 15 articles used tabular presentation of absorption means along with standard errors or LSD tests. One of the sampled articles plotted a regression line but provided no details about the model (or its parameters) used. Nine of the articles (33%) performed some type of linear or nonlinear regression analysis but did not interpret regression parameters in any meaningful way, other than to provide the equation, along with plotted data. Only 2 of the 27 sampled articles (7%) conducted regression analysis on the structured data set ***and*** used the model parameters to help explain herbicide absorption (Burke et al. 2007; Grangeot et al. 2006). If the other 25 manuscripts had analyzed the data similarly, then comparisons among them would have been possible (similar herbicides, plant species, etc).

The purpose of many foliar herbicide absorption studies is to examine herbicide absorption over time. These studies typically rely on destructive sampling of treated plants at various time points after application, requiring independent experimental units for each time point. The time points used in the study are typically of little inherent value, but rather serve to characterize the overall absorption pattern; however, it is common practice for researchers to ignore the time structure of their data in the statistical analysis and make comparisons among treatments at each time point. When treatment effects are analyzed separately by time point, it is common to observe experimental artifacts that are unlikely to represent the true absorption pattern. Additionally, when the time structure is not used in the analysis, valuable information may be ignored (Cousens 1988). In fact, the instructions to authors for most peer-reviewed journals (including *Weed Science* and *Weed Technology*) prohibit the use of LSD tests or other multiple comparison procedures for data that have a quantitative treatment structure. Even so, this approach has been used in most recently published, herbicide absorption studies (Table 1). Using regression analysis to describe herbicide absorption over time allows the researcher to estimate absorption at a particular time of interest, rather than relying solely on data (and associated experimental error) from a single time point.

Our objective was to use previously generated foliar herbicide-absorption data to find an appropriate model to standardize the analysis and presentation of these data in the weed science literature. Most foliar absorption studies have shown rapid, initial absorption, followed by a plateau, or maximum asymptote (including nearly all 27 articles reviewed in Table 1). Therefore, a desirable model used to describe these data over time should have, at minimum, four attributes: (1) it should be continuously increasing, (2) it should be asymptotic in nature to adequately describe the absorption plateau, (3) it should describe the rate of herbicide absorption, and (4) its model parameters should provide relevant, interpretable information to the researcher and readers.

Potential Models to Describe Foliar Herbicide Absorption

Asymptotic regression (AR) functions (also known as exponential rise to maximum functions) have been used previously to describe foliar herbicide absorption by Bukun et al. (2009, 2010), Dodds et al. (2007), and Grangeot et al. (2006). The form used by Bukun et al. (2009, 2010) is a two-parameter function:

$$Absorption = A_{max} \times [1 - \exp(-b - t)] \quad [1]$$

where absorption is expressed as a percentage of the applied dose, A_{max} is the upper limit or the maximum percentage of

DOI: 10.1614/WS-D-11-00034.1

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Table 1. Sample of herbicide-absorption studies, published between 2006 and 2010, and the models used to describe foliar herbicide absorption over time.

Reference	Plant species[a]	Time frame	Herbicide(s)	Model
Belles et al. 2006	Velvetleaf	0–72 h	Glyphosate	Data are presented with plots and curves, but the model is not specified and no parameters are provided
Bukun et al. 2009	Canada thistle	0–192 h	Aminopyralid, clopyralid	$Absorption = A \times [1 - \exp(-bt)]$ Asymptotic regression
Bukun et al. 2010	Canada thistle	1–192 h	Aminocyclopyrachlor	$Absorption = A \times [1 - \exp(-bt)]$ Asymptotic regression
Burke et al. 2007	Browntop millet, palmer amaranth	0–168 h	Glyphosate	$Absorption = A \times e^{-\exp[-(t-k)/b]}$ Gompertz
Dodds et al. 2007	Barnyardgrass	0–96 h	Bispyribac	$Absorption = A \times \exp(bt) - c \times \exp(dt)$ Double exponential rise to maximum
Everman et al. 2009a	Corn, goosegrass, large crabgrass, sicklepod	1–72 h	Glufosinate	No regression performed, means presented in tabular form and separated with Fisher's Protected LSD test results (five time points)
Everman et al. 2009b	Cotton, palmer amaranth, pitted morningglory	1–72 h	Glufosinate	No regression performed, means presented in tabular form and separated with Fisher's Protected LSD test (five time points)
Frihauf et al. 2010	Wheat	1–14 d	Saflufenacil	$Absorption = A \times t/(b + t)$ Two-parameter rectangular hyperbola
Grangeot et al. 2006	Common ragweed	2–48 h	Glufosinate, glyphosate	$Absorption = A \times \{1 - \exp[\log 0.5 \times (t/e)]\}$ Asymptotic regression
Han et al. 2009	Oilseed rape	2–100 h	Zj0273	No regression performed, means plus standard errors plotted (seven time points)
Hatterman-Valenti et al. 2006	Giant foxtail	12–48 h	Fluazifop-*p*	No regression performed, means plus LSD presented in tabular form, averaged over three different time points
Hennigh and Al-Khatib 2010	Barnyardgrass	1–7 d	Nicosulfuron, rimsulfuron, nicosulfuron + rimsulfuron	No regression performed, means plus standard error presented in tabular form (three time points)
Henry et al. 2008	Dallisgrass	0–48 h	Foramsulfuron	$Absorption = A \times \exp(b) + c \times \exp(d) \times \ln[t - \exp(f)]$
Hutchinson et al. 2010	Old world climbing fern	one time point at 9 d	Metsulfuron, triclopyr, glyphosate	No regression performed, only a single time point analyzed
Joy et al. 2009	Grain sorghum	1–7 d	Mesotrione	No regression performed, means plus standard error presented in tabular form (three time points)
Lovelace et al. 2007	Barnyardgrass	0–72 h	Quinclorac	No regression performed, means plus LSD plotted (seven time points)
Lycan and Hart 2006	Creeping bentgrass, annual bluegrass, Kentucky bluegrass, roughstalk bluegrass	0–72 h	Bispyribac	$Absorption = m + bt + ct^2$ Second-order polynomial
Matocha et al. 2006	Palmer amaranth, texasweed	0–72 h	Trifloxysulfuron	$Absorption = A \times \ln(t) + b$ Two-parameter logarithmic
Nandula et al. 2008	Italian ryegrass	24–48 h	Glyphosate	No regression performed, means presented in tabular form and separated with Fisher's Protected LSD test (two time points)
Schuster et al. 2007	Green foxtail, yellow foxtail	1–7 d	Nicosulfuron, rimsulfuron	No regression performed, means plus standard error presented in tabular form (three time points)
Singh and Singh 2008	Redroot pigweed, guineagrass	0–72 h	Glyphosate	No regression performed, means presented in tabular form and separated with Fisher's Protected LSD test (nine time points)
Steele et al. 2008	Sharppod morningglory	0.5–72 h	Glyphosate	No regression performed, means presented in tabular form and separated with Fisher's Protected LSD test (two time points)
Thomas et al. 2007	Cotton	1–7 d	Glyphosate	$Absorption = mt + b$ Linear regression
Troxer et al. 2007	Tobacco	0–72 h	Trifloxysulfuron	$Absorption = A \times \ln(t) + b$ Two-parameter logarithmic
Walker and Oliver 2008	Flowering sicklepod	0–96 h	Glyphosate	No regression performed, means plus LSD presented in tabular form (three time points)
Weinberg et al. 2007	White bean	24–168 h	Tritosulfuron	No regression performed, means plus standard error presented in tabular form (three time points)
Willingham et al. 2008	Alligatorweed	1–48 h	Penoxsulam	No regression performed, means presented in tabular form and separated with Fisher's Protected LSD test (four time points)

[a] Nomenclature for plants not mentioned in text: Velvetleaf, *Abutilon theophrasti* Medik.; browntop millet, *Urochloa ramosa* (L.) Mguyen; palmer amaranth, *Amaranthus palmeri* S. Wats.; barnyardgrass, *Echinochloa crus-galli* (L.) Beauv.; corn, *Zea mays* L.; goosegrass, *Eleusine indica*, (L.) Gaertn.; large crabgrass, *Digitaria sanguinalis* (L.) Scop.; sicklepod, *Senna obtusifolia* (L.) H.S. Irwin & Barneby; cotton, *Gossypium hirsutum* L.; pitted morningglory, *Ipomoea lacunosa* L.; oilseed rape, *Brassica napus* L.; giant foxtail, *Setaria faberi* Herm.; dallisgrass, *Paspalum dilatatum* Poir.; old world climbing fern, *Lygodium microphyllum* (Cav.) R. Br.; grain sorghum, *Sorghum bicolor* (L.) *Moench* ssp. *bicolor*; creeping bentgrass, *Agrostis stolonifera* L.; annual bluegrass, *Poa annua* L.; Kentucky bluegrass, *Poa pratensis* L.; roughstalk bluegrass, *Poa trivialis* L.; texasweed, *Caperonia palustris* (L.) St. Hil.; Italian ryegrass, *Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot; green foxtail, *Setaria viridis* (L.) Beauv; yellow foxtail, *Setaria pumila* (Poir.) Roemer & J.A. Schulte; redroot pigweed, *Amaranthus retroflexus* L.; guineagrass, *Urochloa maxima* (Jacq.) R. Webster; Sharppod morningglory, *Ipomoea cordatotriloba* Dennst.; tobacco, *Nicotiana tabacum* L.; flowering sicklepod, *Senna obtusifolia* (L.) H.S. Irwin & Barneby; white bean, *Phaseolus vulgaris* L.

https://doi.org/10.1614/WS-D-11-00034.1

applied herbicide dose that will be absorbed at large values of t, b is a parameter describing the relative slope of the line as t approaches 0 or the rate at which the herbicide is absorbed following application, and t is time after application. This AR model possesses all four of the desirable attributes described previously. A different parameterization of Equation 1 may take the form:

$$Absorption = A_{max} \times [1 - \exp(-t/e)] \quad [2]$$

where b from Equation 1 is expressed as $1/e$, and all other parameters retain the same interpretation as in Equation 1. Because the e parameter in Equation 2 is the reciprocal of b in Equation 1, both parameters describe the rate with which herbicide absorption reaches the maximum; however, in Equation 1, b has a direct relationship to the rate at which the herbicide is absorbed, whereas the e parameter in Equation 2 has an inverse relationship with the absorption rate. The e parameter in Equation 2 can be interpreted as the time (t) required to reach 63% of the maximum absorption, or $A_{max} \times 0.632$. A large value of e in Equation 2 indicates greater time to reach the maximum absorption level, i.e., slower absorption.

Grangeot et al. (2006) used a modified form of Equation 2 to describe the absorption of two herbicides by common ragweed (*Ambrosia artemisiifolia* L.):

$$Absorption = A_{max} \times \{1 - \exp[(\log 0.5) \times (t/e)]\} \quad [3]$$

where absorption, A_{max}, and t retain the same interpretation as Equation 1; and the e parameter describes the time required to reach 50% of the maximum absorption level (or $A_{max}/2$). This change in the e parameter interpretation will be more intuitive to many researchers compared with the e parameter as defined in Equation 2. The e parameter in Equation 3 is interpreted as the time required for 50% of maximum absorption to occur, which makes it analogous to the LD_{50} or to the herbicide rate required to reduce mean growth of individuals to 50% of the untreated control (GR_{50}), which are common statistics used in many dose response studies. Although describing the rate of absorption in this way may be appealing from an applied perspective, the levels of absorption described by Equation 2 (63% of maximum) and Equation 3 (50% of maximum) are not necessarily valuable to describe an herbicide's absorption pattern. Instead, it may be more informative to estimate the time required to achieve 90% of the maximum absorption (t_{90}) because that will provide a better estimate of the time required for maximum absorption to be nearly complete. That absorption level would be valuable for comparing herbicide formulations or adjuvants for rain fastness or differences between weed and crop species. Although it is possible to estimate the t_{90} value from Equations 1 through 3, it would require less computation by the researcher if the t_{90} value were a parameter in the model itself. The following model is similar to Equation 3, but with t_{90} as a model parameter:

$$Absorption = A_{max} \times \{1 - \exp[(\log 0.1) \times (t/t_{90})]\} \quad [4]$$

where *Absorption* is expressed as the percentage of the applied dose, A_{max} is the maximum absorption at large values of t, t is time, and t_{90} is the time required for 90% of maximum absorption to occur (Figure 1). The A_{max} and t_{90} parameters in Equation 4 have meaningful interpretations in the context of foliar herbicide absorption, and therefore, Equation 4 is a simple but descriptive method for interpreting foliar absorption data. With this model, treatment comparisons are relatively simple because many statistical programs provide a method to conduct statistical tests on model parameters among treatments.

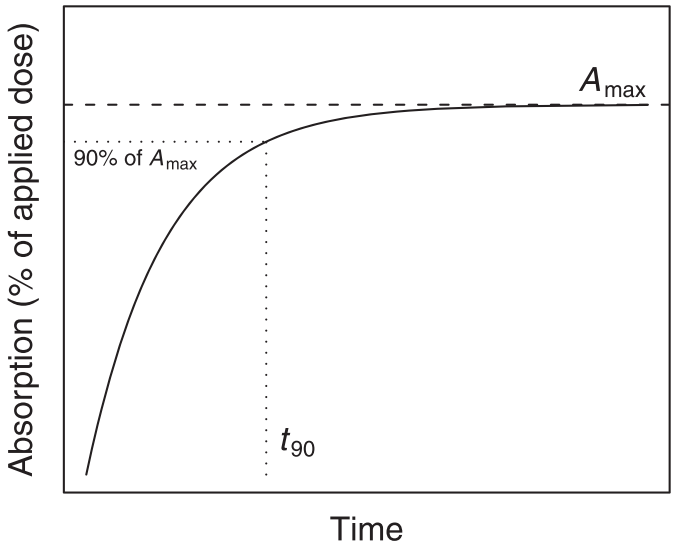

Figure 1. Graphical representation of parameter interpretations for Equation 4 and Equation 9.

Although the t_{90} value will be appropriate to measure the rate of herbicide absorption in most cases, Equation 4 may further be generalized if the researcher prefers to use a different absorption level (t_{33}, for example). If θ describes an arbitrary percentage of A_{max}, then the following equation may be used to incorporate t_θ (or the time required to reach θ absorption) as a model parameter:

$$Absorption = A_{max} \times \langle 1 - \exp\{\log[(100-\theta)/100] \times (t/t_\theta)\} \rangle \quad [5]$$

Rectangular hyperbolic (RHB) models also meet all four of the desirable attributes described previously. At least one form of the RHB model is familiar to many weed scientists because it is commonly used to describe the relationship between weed density and crop yield (Cousens 1985). In the context of foliar herbicide absorption, the model may be expressed as the following:

$$Absorption = (b \times t)/\{[1 + (b \times t)]/A_{max}\} \quad [6]$$

where absorption, A_{max}, b, and t all retain the same interpretation as in Equation 1. A second parameterization of the RHB model is the Michaelis-Menten model, which is commonly used to describe enzyme kinetics. This parameterization was previously used by Frihauf et al. (2010) to describe herbicide absorption over time:

$$Asorption = (A_{max} \times t)/(e + t) \quad [7]$$

where absorption, A_{max}, and t retain the same interpretation as in Equation 1, and e indicates the time required for absorption to reach $A_{max}/2$ or half of the maximum absorption level. The e parameter in Equation 7 has the same interpretation as the e parameter in Equation 3. Like the AR equation, the RHB model may be modified so that the e parameter indicates the time required to absorb an arbitrary

https://doi.org/10.1614/WS-D-11-00034.1

Table 2. Bias-corrected Akaike information criterion (AIC*c*), adjusted R^2, and evidence ratios for foliar herbicide-absorption data sets fit to asymptotic regression (AR) and rectangular hyperbolic (RHB) nonlinear-regression models.

	AIC*c*		R^2_{adj}			
Data set	AR	RHB	AR	RHB	Best model	Evidence ratio
Bukun et al. 2009	401.7	401.3	0.730	0.732	RHB	1.22
Bukun et al. 2010	409.6	409.6	0.664	0.664	—	1.00
Kniss 2006	256.0	267.9	0.923	0.897	AR	381
Kniss et al. 2011	1,153.7	1143.4	0.932	0.936	RHB	172
Everman et al. 2009a	160.1	159.8	0.975	0.975	RHB	1.14
Everman et al. 2009b	177.4	174.6	0.968	0.971	RHB	4.22
Frihauf et al. 2010	76.6	57.0	0.988	0.997	RHB	17,520
Hennigh and Al-Khatib 2010	148.3	107.0	0.994	0.999	RHB	8.8×10^8
Schuster et al. 2007	326.4	329.5	0.963	0.954	AR	4.68
Weinberg et al. 2007	203.2	187.6	0.986	0.993	RHB	2,380

percentage of A_{max}. An RHB model that is analogous to Equation 5 is expressed as follows:

$$Absorption = (A_{max} \times t)/[(10/\theta) \times t_0 + t] \quad [8]$$

where all parameters retain the same interpretation as in Equation 5. Therefore, if the t_{90} value is of interest to the researcher ($\theta = 90$), then Equation 8 becomes the following:

$$Absorption = (A_{max} \times t)/(0.11 \times t_{90} + t) \quad [9]$$

Equations 1 though 9 all describe the maximum level of foliar herbicide absorption in terms of the A_{max} parameter, and thus, maximum uptake can be statistically compared among treatments with a likelihood ratio test of the A_{max} parameter among curves, regardless of the model. Equations 1 and 6 describe the rate at which the herbicide is absorbed in terms of the parameter *b*, which has a direct relationship to the herbicide absorption rate. Conversely, Equations 2, 3, and 7 describe the absorption rate in terms of the parameter *e*, or the time required to reach some percentage of A_{max}. Equations 5 and 8 have the model parameter for determining absorption at any arbitrary time (such as t_{33} or t_{80}); whereas Equations 4 and 9 (the AR and RHB model, respectively) use t_{90} as a model parameter (Figure 1). In many cases, the t_{90} value will provide a useful and descriptive parameter to compare the rates of herbicide absorption, and therefore, Equations 4 and 9 have been used in the following sections. However, individual researchers may easily choose a different level of absorption by using the more general Equations 5 and 8.

Comparison of Models for Herbicide Absorption

Model Comparison Procedures. To determine which model best describes foliar herbicide absorption, 10 herbicide-absorption data sets were analyzed using the AR model (Equation 4) and the RHB model (Equation 9). Raw data were used for four of the data sets (Bukun et al. 2009, 2010; Kniss 2006; Kniss et al. 2011). For the remaining 6 data sets, treatment means reported in the published articles were used (Everman et al. 2009a,b; Frihauf et al. 2010; Hennigh and Al-Khatib 2010; Schuster et al. 2007; Weinberg et al. 2007). For each data set, the A_{max} parameter was constrained to a maximum value of 100 (indicating 100% absorption of the applied dose), and the t_{90} parameter was constrained to positive values. Data were analyzed using the drc package in R (R Development Core Team 2009; Ritz and Streibig 2005).[1]

The bias-corrected Akaike information criterion corrected for small sample size (AIC*c*) were then calculated for both models for all data sets (Ritz and Spiess 2008; Spiess and Neumeyer 2010). The AIC*c* is similar to the more common AIC, but accounts for sample size; for large sample sizes, the AIC*c* will be equal to the AIC. Smaller AIC*c* values indicate the model that better describes the data set. For the model with the smallest AIC*c* for each data set, the relative likelihood of that model being correct was compared with the second model and is presented as a weight of evidence ratio (Spiess and Neumeyer 2010). The evidence ratio uses Akaike weights to describe how much more likely one model is to be a better fit to these data compared a second model; for example, an evidence ratio of 1 means the two models provide an equally good fit to these

Table 3. Model parameters (with standard errors) for asymptotic regression (Equation 4) and rectangular hyperbolic (Equation 9) models fit to four foliar herbicide-absorption data sets.

		Asymptotic regression		Rectangular hyperbola		
Data source	Treatment	A_{max}	t_{90}	A_{max}	t_{90}	Observed maximum[a]
		No. (SE)				
Bukun et al. 2009	Clopyralid	78 (3.0)	21 (7.8)	79 (4.5)	15 (23.0)	89
	Aminopyralid	57 (4.5)	70 (19.3)	66 (7.3)	218 (91.9)	91
Bukun et al. 2010	DPX–KJM44	57 (2.8)	4.3 (87.3)	57 (4.9)	0.1 (33.9)	86
	DPX–MAT28	84 (2.8)	4.3 (89.5)	84 (4.8)	0.1 (22.0)	98
Kniss 2006	Susceptible	73 (3.5)	44 (5.6)	96 (8.1)	203 (42.9)	70
	Tolerant	69 (3.4)	43 (5.5)	91 (7.8)	197 (42.3)	67
Kniss et al. 2010	NIS + N	79 (3.0)	2.5 (0.66)	85 (4.1)	4.9 (2.07)	90
	NIS + N + MCPA	87 (2.7)	1.9 (0.56)	90 (3.8)	2.4 (1.56)	94
	MSO	90 (2.2)	0.32 (27.0)	91 (3.6)	0.23 (1.26)	97
	MCPA	58 (4.7)	7.7 (2.08)	65 (6.1)	22.8 (7.73)	76
	MSO + MCPA	90 (2.8)	2.0 (0.52)	93 (3.8)	2.7 (1.52)	95

[a] Observed maximum indicates the maximum value obtained by any single observation in the study.

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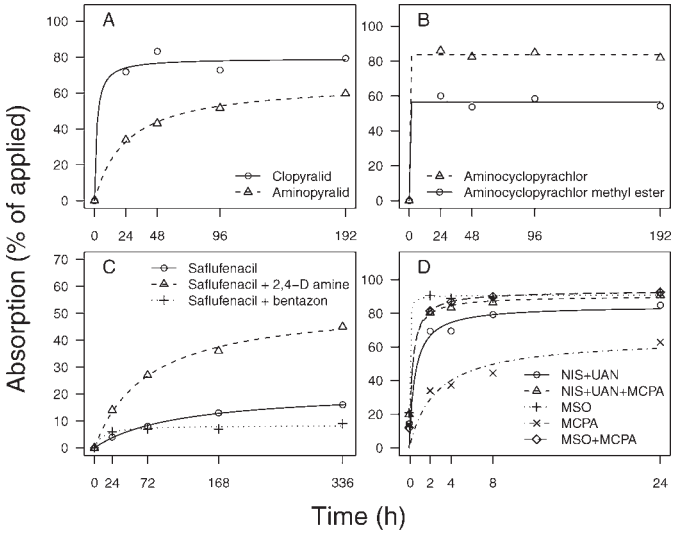

Figure 2. Previously published herbicide-absorption data reanalyzed with the rectangular hyperbola model (Equation 9). (A) Aminopyralid and clopyralid absorption in Canada thistle (from Bukun et al. 2009). (B) Absorption of two formulations of aminocyclopyrachlor in Canada thistle (from Bukun et al. 2010). (C) Saflufenacil absorption in winter wheat as influenced by two tank-mix herbicides (from Frihauf et al. 2010). (D) Imazamox absorption in feral rye as influenced by MCPA-ester and adjuvants (from Kniss et al. 2011).

experimental data, and an evidence ratio of 2 would indicate that the first model is twice as likely to be the correct model.

Model Comparison Results. For 5 of 10 data sets, the AIC*c* values were similar, with evidence ratios (or the weight of evidence for one model over the other) less than 5 (Table 2). There appears to be no strong evidence that one model was superior compared with the other for these five data sets. Burnham and Anderson (1998) suggest that a difference in AIC less than 10 essentially means that models provide similar fits to a particular data set. At this cutoff value, there was sufficient evidence to choose one model over the other for the remaining five data sets. Of these five data sets, the RHB model was selected as the better model in four cases (Frihauf et al. 2010; Hennigh and Al-Khatib 2010; Kniss et al. 2011; Weinberg et al. 2007), whereas the AR model was selected in one case (Kniss 2006). Based on these results, there is not sufficient evidence that one of these models provides a better description of foliar herbicide absorption in general. This may be due to many factors, including the multitude of herbicides, adjuvant systems, plant species, humidity, and temperatures involved in these experiments. It is possible that, for some species and herbicide combinations, the underlying absorption pattern is best described by an AR model, whereas for others the RHB model is more appropriate.

In each case, the adjusted coefficient of determination $\left(R^2_{adj}\right)$ values were greater for the model with the lowest AIC*c*; however, in many cases the R^2_{adj} values were the same to two decimal places, even when there was substantial evidence for one model based on the AIC*c* (Table 2). These results are similar to previous reports that, even when the AIC*c* indicated evidence in favor of one model by 16 orders of magnitude, the R^2_{adj} only differed in the third to fifth decimal place (Spiess and Neumeyer 2010). For that reason, researchers should be strongly discouraged from using R^2 values for model comparisons or for assessing model fit in herbicide-absorption studies.

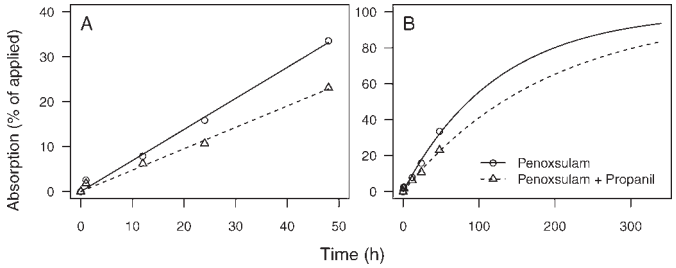

Figure 3. Penoxsulam absorption in alligatorweed when applied alone or with propanil (as reported by Willingham et al. 2008) reanalyzed with (A) linear regression and (B) asymptotic regression (Equation 4).

When these models were fit to raw data sets, standard errors associated with the RHB model parameter estimates were consistently greater than those associated with the AR model (Table 3). The RHB model also provided estimates of A_{max} that were always greater than or equal to the AR model. Even though the estimates of A_{max} were consistently higher for the RHB model, the model with the lowest AIC*c* provided A_{max} estimates that were more similar to the maximum absorption observed in these data sets. For example, the AR model was far more likely to be the better model for the Kniss (2006) data set compared with the RHB model based on AIC*c*, and the AR model provided A_{max} estimates of 69 to 73%, which were very similar to the maximum absorption observed in that study (67 to 70%); conversely, the RHB model provided A_{max} estimates of 91 to 96%, far greater than any absorption value that was observed in the study. Similarly, the RHB model provided a better description of the Kniss et al. (2011) data set, and the A_{max} estimates were more similar to the observed maximum absorption values for each treatment compared with the AR model.

Designing Herbicide Absorption Studies

Radiolabeled herbicides allow relatively simple quantification of absorption and translocation in plants and remain the most common tool to study herbicide behavior. Although this section focuses on use of radiolabeled herbicides, many of the same concepts (and specifically the analysis methods described in previous and subsequent sections) apply to other experimental methods as well. It is always best to select uniform plants of the same age. A common first step is to cover one or several of the youngest fully expanded leaves, and overspray the entire plant with the appropriate herbicide/surfactant combination. In rare cases, overspraying with nonradiolabeled herbicide may be undesirable. For example, subtle absorption or translocation differences between resistant and susceptible weed biotypes may be masked by a high-concentration overspray of a self-limiting herbicide, such as glyphosate (Shaner 2009). The choice to overspray or not should be made by the researcher based on the specific study objectives; however, absorption and translocation patterns observed without overspraying could yield artificial results.

This spray solution used for overspraying can be used to make the radiolabeled treatment solution, provided that the addition of the radiolabeled herbicide does not significantly change the herbicide concentration. This will depend largely on the compound's specific activity (kBq $mmol^{-1}$). Radio-

https://doi.org/10.1614/WS-D-11-00034.1

Table 4. Annotated R code to analyze foliar glyphosate absorption in two biotypes of common lambsquarters.[a,b]

Step 1: Load the required packages

```
Code:   # Load the drc library in order to fit dose response models
        library(drc)
        # Load the qpcR library to calculate AICc and adjusted R-squared
        library(qpcR)
```

Step 2: Create the functions for drm to use when fitting the AR and RHB models

```
Code:   # Create the asymptotic regression function for use by drm()
        # θ set by default to 90%
        KAR.fct<-function(t,parm,theta=90){
          parm[,1]*(1-exp((log((100-theta)/100)*t/parm[,2]))}
        ssKAR.fct<-function(data){Amax<-max(data[,1])
                                  t.theta<-0.2*max(data[,2])
                                  return(c(Amax,t.theta))}
        KAR.names<-c("Amax","t90")
        KAR.2<-list(KAR.fct,ssKAR.fct,KAR.names)

        # Create the rectangular hyperbola function for use by drm()
        # θ set by default to 90%
        KRH.fct<-function(t,parm,theta=90){
          (parm[,1]*t) / ((10/theta)*parm[,2]+t)}
        ssKRH.fct<-function(data){Amax<-max(data[,1])
                                  t.theta<-0.2*max(data[,2])
                                  return(c(Amax,t.theta))}
        KRH.names<-c("Amax","t90")
        KRH.2<-list(KRH.fct,ssKRH.fct,KRH.names)
```

Step 3: Load the data set from a .csv file

```
Code:   # Load the data set from a .csv file
        read.csv('cheal.csv',header=T)->cheal
```

Step 4: Fit a linear model, and the two non linear models to the data set

```
Code:   # Fit a linear model to the data
        lm(abs~time.h*trt,data=cheal)->cheal.lm

Code:   # Fit the asymptotic regression model to the data
        drm(abs~time.h,trt,data=cheal,fct=KAR.2,
            upperl=c(100,NA),lowerl=c(0,0))->cheal.kar
        # Print the model parameters for the AR model
        summary(cheal.kar)

Output: Parameter estimates:

                Estimate Std. Error t-value p-value
        Amax:1  72.5369     3.5025 20.7102       0
```

[a] Abbreviations: AR, asymptotic regression; RHB, rectangular hyperbolic; .csv, comma separated values; AIC*c*, bias-corrected Akaike information criterion.
[b] Abbreviations within coding are defined at the R Foundation for Statistical Computing (Wien, Austria) website: http://www.R-project.org.

labeled herbicide can be added to a few hundred microliters of spray solution. It is critical at this point to accurately determine the amount of radioactivity per unit volume in the treatment solution. All ensuing calculations will be based on the amount of radioactivity applied per plant.

When choosing time points for herbicide absorption studies, the most precise parameter estimates will be obtained when half of the time points occur near t_θ and the remaining half occur at the longest practical time after treatment (Currie 1982). Therefore, optimal time points will depend on many different factors, but researchers should strongly consider using at least six time points in addition to a 0-h time point. When resources are limited, it will often be better to reduce the number of replications and increase the number of time points.

Because of differences in the absorption patterns of various herbicides as influenced by plant species, adjuvant systems, and environmental conditions, there is no predetermined set of time points that can be recommended for use in all herbicide absorption studies. Under some experimental conditions, herbicides may reach A_{max} within as little as 2 h, whereas for others, it may take more than 300 h (Figure 2). Therefore, it is up to the researcher to conduct preliminary work to determine the optimal time points for a particular set of treatments. Previously published herbicide absorption studies are instructive in this regard (Figures 2 and 3).

Bukun et al. (2009, 2010) used time points of 0, 24, 48, 96, and 192 h after treatment to quantify absorption of clopyralid, aminopyralid, and aminocyclopyrachlor in Canada thistle [*Cirsium arvense* (L.) Scop.]. This series of time points

https://doi.org/10.1614/WS-D-11-00034.1

Table 4. Continued.

```
        Amax:2  69.0105     3.3839 20.3938       0
        t90:1   44.3147     5.5505  7.9839       0
        t90:2   43.0325     5.4821  7.8496       0

        Residual standard error:
         6.351897 (34 degrees of freedom)

Code:   # Fit the rectangular hyperbola model to the data
        drm(abs~time.h,trt,data=cheal,fct=KRH.2,
            upperl=c(100,NA),lowerl=c(0,0))->cheal.krh
        # Print the model parameters for the RHB model
        summary(cheal.krh)

Output: Parameter estimates:

               Estimate Std. Error  t-value p-value
        Amax:1  95.7556     8.1591  11.7361   0e+00
        Amax:2  90.8595     7.8785  11.5327   0e+00
        t90:1  202.5639    42.8718   4.7249   0e+00
        t90:2  196.6950    42.6690   4.6098   1e-04

        Residual standard error:
         7.427245 (34 degrees of freedom)
```

Step 5: Compare the model fit using AICc

```
Code:   # Calculate the AICc for all three models
        data.frame(AICc(cheal.kar),AICc(cheal.krh),AICc(cheal.lm))->AICc.table
        colnames(AICc.table)<-c("AR model","RHB model",
                                "Linear model")
        AICc.table

Output:   AR model RHB model Linear model
            255.993 267.8795     316.3654

Code:   # Calculate the Akaike weights evidence ratio
        evidence(cheal.kar,cheal.krh)

Output: 381.18
```

Step 6: Compare the maximum absorption and rate of absorption using the most appropriate model

```
Code:   # Compare the Amax parameter between biotypes
        compParm(cheal.kar,"Amax","-")

Output: Comparison of parameter 'Amax'

            Estimate Std. Error t-value p-value
        1-2  3.52639    4.87011 0.72409   0.474

Code:   # Compare the t90 parameters between biotypes
        compParm(cheal.kar,"t90","-")

Output: Comparison of parameter 't90'

            Estimate Std. Error t-value p-value
        1-2  1.28217    7.80140 0.16435  0.8704
```

appears appropriate for describing the absorption pattern of aminopyralid (Figure 2A). Based on the aminopyralid absorption curve, both the t_{90} and A_{max} parameters can be estimated reliably. However, the A_{max} parameter appears to have been reached before the 24-h time point for clopyralid (Figure 2A) as well as for both formulations of aminocyclopyrachlor (Figure 2B). In these cases, robust estimates of A_{max} can be obtained, but estimating the t_{90} parameter is much more difficult because there are no data points to describe the shape of the curve as it approaches A_{max}. This is particularly evident in Figure 2B, where the absorption plateau occurs before the first nonzero time point. Additional time points in the 0- to 24-h after-treatment range would be desirable to better define the absorption pattern of aminocyclopyrachlor in Canada thistle. Based on Figure 2B, it appears that time points taking place after 24 h after treatment were largely unnecessary.

https://doi.org/10.1614/WS-D-11-00034.1

Frihauf et al. (2010) used a similar range of time points as Bukun et al. (2009, 2010), but that range appears appropriate for studying saflufenacil absorption in winter wheat (*Triticum aestivum* L.)(Figure 2C). When saflufenacil was applied alone or in combination with 2,4-D amine, absorption continued to increase until the last time point of 336 h after treatment. The rectangular hyperbola model (Equation 9) estimated t_{90} values of greater than 600 h for those treatments. In contrast, when saflufenacil was applied with the rapidly acting photosystem II inhibitor bentazon, t_{90} was estimated to occur 98 h after treatment. The A_{max} parameter was 21.5, 53.5, and 8.4% of the applied dose for saflufenacil alone, saflufenacil plus 2,4-D amine, and saflufenacil plus bentazon, respectively. These estimates illustrate the effect that a rapidly acting herbicide, such as bentazon, can have on absorption of coapplied herbicides, as well as the safening effect of 2,4-D amine that Frihauf et al. (2010) reported. Saflufenacil applied alone to winter wheat exhibited self-limiting effects with respect to absorption, whereas the safening effect of 2,4-D allowed more saflufenacil to enter the plant. In this case, much of this effect would have been missed if the time points had ranged only from 0 to 24 h.

Kniss et al. (2011) used a much shorter range of time points than did Bukun et al. (2009, 2010) and Frihauf et al. (2010) to evaluate the effect of MCPA ester and spray additives on imazamox absorption in feral rye (*Secale cereale* L.) (Figure 2D). In this study, the shorter period was appropriate because the t_{90} values were estimated to be < 23 h for all treatments. A 0.5- or 1-h point would have even been desirable for this particular study because one treatment (methylated seed oil [MSO]) had reached t_{90} before the 2-h point; however, including very short time intervals can be problematic because it could take 0.5 h or longer just to make the initial applications if the experiment includes a large number of experimental units.

Willingham et al. (2008) also uses a relatively short period (up to 48 h after treatment) to compare penoxsulam absorption in alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.] as influenced by a propanil tank mixture. For the period analyzed, a linear regression appears to describe the data quite well (Figure 3A). Indeed, if the only research question were to determine whether propanil changed the absorption rate of penoxsulam, a linear regression analysis over this time would be adequate. Statistical comparison of slopes would most likely show a difference between the two treatments. However, because the absorption of penoxsulam never reached a plateau in either treatment, it is impossible to know whether maximum absorption was affected. If more points had been included in this study, similar to that of Frihauf et al. (2010), the full absorption pattern could have been described, and A_{max} could be estimated. The asymptotic regression model appears to describe the initial absorption of Willingham et al. (2008) rather well, but the lack of data at later times makes this model rather speculative (Figure 3B).

Determining herbicide absorption by assuming that any radioactivity not recovered in the leaf wash has been absorbed works in most cases; however, there are examples where that method could significantly overestimate herbicide absorption. Herbicide losses from volatility would not be accounted for unless absorption was verified by mass balance. Mass balance simply means accounting for all the sources of radioactivity (leaf wash, all plant parts, and root exudation) and comparing that to the amount of radioactivity applied at the start of the experiment. Determining a mass balance means that herbicide volatility would be detected when the absorption predicted by subtracting the leaf wash from the applied radioactivity is significantly different from the sum of radioactivity found in various plant parts. The mass balance for any experiment should account for at least 80% of the applied radioactivity to be considered a valid experiment. Recovery of less than 80% indicates potential issues with experimental techniques. It is important for researchers to report the total recovery from all sources as a percentage of the applied dose.

A Framework for Analysis of Herbicide Absorption over Time

A stepwise framework is provided to analyze herbicide absorption data in the statistical language R, using a study investigating two common lambsquarters (*Chenopodium album* L.) biotypes with differential susceptibility to glyphosate. The objective of the study was to determine whether differences in glyphosate susceptibility could be attributed to differential herbicide absorption among biotypes. Before proceeding with data analyses, two packages must be loaded. The drc package is used to conduct the nonlinear regressions (Ritz and Streibig 2005) and the qpcR package is used to calculate the AIC*c* for each model (Ritz and Spiess 2008). These packages are loaded with the "library()" function in R (Table 4; step 1).

Step 2 (Table 4) provides the R code necessary to create the AR (Equation 4) and RHB (Equation 9) functions so they may be used along with the "drm()" function in the drc package. These functions as written use a θ value of 90 (t_{90} as a model parameter) by setting "theta=90" in the first line of each function definition. If a different value of θ is deemed more appropriate for any reason, the value may be changed in this line of code. Step 3 simply loads data from a comma–separated value spreadsheet file into R. A linear model is then fit to these data using the "lm()" function (Step 4). This is an important step because there is evidence in the literature that a linear model may be sufficient to describe foliar absorption under some circumstances (Willingham et al. 2008). The AR and RHB models are then fit to these data using the "drm()" function, and model parameters and standard errors are printed with the "summary()" function. By default, the "summary()" of a drc object will provide the residual standard error for the model. The lower residual standard error of the AR model (6.35) is a first indication that it provides a better fit to this particular data set compared with the RHB model (7.43).

To further compare models, the AIC*c* for each model was calculated. Step 5 (Table 4) provides code for doing this in a manner that can be printed in an easily readable way—first, by creating a data frame with the AIC*c* for each model, then, by assigning column names to each, and finally, by printing the table. The output shows rather convincingly that both of the nonlinear models provide a better fit to these particular data compared with the linear model. As might be expected based on the residual standard error from the models, the AR model has the smallest AIC*c* value as well. The evidence ratio based on Akaike weights is 381, meaning the AR model is 381 times more likely to be the appropriate model compared with the RHB model. It is important to keep in mind that the AIC*c* and associated evidence ratio do not indicate that the AR model is the "best" model to describe these data; rather, it

https://doi.org/10.1614/WS-D-11-00034.1

provides information to select the best model among the candidate models considered. Additional models may be tested by the researcher in a similar manner.

Once the decision is made as to the most appropriate model, parameters can then be interpreted and tested statistically. The A_{max} parameters were estimated to be 72.5 and 69.0 for the susceptible and tolerant biotypes, respectively (Table 4, step 4). The two biotypes would be estimated to absorb a maximum of between 69 and 73% of the applied dose. The t_{90} parameters were estimated to be 44.3 and 44.0 for the susceptible and tolerant biotypes, respectively. This indicates that the time required for 90% of maximum absorption to occur would be approximately 44 h for both biotypes. A likelihood ratio test can be conducted with the ''compParm()'' function to compare parameters statistically among biotypes (step 6). The P values for both comparisons are not significant ($P = 0.47$ and $P = 0.87$ for the A_{max} and t_{90} parameters, respectively). Based on this analysis, the final conclusion of this study is that foliar absorption of glyphosate does not differ between the two biotypes, and thus, glyphosate absorption does not explain differences in whole-plant susceptibility.

Summary

There is a documented need to standardize the analysis of herbicide absorption studies that incorporate the time structure of the data sets. In this article, two potential nonlinear regression models have been proposed to fill this need. Because personal computers have become more powerful and statistical software programs have become more user friendly, nonlinear regression has become a familiar tool to many weed science researchers. Based on the analysis of 10 previously published data sets, it was demonstrated that one or both of these nonlinear regression models provides useful information for researchers and practitioners through directly interpretable model parameters. These models also allow for a statistical comparison of model parameters among treatments within an experiment. Finally, a stepwise framework for model fitting and data interpretation has been provided using a real foliar herbicide-absorption data set, and the R code has been provided. If this proposed framework for analysis of herbicide absorption studies becomes a standard practice, the body of literature surrounding herbicide absorption will be improved compared with the diversity of methods that are currently being used. Additionally, a standardized method of analysis allows for more-meaningful comparisons of studies conducted by different researchers or at different times (Seefeldt et al. 1995).

Source of Materials

[1] R statistical software, R Foundation for Statistical Computing, Wien, Austria.

Acknowledgments

We gratefully acknowledge Bekir Bukun and John Frihauf for providing data to be used in the model comparison portion of this article. We would also like to thank the associate editor and two anonymous reviewers for comments and suggestions that have substantially improved the utility of this article.

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Received March 11, 2011, and approved June 28, 2011.