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Special Topics

Estimation of Dose–Response Models for Discrete and Continuous Data in Weed Science

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Dose–response analysis is widely used in biological sciences and has application to a variety of risk assessment, bioassay, and calibration problems. In weed science, dose–response methodologies have typically relied on least squares estimation under the assumptions of normal, homoscedastic, and independent errors. Advances in computational abilities and available software, however, have given researchers more flexibility and choices for data analysis when these assumptions are not appropriate. This article will explore these techniques and demonstrate their use to provide researchers with an up-to-date set of tools necessary for analysis of dose–response problems. Demonstrations of the techniques are provided using a variety of data examples from weed science.
Key words: Alternative model estimation, bioassay, maximum likelihood, nonlinear models, treatment comparison.

El análisis de respuesta a dosis es ampliamente usado en las ciencias biológicas y tiene aplicación a una variedad de problemas de evaluación de riesgo, bioensayos y calibración. En la ciencia de malezas, las metodologías de respuesta a dosis han dependido típicamente de la estimación de los mínimos cuadrados bajo la premisa de que los error son normales, homocedásticos e independientes. Sin embargo, los avances en las habilidades computacionales y la disponibilidad de software han dado a los investigadores más flexibilidad y opciones para analizar datos cuando estas premisas no son apropiadas. Este artículo explora estas técnicas y demuestra su uso con la intención de brindar a los investigadores un grupo actualizado de herramientas necesarias para el análisis de problemas de respuesta a dosis. Se proveen demostraciones de las técnicas usando una variedad de ejemplos de datos para la ciencia de malezas.

The concept of dose–response in biology is an intuitive extension of physiological and biochemical processes. Increasing exposure of a biological system, either physically or temporally, to doses of an external factor can result in corresponding changes in the response of the system. Initial work on quantifying and interpreting dose–response data was carried out in the early to mid 20th century (see, e.g., Bliss 1934; Finney 1971; Fisher 1935; Reed and Berkson 1929); however, considerable advances in the statistical methods, computational ability, and available software have been made in recent years (see, e.g., Huet et al. 2004; Knezevic et al. 2007; Littell et al. 2006; Pinheiro and Bates 1995; Ritz 2010; Ritz and Streibig 2008; Ritz et al. 2006). These advances are designed to better address certain statistical issues that often arise when conducting dose–response analyses, such as the handling of discrete responses, nonconstant variance (heteroskedastic errors), and unexpected levels of variability (underdispersion and overdispersion). When present, these problems can lead to underestimation of model error terms and, hence, result in unreliable statistical tests and inference. This article will describe and illustrate dose–response analyses addressing these concerns using examples from weed science with applicable program codes.

Methodology

To lay the groundwork for the discussion that follows, a brief outline of the statistical models and assumptions is provided. The concept of curve-fitting is relatively straightforward; however, the process does require attention to specific statistical details and complexities. Although these will be addressed here, more in-depth explanations on nonlinear estimation can be found in references, such as Bates and Watts (2007), Ritz and Streibig (2008), or Seber and Wild (2003). Furthermore, for specific issues regarding dose–response models, experimental design and replication, or selection and range of doses, the reader is referred to Davidian and Giltinan (1998), Nielson et al. (2004), Ritz (2010), Seefeldt et al. (1995), and Streibig et al. (1993).

Model Form. The dose–response model can be specified as a nonlinear function given by:

$$E(y_{ij}) = f(dose_i, \theta) \quad [1]$$

where $E(y_{ij})$ is the expected value for the measured responses y_{ij} at the jth dose, $j = 1$ to k, and for the ith replicate, $i = 1$ to r. The model, $f(dose_i, \theta)$, is a nonlinear function of a vector of one or more unknown parameters θ, which relates the applied dose to the expected response. The dose–response model generally describes an increasing or decreasing pattern across an ordered sequence of dose values, often following an S-shaped or gradually changing, asymptotic trend (see, e.g., Ritz 2010; Shafii and Price 2001).

In classical dose–response analysis, the response is measured as the probability of an event, such as mortality and $f(dose_i, \theta)$ is assumed to follow a cumulative density function (CDF) referred to as the *tolerance distribution* (Finney 1971). A normal CDF (Probit analysis) or logistic CDF (logit analysis) may be used, for example, with symmetric data distributions, whereas a Gompertz CDF (Gompit analysis) might be used with asymmetric data. Although scaled CDF functional forms

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may occasionally be used in weed science, the responses are more likely to be measured as physical attributes, such as heights, weights, or counts. Although much of the classical dose–response methodology can be retained or adapted to these scenarios, the selection of a dose–response function should be based on its ability to reflect relevant aspects of the dose–response relationship.

To that end, the specified dose–response form should consider the shape and symmetry of the data, the parsimony (number of parameters) of the model, and, most important for a weed science application, a relevant biological interpretation of those parameters. Ritz (2010) has provided a general framework of four common dose–response model forms that meet these requirements. One example commonly used in weed science is the log-logistic form:

$$f\left[dose_j,\ \theta=(C,\ D,\ B,\ I_{50})\right]= C+(D-C)/\langle 1+\exp\left\{B\left[\log\left(dose_j\right)-\log(I_{50})\right]\right\}\rangle. \quad [2]$$

Here, parameters C and D scale the model to accommodate lower and upper asymptotes, representing the theoretical minimum and maximum response levels, respectively. The parameter B is a rate-related parameter, describing how rapidly the response changes within the range from C to D. Changes in the sign of B determine the direction of the sigmoidal curve, with negative values resulting in an increasing pattern and positive values describing a decreasing one. The parameter I_{50} measures the dose at which the response is midway between the asymptotes C and D. This is a common index obtained from dose–response analyses and is frequently referred to as the *median lethal dose* (LD_{50}) for mortality data, or *median effective dose* (ED_{50}) for toxicology data. Each of these parameters describes an important aspect of the dose–response relationship, and together, they provide a complete, biologically relevant picture of the dose–response process.

Another common model form is the Weibull-I function given by Equation 3:

$$f\left[dose_j,\ \theta=(C,\ D,\ B,\ I_{50})\right]= C+(D-C)e^{-\exp\langle B\left\{\log\left(dose_j\right)-\log(I)\right\}\rangle} \quad [3]$$

where parameters C, D, and B have similar interpretations as the log-logistic model, and parameter I is the inflection point of the curve. The Weibull model is useful in situations with asymmetric data and defines a response that declines or increases at a different rate than the log-logistic curve.

An important feature of these models is the use of a logarithmic function on the dose. Although a logarithmic transformation of dosage is often suggested for asymmetric data, its use will also ensure that the asymptotic behavior of the model conforms to the true positive range of doses. Rather than transforming the dose values before analysis, however, the logarithmic transformation is implicitly incorporated into the dose–response model. The transformation process often involves the addition of an arbitrary, small constant value to each dose to avoid problems with the logarithmic function where $dose = 0$. This can distort the resulting estimation depending on the value of the added constant and should be avoided (Ritz 2010). Although computational problems in the models can still occur at control doses, these situations can be conditionally circumvented through the estimation software (see Appendix 1a).

At times, it is of interest to estimate the dose at which a desired response level occurs. The log-logistic model, for example, naturally estimates the dose I_{50}, which gives a response at 50% of the distance between the upper and lower asymptotes D and C. Other percentages may be required, however, say, at 90% or 10%. These estimates can be found through algebraic manipulation of the dose–response function to solve for the desired dose as a function of the parameter estimates. Some software packages will provide options for producing those values and their standard errors or, alternatively, they may be programmed by the user. However, closed-form solutions may not exist for all model forms, such as the Brain-Cousens hormesis model (Brain and Cousens 1989) and that iterative solutions will be required in these cases. In addition, inferences for doses at the extremes of the model range, e.g., 1% or 99% of the potential response will be less reliable than intermediate values because of the shallow slope of the dose–response function near the asymptotes. Caution should be exercised in using estimates from these regions.

Although the models above can be successfully used in many dose–response problems, the data structure observed in some cases may not be adequately described by the S-shaped patterns of Equations 2 or 3. Responses that simply decline to a lower asymptote, for example, may be best described using an exponential model form, whereas data patterns showing an initial increase in response at low doses may better fit a hormesis model, such as that described by Brain and Cousens (1989). Although a different model form may be more appropriate in those cases, the selected model should still meet biological and theoretical expectations, have interpretable parameters, and be applicable to the data under consideration.

Model Estimation. A common approach for dose–response parameter estimations with continuous responses relies on the technique of iterative, nonlinear least squares (see, e.g., Bates and Watts 2007). The estimated model can be generally specified as in Equation 4:

$$y_{ij}=f\left(dose_j,\ \theta\right)+\varepsilon_{ij}, \quad [4]$$

where y_{ij}, $f(dose_j, \theta)$, and θ are as defined earlier, and the additive error term ε_{ij} is assumed to be distributed as $N(0, \sigma^2)$. As with any least squares algorithm, the goal is to minimize the sum of squared differences between the estimated model and the observed data. For the nonlinear model, this is accomplished by iteratively adjusting the parameter values in a directed manner until the sum of squared errors is reduced to a level below a preset minimum. Reaching that minimum is referred to as *convergence*. For the continuous response, this can be considered a reasonable approach given the response follows the stated conditions of normality.

However, the nature of dose–response data often makes it difficult or impossible to meet these conditions. These data, for example, may be expected to exhibit some variability at

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low to moderate doses but have low to no variability at high dose levels, thereby violating the condition of a constant variance σ^2. This condition of heteroskedastic errors can lead to biased estimates for model error terms and subsequently unreliable statistical inferences. Heteroskedasticity has typically been addressed through weighting or Box-Cox type data transformations with the goal of scaling the variance as a function of the mean response at each dose (Box and Cox 1964). This can be done through either a transform-both-sides (TBS) method (Seber and Wild 2003) or approximated in nonlinear regression by modeling the variance as a power function of the mean (Davidian and Giltinan 1998).

Although these methods can often effectively address the problem, they may overlook the underlying data structure causing the heteroskedastic response. One means of incorporating such structure is through a random-effects, mixed model where the parameter vector θ in Equation 4 becomes a function of fixed and random effects because of "subject" level variation within the data. Specifically, Equation 4 is now defined as (Pinheiro and Bates 1995) follows:

$$y_{ij} = f\left(dose_{ij},\ \theta\right) + \varepsilon_{ij}, \qquad \theta_{ij} = A_{ij}\theta + G_{ij}\phi_i, \qquad [5]$$

where θ_{ij} is a subject-level parameter vector made up of the fixed effects common across subjects, θ, and random effects because of subject variation ϕ_i. This later term is typically assumed to be normally distributed with a mean = 0 and variance–covariance matrix V. A_{ij} and G_{ij} in Equation 4 are design matrices assigning the fixed and random effects to the observations according to the treatment and experimental design structure. In practical terms, subjects represent a natural clustering or grouping of observations. For example, clustering may be due to experimental design structures, such as blocks or hierarchal main plot groups, among others. Grouping may also occur from subpopulation sampling across environmental (years, locations, and the like) or biological (biotype, varietal, and other) effects.

When subject-level variation exists in the dose–response relationship, it can be manifested in the data as a heteroskedastic error structure. In the log-logistic model, for example, subject-level variation in the parameter for the maximum response level D could result in an increasing level of variability as response level increases. Incorporation of an additional random effect for the parameter D would account for this subject level variability and reduce the heteroskedastic nature of the estimated errors.

Random effects can be applied to multiple parameters as well, although numerous random effects can induce computational problems for estimation algorithms. The potential covariance between random effects must also be evaluated in these cases. Dealing with these more complex model forms may require further programming steps as well as reparameterization of the variance covariance structure. Readers should refer to Littell et al. (2006) for additional details pertinent to that process.

Estimation of the mixed-effects model is accomplished through maximum-likelihood techniques. In maximum-likelihood estimation (MLE), parameter values are identified such that the joint probability or likelihood of the observed data is maximized. A general form of the likelihood is given by Equation 6:

$$L\left(\theta,\ \phi|y_{ij}\right) \propto \Pi\, g\left(\theta|y_{ij}\right) h\left(\phi|y_{ij}\right), \qquad [6]$$

where $g(\theta|y_{ij})$ and $h(\phi|y_{ij})$ are the probability distributions of the data and random effects, respectively. As with the nonlinear least-squares procedure, iterative adjustments to parameter values are made until the likelihood converges on a maximum value. For theoretical, analytical, and computational reasons, it is usually more convenient to perform this optimization on the log of the likelihood function, although the convergence criteria remain the same. The value of the log-likelihood function at the point of convergence is also an important metric used in model assessment and inference, which will be discussed in more detail below.

In the case considered thus far, $g(\theta|y_{ij})$ would be considered as a normal distribution with a mean of $f(dose_j, \theta)$ and a variance of σ^2. It should be noted, however, that the MLE for the variance is biased in this case. The random effects may take on any proper distributional form, although most estimation software makes the assumption that they are distributed normally, i.e., $h(\phi|y_{ij}) = N(0, V)$.

The function $g(\theta|y_{ij})$ can take on any legitimate probability distribution consistent with the response y_{ij}. Such models are referred to as *generalized nonlinear models* or *generalized nonlinear mixed models* if they also contain random effects. Weed science research often generates nonnormal data types, primarily in the form of discrete counts. Hence, the flexibility to specify a nonnormal likelihood in Equation 6 is an enticing aspect of modern model-estimation techniques. Use of discrete data, however, comes with special considerations. Unlike the continuous, normally distributed data, discrete data distributions are typically characterized by functional relationships or dependencies between their mean and variance parameters. Although this can inherently help account for response heteroskedasticity, as described above, these data types often exhibit additional variability beyond what is expected from the mean–variance relationship. Such variation is referred to as *overdispersion*, and like heteroskedasticity, this condition can lead to biased error estimation and unreliable statistical inference. Some common discrete distributions encountered in weed science and their associated corrective measures for overdispersion are discussed below.

Discrete data can arise from a variety of processes. Such data, for example, can occur when the response consists of counts, such as the number of plants, shoots, leaves, and the like, observed over a predefined spatial frame or time period. These counting situations result in nonnegative integer data ranging from zero to some large, unspecified, upper bound. Counting data are often considered to be derived from a Poisson process. Functionally, the Poisson distribution is given by Equation 7:

$$g\left(\lambda|y_{ij}\right) = \left(\lambda^{y_{ij}} e^{-\lambda}\right) / \left(y_{ij}!\right), \qquad [7]$$

where y_{ij} are the observed counts from the ith dose of the jth replicate, and λ is a parameter defining both the mean and variance of the distribution, i.e., $E(y_{ij}) = f(dose_i) = \mathrm{var}(y_{ij}) = \lambda$. Using Equation 7 as a basis for the likelihood, MLE can then be performed to estimate $\lambda = f(dose_j, \theta)$.

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Poisson data, however, often show higher levels of variability than would be expected from the implied *mean = variance* relationship. With replicated data, this condition is one that can be easily assessed graphically by plotting the sample variance of the count data at each dose against their corresponding means. Variances larger than the mean are symptomatic of overdispersion and will require remediation. For overdispersion in Poisson data, it is often recommended that an alternative distributional form to the data, such as the negative binomial distribution, be applied:

$$g(p,\ k) = \binom{k+r-1}{k} p^k (1-p)^r. \quad [8]$$

In this general specification, p is the probability of observing a single event and $g(p, k)$ is the probability of observing k such events out of $k + r - 1$ trials. This distribution will theoretically approach the Poisson distribution for large values of k but exhibits a higher degree of variability than the Poisson at smaller values of k. Hence, it is considered a logical substitute for the Poisson when overdispersion is present. To make the conversion from the Poisson distribution to the negative binomial form, the parameter p is defined as $p = k/(k + \lambda)$ where $\lambda = f(dose_j, \theta)$ from the Poisson distribution, and the value k becomes an additional parameter to be estimated in the negative binomial likelihood function.

Alternatively, if overdispersion appears to be due to clustering or grouping of subpopulations, the mixed modeling approach defined above could be employed with the addition of random effects to the Poisson likelihood function. As before, the random effects distribution is typically assumed to be normal with a mean of zero and a variance–covariance matrix of V.

Another common discrete data form is the binary response. In this case, the data are recorded as Yes/No responses relative to an observed condition. Observations are made a fixed number of times (*trials*; N_{ij}) per experimental unit, and the number of positive responses y_{ij} is recorded. Examples might be the number of surviving plants out of a given total number of plants treated or the number of seeds germinated out of the total number placed in a petri dish. These types of data are often expressed as proportions or percentages of the total N_{ij}; however, they are more accurately described as *binomial count data*. The binomial distribution is given by Equation 9:

$$g(p_j,\ N_{ij}) = \binom{N_{ij}}{y_{ij}} p_j^{y_{ij}} (1-p_j)^{N_{ij}-y_{ij}}, \quad [9]$$

where p_j is the probability of a positive response in the jth dose, and y_{ij} and N_{ij} are as defined above. As with the Poisson distribution, the mean and variance of the binomial are related, where $E(y_{ij}) = N_{ij}p_j$ and $\text{var}(y_{ij}) = N_{ij}p_j(1 - p_j)$. In practice, MLE can be used by taking Equation 9 as the foundation of the likelihood function and assuming $p_j = f(dose_j, \theta)$. This, of course, requires that the dose–response function and its associated parameters θ conform to the defined (0.0, 1.0) range of the parameter p_j.

Problems related to overdispersion can also occur with binomial data and can be corrected with methods similar to the Poisson distribution. Alternative distributions, which modify the binomial likelihood function to correct for additional variability, would be the quasi-binomial (Consul 1974), where the probability of a positive result is adjusted to a value of $p_j^* = p_j + \beta y_{ij}$ or to the beta-binomial, where probability p_j is modeled as a random variate from a beta distribution before entry into the binomial likelihood function. Additionally, random effects models for binomial data are also applicable if the data structure supports it, as outlined earlier.

Occasionally, situations may arise where binomial or Poisson data display more zero values than are expected from their respective probability distributions. A special class of models called *zero inflated* models exist to handle these scenarios. These models estimate the probability of a zero occurring as function of some underlying latent structure in the data. An example of such a structure might be the classification of plant age, where young plants are more susceptible to a herbicide treatment than older plants and are, therefore, more likely to result in zero count values. For information on the estimation of these model types, the reader is referred to more specific literature on this topic, such as Cameron and Trivedi (1998) or Littel et al. (2006).

Other estimation techniques, such as parametric and nonparametric Bayesian methods are also available for the situation outlined above; however, they will not be addressed here (for more information, see Davidian and Giltinan 1998; Mukhopadhyay 2000; Shafii and Price 2006).

Model Assessment. Convergence of the iterative methods above is a necessary step for successful model estimation. Lack of convergence can be due to several factors, such as poor starting values for parameters, incorrect model specifications, or incorrect model forms for the data at hand. Adjustment or correction of these factors will usually improve model convergence. Convergence issues may also arise when the scale of the parameters, response, and dose values differ excessively. A response measured in thousands of grams, in conjunction with dosages measures in hundredths of milligrams, for example, may cause problems. Rescaling these variables to kilograms and micrograms, respectively, could help eliminate convergence problems. If lack of convergence persists, however, the user should consult the software documentation and appropriate literature for further information on modifying specific settings of the optimization routines being used.

Although model convergence is necessary, it is not a sufficient criterion for judging model adequacy. It is quite possible for convergence to occur on parameter solutions that describe the data poorly. For that reason, a good initial step in model assessment is to overlay a plot of the predicted model on the observed data points. A proper model fit will be centered on the data and follow the data pattern well. In conjunction with that plot, the user should examine the parameter estimates to judge whether the estimated values are biologically relevant or possible. Negative values for asymptotic parameters, for example, would be an immediate indication of an estimation problem. As with convergence issues, changing starting values and rechecking model specifications can help correct this type of problem.

Parameter estimates may be evaluated numerically as well. In nonlinear regression, an approximate t-test can be carried

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out by dividing the parameter estimates by their respective standard errors. Most nonlinear regression software will produce these tests in addition to the associated P-values. Although these tests are approximate, they do provide a means for assessing the significance of each parameter in the model. Nonsignificant estimates are an indication that the model is overparameterized and that the model should be rewritten without the specified parameter or another model form with fewer parameters should be selected. Parameter estimates in nonlinear regression can also be correlated with one another. These correlations should be evaluated to assess redundancies within the model. A value of 0.99 or more is an indication that the data cannot support all the parameters in the model. Models resulting in high correlations should be reparameterized or expressed in an alternate form to reduce the problem or avoid redundancy in the model. Parameters expressed as exponents or divisors in model functions often display correlation or significance problems. Writing these expressions in terms of log functions can greatly improve model estimation. Further diagnosis of parameter estimates can be gained through inspection of their gradient values. Gradient values measure the curvature of the likelihood surface at the estimated solution. Ideally, these values should be low (≤ 0.0001), demonstrating that the solution is at a true optimum. Problems with large gradient values can usually be corrected through reestimation of the model using the last value of the estimated parameters as the starting values or increasing the number of iterations used.

The diagnostics above help ensure that the iterative optimization process has performed well. Further diagnostics concerning overall model fit should also be evaluated. Plots of residual values against dose levels or predicted values can be useful for assessing heteroskedasticity or overdispersion. Fanning or spreading patterns in residual plots are indicative of these conditions. Residual patterns that display systematic trends are characteristic of poor parameter estimates or an incorrect model form.

Model performance can be quantified as well. In linear regression, it is common to report the coefficient of determination or R^2. In dose–response modeling, however, this metric has no applicability. Because the estimated models and methods in dose–response are, in fact, nonlinear, the R^2 metric, a measure of linear association, is inappropriate and should not be used in either dose–response modeling or any other nonlinear regression application. Other numeric metrics related to model fit are available, however. Information criteria or IC, for example, can be derived from the final log-likelihood value and are often reported by the modeling software. Although the value of the IC statistic has little meaning on its own, it is useful for comparing the competing model forms. Several variations of IC, such as Akaike IC (AIC), Akaike IC adjusted for small sample size ($AAIC_c$), or the Bayesian IC (BIC), have been developed to adjust the log-likelihood statistic for the number of parameters estimated and sample sizes. Regardless of the IC form, however, the interpretation of the values is the same, where models having lower IC values are preferred over alternate models. Comparison of IC values is not appropriate, however, when assessments are made across different data sets or likelihood types, e.g., Poisson distribution to negative-binomial distribution.

Additional numeric tests that directly assess the goodness-of-fit or lack-of-fit of a specific model can be used when the data are replicated. Such tests may be provided by the software directly or, alternatively, can be computed manually by the user. For continuous, normally distributed data, a lack of fit test can be computed using the sum of squares for error (*SSE*) value from the nonlinear, regression SSE_R and the *SSE* obtained from fitting the data directly to the dose levels in a one-way ANOVA (Bates and Watts 1988). The ANOVA SSE is referred to as the *pure error* or SS_{PE}. An *F* test for lack-of-fit can then be computed from Equation 10:

$$F_{(df_{ER}-df_{PE},df_{PE})} = [(SS_{ER}-SS_{PE})/(df_{ER}-df_{PE})]/(SS_{PE}/df_{PE}), \quad [10]$$

where df_{ER} and df_{PE} degrees of freedom are from the regression and ANOVA error terms, respectively. For MLE-based models, a χ^2 test can be formed from the differences in the log-likelihood values of a saturated model (LL_{Sat}) and the model being fit (LL_M):

$$\chi^2_{(df_{Sat}-df_M)} = -2(LL_{Sat}-LL_M). \quad [11]$$

Here, LL_{Sat} is the log-likelihood statistic formed assuming $E(y_{ij}) = y_{ij}$. The goodness-of-fit χ^2 has $df_{Sat} - df_M = N - p$ degrees of freedom. Although these tests provide numerical assessments of model fit, they can have limited statistical power. In addition, they will not necessarily detect heteroskedasticity or overdispersion. To ensure an adequate model fit to the data, a full suite of model assessment methods should be carried out for each dose–response estimation conducted.

The lack-of-fit and goodness-of-fit tests above can also be adapted for testing higher-order treatment structure in the data. Comparison of dose–response relationships across herbicide treatments, varieties and biotypes, or management schemes, for example, is often an important objective in weed science. The process should begin with complete estimation and assessment of each treatment separately. Following these procedures and, given that a common model form is applicable to each

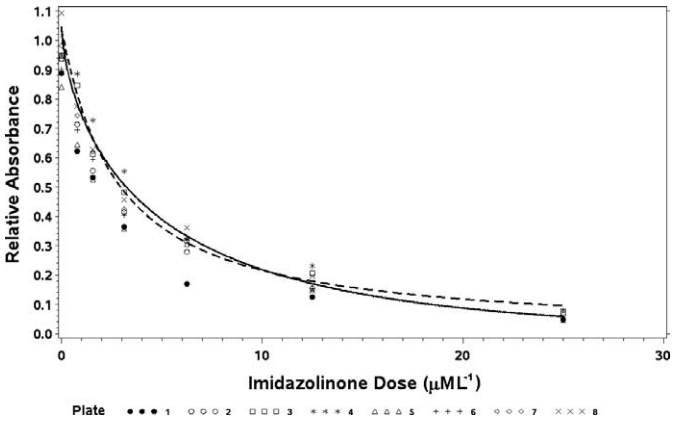


Figure 1. Relative acetolactase synthase (ALS) response of wheat cultivar Above at seven increasing doses of imidazolinone. Assays were conducted on eight independent microtiter plates. Solid line is the estimated Weibull-*I* model, whereas the dashed line is the estimated log-logistic model (from Rainbolt et al. 2005).

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treatment, a full model assuming a unique set of parameter values for each treatment is constructed. Likewise, a corresponding "reduced" model is also constructed, where one or more parameters are assumed to be common across treatments. The degree of change in the *SSE* or likelihood values between the two models is then tested for significance. A nonsignificant result indicates that the reduced model form is adequate to explain the dose–response relationship and no treatment effect is present relative to the parameters in question. Specifically, tests for continuous normal data can be formed using Equation 12:

$$F_{(df_{Reduced}-df_{Full},\, df_{Full})} = [(SS_{Reduced}-SS_{Full})/(df_{Reduced}-df_{Full})]/(SS_{Full}/df_{Full}), \quad [12]$$

whereas tests based on MLE can be derived as in Equation 13:

$$\chi^2_{(df_{Full}-df_{Reduced})} = -2(LL_{Full}-LL_{Reduced}). \quad [13]$$

These tests are analogous to those used in dummy variable regression techniques common in linear models.

Application

In the examples that follow, demonstrations of the methods outlined above will be given with relevant software codes. Although many software packages exist that are capable of estimating dose–response curves, the SAS (SAS 2009) software system (SAS Institute, Cary, NC 27513-2414) and R Statistical Language software (R Development Core Team 2011; R Foundation for Statistical Computing, Wien, Austria) are used

Table 1. Estimated parameters, standard errors, and correlations for dose–response models fit to (A) a log-logistic model using least-squares estimation, (B) a Weibull-*I* model using least-squares estimation, (C) a Weibull-*I* model using a Box-Cox equivalent transformation and maximum-likelihood estimation, and (D) a Weibull-*I* mixed model assuming the parameter *D* is random using maximum-likelihood estimation.ln (s) and ln(sD) are the estimated random effects for model variance and parameter D, respectively.

A

Parameter	Estimate	SE
D	0.9411	0.0213
B	1.0348	0.0614
I	2.7483	0.1979

Approximate correlations

	B	*I*
D	−0.40	−0.74
B		0.38

B

Parameter	Estimate	SE
D	0.95	0.0217
B	0.6644	0.0408
I	5.0971	0.3332

Approximate correlations

	B	*I*
D	−0.50	−0.63
B		0.18

C

Parameter	Estimate	SE	Gradient	
D	0.9499	0.03272	0.000221	
B	0.6477	0.03125	−0.00000701	
I	5.1597	0.3926	0.000012	
ln(*s*)	−2.2803	0.1632	−0.00005	
λ	0.3357	0.1246	−0.0001	

Approximate correlations

	B	*I*	ln(*s*)	λ
D	−0.69	−0.85	−0.09	0.09
B		0.82	−0.01	0.02
I			0.06	−0.10
ln(*s*)				−0.81

D

Parameter	Estimate	SE	Gradient
D	0.948	0.03108	−0.00019
B	0.6662	0.02589	−0.00013
I	5.141	0.2166	−0.00005
ln(*s*)	−3.2103	0.1021	0.000488
ln(*sD*)	−2.5414	0.2784	−0.00003

Approximate correlations

	B	*I*	ln(*s*)	ln(*sD*)
D	−0.21	−0.28	0.00	0.01
B		0.13	0.00	−0.02
I			0.00	−0.02
ln(*s*)				−0.04

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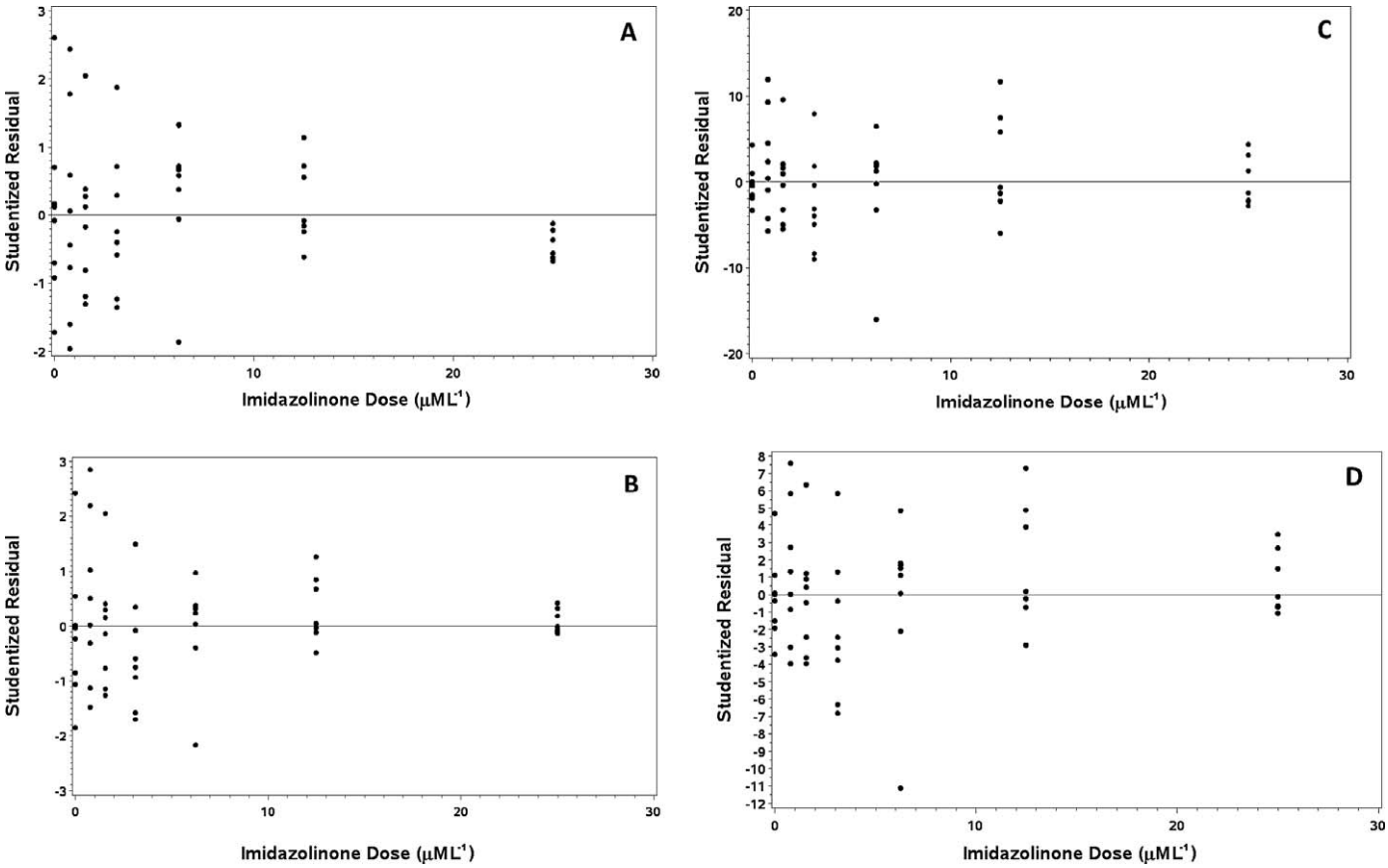


Figure 2. Studentized residuals from estimated dose–response models fit to the relative acetolactase synthase (ALS) response of wheat cultivar Above at seven increasing doses of imidazolinone. Four model residuals sets are shown: (A) log-logistic model using a least-squares estimation, (B) Weibull-*I* model using a least-squares estimations, (C) Weibull-*I* mixed model assuming a Box-Cox transformation using maximum-likelihood estimation, and (D) Weibull-*I* mixed model assuming a random upper-asymptote fit using maximum-likelihood estimation (from Rainbolt et al. 2005).

and demonstrated. Specifically, codes for the SAS procedures PROC NLIN and PROC NLMIXED, as well as for the R packages drc (Ritz and Streibig 2005) and nlme (Pinheiro et al. 2011), are provided in the accompanying appendices. Sample data files can be found on the authors' website at http://www.uiweb.uidaho.edu/ag/statprog/index.html.

Example 1: Continuous Normal Data. This data set is from Rainbolt et al. (2005) and measures the colorimetric response related to the presence of the enzyme acetolactase synthase (ALS) in winter wheat (*Triticum aestivum* L.) at varying doses of the herbicide imidazolinone. ALS activity (spectrophotometric absorbance) was recorded from a dilution series of seven imidazolinone solutions placed in microtiter plate cells with wheat stem segments. Each treated cell was paired with an adjacent untreated control cell containing only the zero-dose solution and the plant material. After incubation and further chemical treatment, the absorbance for each treated cell was expressed as a relative percentage of the paired control cell. Several wheat cultivars were tested in this manner using a blocked design with one complete cultivar–dose series appearing once on each of four separate microtiter plates. The experiment was run twice giving a total of eight independent dose series (plates) per cultivar.

The use of percent-of-control data is not encouraged in dose–response modeling; however, its application here is due to the designed pairing of the control and treated cells and the inherent relative nature of spectrophotometric absorbance data. For this example, the relative ALS response is assumed to be continuous and normally distributed as $N[\mu_j = f(dose_j, \theta), \sigma^2]$. Program codes for the ALS example may be found in Appendix 1.

A plot of the cultivar 'Above' is shown in Figure 1. This demonstrates a typical dose–response pattern, which begins at a high response level and smoothly declines to a lower asymptote. With these data, it is reasonable to assume that the lower asymptote is zero because no ALS response is anticipated at very high doses. As a first attempt at modeling the ALS dose–response, a three parameter log-logistic model following Equation 2 with $C = 0$ was fitted to the data. The iterative least-squares solution converges and provides significant parameter estimates with minimal correlations (Table 1a). The parameter estimates make biological sense in terms of the problem, with a maximum of $D = 0.94$ and a midpoint or $I = ED_{50} = 2.74$ μM L^{-1}. A further ANOVA-type lack-of fit test gives a value of $F_{4,49} = 0.6875$ and a corresponding nonsignificant P value of 0.6041, demonstrating no lack-of-fit.

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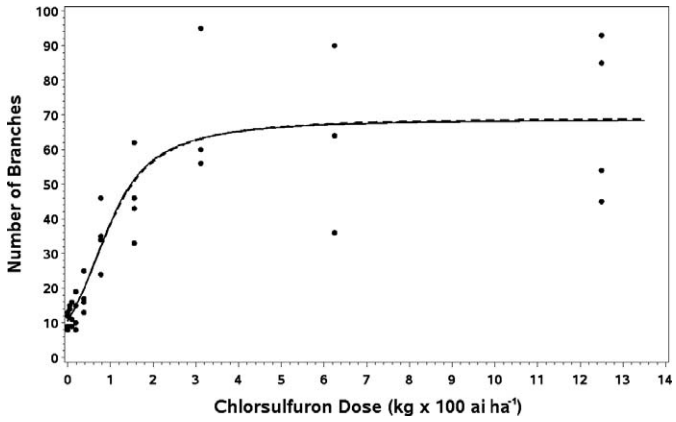


Figure 3. Estimated log-logistic models for the branching response of dry pea to increasing doses of chlorsulfuron. Solid line is the estimated curve for an assumption of negative binomial data, whereas the dashed line is a Poisson distributional assumption for the same data.

To this point, the estimation has shown a converged model with significant parameter estimates and no lack-of fit. Despite these good signs, however, an examination of the predicted curve (dashed line, Figure 1) and residuals (Figure 2a) reveal some deviation of the fitted model from the observed data. The estimated log-logistic model is overpredicting the response at higher doses. This implies that the model does not approach the lower asymptote rapidly enough. As an alternative, the Weibull-I model in Equation 3, with a lower asymptote of 0, can potentially provide for a higher rate of decline for the response. An estimation of this alternative model (Table 1b) also converges quickly, shows no lack-of fit ($F_{4,49} = 1.023$; P = 0.4049), and has similar significant relevant parameter estimates. The fitted Weibull-I predicted curve and residuals are given in Figure 1 (solid line) and Figure 2b, respectively. The Weibull-I curve follows the data better at high doses, and the resulting residuals are more centered on zero. Hence, the Weibull-I appears to be a more appropriate model form.

The residual plot, however, does show a fanning pattern, indicating the presence of heteroskedasticity, where the response becomes less variable with higher dose levels. Using maximum-likelihood estimation to apply an approximately equivalent Box-Cox–type TBS variance adjustment (Davidian and Giltinan 1998) improves the estimation and produces a better random pattern of residuals (Figure 2c). The parameter values from this model are significant and biologically relevant (Table 1c), and the model shows no significant lack-of-fit ($F_{4,49} = 0.9590$; P = 0.4384). In this estimation $\ln(\sigma)$ is the estimated variance (equivalent to the MSE in least squares), and λ is the Box-Cox parameter. This transformed model appears to provide a good description of the ALS dose–response.

An alternative method of correcting for heteroskedasticity would be to model the data variability using the underlying experimental structure. Recalling that the data comprise eight separate and independent series (plates), the apparent change in variability can potentially be attributed to the random effect of plates (Figure 1). Specifically, the larger variances observed at low doses may be attributable to plate-to-plate differences in the upper asymptote D. Hence, a maximum-likelihood estimation of Equation 6, assuming $g(\theta|y_{ij}) = N[f(dose_j, \theta), \sigma^2)$ and $h(\phi|y_{ij}) = N(0, \sigma_D^2)$ can be carried out where $f(dose_j, \theta)$ is the three parameter Weibull-I function and σ_D^2 is the random effect of plates on the upper asymptote D. Parameter estimates and the corresponding residual plot from this mixed, nonlinear model are shown in Table 1d and Figure 2d, respectively. These estimates, in addition to the variance component, σ_D^2 (estimated on a log scale) are all significant with minimal gradient values. An ANOVA-type lack-of-fit test (using the fixed-effects model components) is nonsignificant ($F_{4,49} = 1.024$; P = 0.4043).

Table 2. Estimated log-logistic parameters, standard errors, and associated correlations for dry pea data. Estimated ED_{90} values are shown as well. Estimates are shown for data assuming (A) a Poisson distribution, and (B) a negative binomial distribution.

		A		
Parameter	Estimate	SE	Gradient	
D	69.1896	3.3995	$-4.66e^{-07}$	
I	1.0623	0.1284	$8.66e^{-06}$	
B	1.9605	0.3187	$-7.04e^{-07}$	
C	11.7473	0.9964	$-2.83e^{-07}$	
ED_{90}	3.2437	0.7319		
Approximate correlations				
	I	*B*	*C*	
D	0.70	−0.53	−0.15	
I		−0.50	0.09	
B			0.43	
		B		
Parameter	Estimate	SE	Gradient	
D	68.6799	6.6433	$-3.28e^{-07}$	
I	1.0406	0.2261	$-2.35e^{-06}$	
B	2.0308	0.5525	$-5.27e^{-06}$	
C	11.813	1.2612	$1.47e^{-06}$	
k	21.4662	7.8853	$5.32e^{-07}$	
ED_{90}	3.1971	1.4507		
Approximate correlations				
	I	*B*	*C*	*k*
D	0.74	−0.54	−0.16	0.01
I		−0.65	−0.03	0.02
B			0.43	−0.03
C				−0.01

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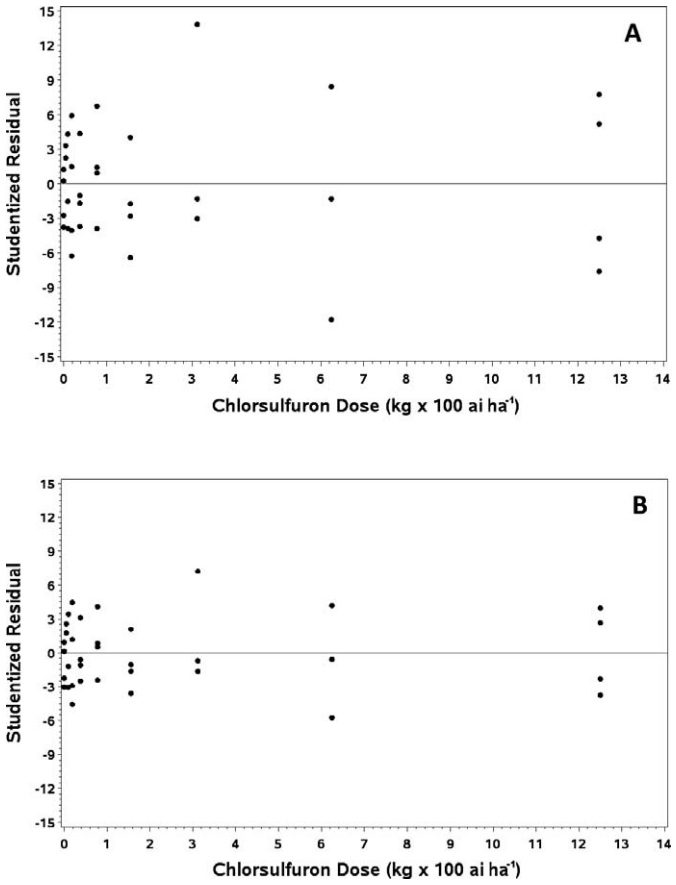


Figure 4. Studentized residuals from the log-logistic models estimating the branching response of dry pea to increasing doses of chlorsulfuron. Residuals are shown for two distributional assumptions: (A) the Poisson, and (B) the negative binomial.

Like the Box-Cox–transformed model, this set of residuals are more evenly scattered about zero.

Both the TBS and mixed-effects Weibull models provide a good description of the ALS data. The mixed-effects model is preferred, however, because it meets the specific experimental situation and provides smaller standard errors for the parameter estimates of B and I. In addition, the estimated, residual-variance of the mixed-effects model, given by $\exp[\ln(\sigma)]$, is much smaller, indicating that this model accounts for more data variability than the does the TBS model.

Example 2: Poisson Data. The data for this example relates to potential drift injury to dry pea from application of the herbicide chlorsulfuron. Chlorsulfuron damage in dry pea (*Pisum sativum* L.) can be manifested as an increase in stem branching. Hence, the data recorded here indicate the number of branches formed per plant at 10 chlorsulfuron doses ranged from 0 to 1.1 kg ai ha^{-1}. Four plants (replications) per dose were observed (one replication is missing from each of the doses 0.0312 and 0.0625 kg ai ha^{-1}). Program codes for example 2 are found in Appendix 2.

The data are shown in Figure 3 where the branch counts demonstrate an increasing sigmoidal pattern with increasing dose levels. A four-parameter log-logistic model is a good

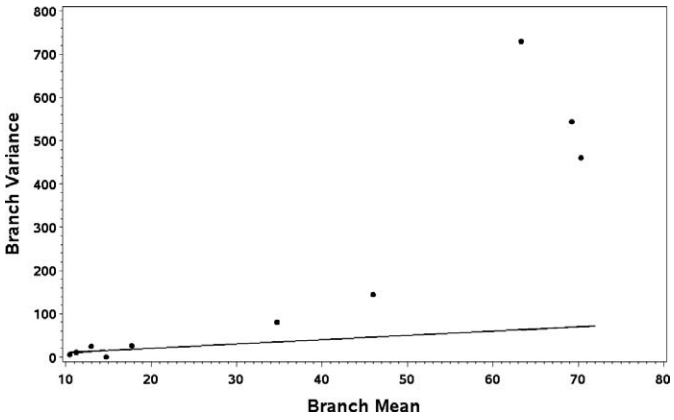


Figure 5. The mean–variance relationship of the branching response of dry pea. The solid reference line represents the expected equal mean–variance relationship of a Poisson distribution.

starting place for these data. A lower asymptote is reasonable in this case because plants exposed to no chlorsulfuron are expected to show some natural branching, whereas an upper limit to the potential for branching might be anticipated at the highest doses. A large difference in response and dose magnitudes exists, i.e., branch numbers range from 0 to 100, whereas the doses range from 0 to 0.125. To prevent potential convergence problems and make the unit magnitudes more comparable, the dose levels are multiplied by 100 before estimation. Final results may be rescaled to the original units following estimation.

Because the data are discrete positive counts, the Poisson likelihood function given in Equation 7 and maximum-likelihood estimation are used to estimate the log-logistic model. This model converged successfully, and all parameter estimates are significant with small gradient values (Table 2a). The estimated function begins at a lower asymptote of a C of about 11 branches, i.e., the number of branches that might be expected to form naturally on this variety of dry pea. The response then increases to an estimated maximum D of 69 branches. The inflection point, I or ED_{50}, is a dose of 0.0106 kg ai ha^{-1} (the estimate divided by 100) and corresponds to an expected number of branches ($n = 40$) halfway between those limits. Additionally, a dose of 0.0324 kg ai ha^{-1} was estimated to give a response of 90% of the distance between D and C.

The predicted curve (dashed line, Figure 3) follows the center of the data pattern well; however, the residuals shown in Figure 4a have a spreading pattern with increasing dose, indicating overdispersion. A plot of sample variance and mean values, computed at each dose, illustrate the problem (Figure 5). The data are well above the reference line for the expected Poisson equality between the mean and variance. Additionally, a goodness-of-fit test based on the log-likelihood is significant ($\chi^2_{34} = 101$; $P < 0.0001$). All these diagnostics point to the Poisson distribution being inadequate to describe the variability of these data. Such patterns are common with these types of count data.

Corrective measures using a Box-Cox TBS transformation or change in response distribution are warranted. A switch to a negative binomial distribution is shown here (SAS code, Appendix 2). The converged estimation under this likelihood model results in significant parameter estimates with small gradient values (Table 2b). The parameter k, here, is an

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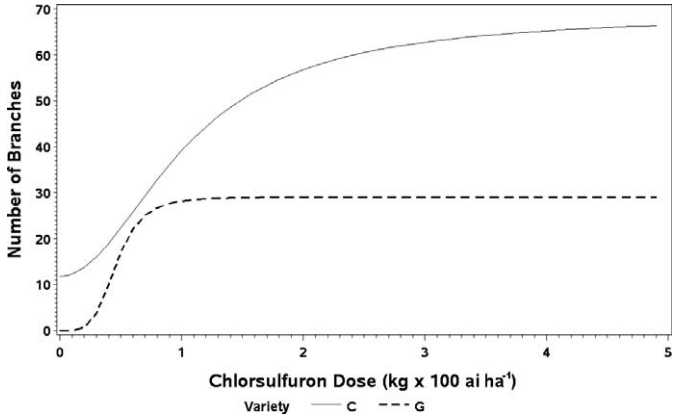


Figure 6. Estimated log-logistic models for the branching response of two dry pea varieties to increasing doses of chlorsulfuron. Solid line is the estimated curve for variety *C*, and dashed line is the same for variety *G*.

additional parameter estimated for the negative binomial distribution. The predicted curve (solid line, Figure 3) is almost identical to that of the Poisson distribution, and the corresponding residuals show a random pattern across the dose (Figure 4b). Although a goodness-of-fit test is still significant ($\chi^2_{33} = 65.4$; $P < 0.0001$), the improvement in the residual structure justifies the use of this distribution. Further extension of the model to incorporate random effects cannot be supported by the data because there is no subject-level grouping information available.

Example 3: Treatment Comparisons. Comparison of dose–response series across structured treatment designs is a common objective of dose–response modeling. Such structure may reflect differing herbicides, locations, biotypes, cultivars, and so forth. In the preceding demonstration, the number of branches induced by the application of chlorsulfuron was examined for one variety of dry pea. Although that experiment was primarily designed to examine the sensitivity of dry pea to chlorsulfuron, it was also of interest to assess potential varietal effects on this dose–response. To this end, an additional dose–response series for a second variety *G* was also tested. Following the procedures and models in Example 2, a log-logistic dose–response model for this variety was also estimated (SAS codes in Appendix 3). As with the first variety *C*, the second variety was corrected for the presence of

Table 3. Estimated log-logistic parameters and standard errors for two dry pea varieties, (A) variety *C*, and (B) variety *G*.

Parameter	Estimate	SE	Gradient
	A		
D	68.6799	6.6433	$-3.28e^{-07}$
I	1.0406	0.2261	$-2.35e^{-06}$
B	2.0308	0.5525	$-5.27e^{-06}$
C	11.813	1.2612	$1.47e^{-06}$
k	21.4662	7.8853	$5.32e^{-07}$
	B		
D	29.0672	1.8999	$3.56e^{-06}$
I	0.4621	0.03663	$5.70e^{-05}$
B	4.4317	0.7788	$-5.40e^{-06}$
C	0	—	—
k	23.1222	13.784	$5.30e^{-07}$

Table 4. Estimated relative potencies (RP), standard errors, and 95% confidence bounds for two dry pea varieties *C* and *G*. RP values represent the equivalent dose (kg ai ha^{-1}) for variety *C* relative to variety *G* to give the same number of branches per plant.

			95% Bounds	
RP	Value	SE	Lower	Upper
RP, ED_{50}	2.2519	0.5208	1.215	3.2888
RP, 15 branches	0.5525	0.1472	0.2594	0.8456
RP, 18 branches	0.7163	0.1473	0.4231	1.0095
RP, 21 branches	0.8065	0.1481	0.5118	1.1013
RP, 24 branches	0.8362	0.1543	0.529	1.1434
RP, 27 branches	0.767	0.1943	0.3802	1.1539

overdispersion assuming a negative binomial data distribution. The resulting estimated parameters and standard errors for both varieties are shown in Tables 3a and 3b. Because the variety *G* was slower in development, it showed no initial branching with no herbicide application. Hence, the lower asymptote was set to zero for that variety (Figure 6).

Treatment differences can occur in one or more aspects of dose–response models, e.g., asymptotes, rates, ED values, and the like. Because of the potential dependency of parameters on one another, however, comparison of only one aspect of the dose–response may not give a complete picture of the treatment effects. For the example of the dry peas, an initial comparison of all aspects of the dose–response curve was carried out using a full model dummy variable technique. As might be expected, all parameters are significantly different. Of particular interest for herbicide applications is the comparison of ED values. The difference in ED_{50} here, for example, is nearly twofold, with variety *C* having the higher dose to reach the response halfway between asymptotes *C* and *D* (Table 3). That is deceiving, however, because the asymptotes and rate parameters of each variety differ. The response level for variety *C* at its ED_{50}, 40 branches, is not even obtainable by variety *G*. Ritz et al. (2006) have cautioned against such direct comparisons with nonsimilar dose–response models and suggested the use of the ratio of ED values (relative potency, RP) as a better measure. Furthermore, the comparison should only be made where it is supported by the data. That is, between the maximum lower

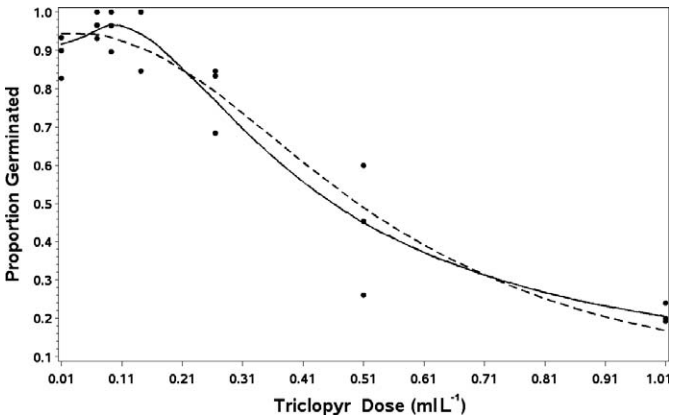


Figure 7. Estimated dose–response models (dashed line: log-logistic model, solid line: Brain-Cousens hormesis model) for the proportion of germinated garden lettuce seed after treatment with seven doses of triclopyr.

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Table 5. Estimated dose–response models for garden lettuce seed germination following seven doses of triclopyr herbicide. The dose–response forms estimated were (A) the log-logistic model, and (B) the Brain-Cousens hormesis model.

A			
Parameter	Estimate	SE	Gradient
D	0.9433	0.0135	$7.661e^{-06}$
B	2.3510	0.3005	$-1.32e^{-07}$
I	0.5262	0.0409	$5.729e^{-06}$
Approximate correlations			
	B	*I*	
D	−0.23	−0.25	
B		−0.06	
B			
Parameter	Estimate	SE	Gradient
D	0.9012	0.0267	0.000032
B	2.0369	0.2073	$-5.95e^{-07}$
I	0.3055	0.0613	0.000032
f	1.6223	0.8192	$3.07e^{-06}$
Approximate correlations			
	B	*I*	*f*
D	0.19	0.63	−0.75
B		0.68	−0.65
I			−0.96

asymptote and the minimum upper asymptote. Within that range, the RP may change, depending on the response level being assessed. For the dry peas, the RP of chlorsulfuron on variety *C* compared with variety *G* increases and decreases systematically ranging from RP = 0.5 to RP = 0.84 within the comparable response level range of 15 to 27 branches (Table 4). Only the RP value at the response of 15 branches has confidence bounds not encompassing zero, indicating a significant difference in varieties at the estimated dose of 0.55 kg ai ha^{-1}. This is quite different from the direct comparison of ED_{50} values. A complete understanding of the differences in varietal response requires examination of all parameters.

Example 4: Binomial Data. An experiment was carried out applying seven doses of the herbicide triclopyr to garden lettuce (*Lactuca sativa* L.) seeds (data developed in conjunction with Ranft et al. 2010). For each dose, 30 seeds were treated in a petri dish. Subsequent germination of each seed in the dish was observed after 10 d and recorded as *germinated* or *not germinated*. Four petri dishes (replications) of each dose were used. Given the early stages of growth and controlled environment of the petri dish, it is assumed that seeds germinated independently of one another. The response was then recorded as the number of germinated seeds (y_{ij}) out of the total in each dish. The totals varied from the 30 initial seeds used, however, because of fungal contamination (N_{ij} = 15 to 30). Example program codes are given in Appendix 4.

An estimated three-parameter, log-logistic model was fitted to the data assuming a zero germination response at very high doses. The estimated curve and associated parameter estimates are given in Figure 7 and Table 5a, respectively. Although the model converged and found significant parameter estimates, the corresponding residual pattern in Figure 8a shows overprediction (negative residuals) at the lowest doses and underprediction (positive residuals) at the highest dose, 1.0 ml L^{-1}. A goodness-of-fit test is significant (P = 0.0373), indicating that the model does not match the data well.

A closer inspection of the data pattern, particularly at low doses, suggests a slight increase in the proportion germinated

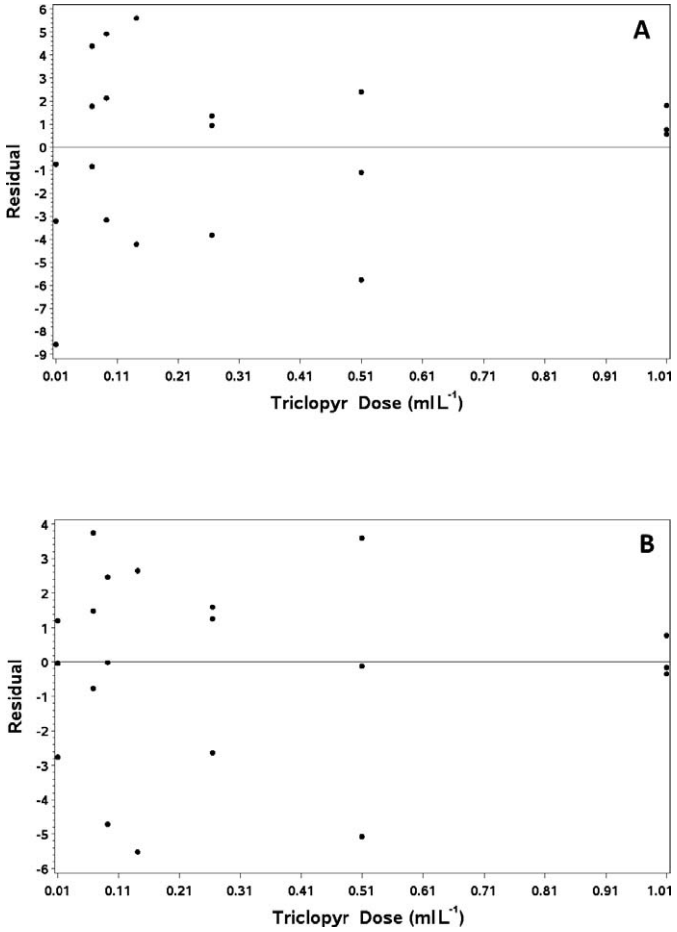


Figure 8. Residuals from the estimated dose–response models for the proportion of germinated garden lettuce seed after treatment with seven doses of triclopyr. The residuals shown are from (A) the log-logistic model, and (B) the Brain-Cousens hormesis model.

before the overall declining response begins. A subsequent remodeling of the data assuming a Brain-Cousens hormesis model (Brain and Cousens 1988) is shown in Figure 7, with the corresponding residuals shown in Figure 8b. The fitted model now correctly describes the initial increase in germination at low doses. In addition, the model now fits the observed data at the highest dose better than the log-logistic. The residual pattern is more centered around zero at those points. All estimated parameters are significant with small gradient values (Table 5b). The goodness-of-fit test is now nonsignificant at the $\alpha = 0.05$ level of significance (P = 0.0756). In this case, the addition of another parameter to account for hormesis helped improve the model fit and, hence, more accurately described the data.

Discussion

Although dose–response has application to a wide array of research objectives in weed science, the effectiveness of dose–response analyses is reliant on appropriate estimation, diagnostic, and corrective methods. Data displaying heterogeneity and underdispersion or overdispersion, for example, must be addressed to produce reliable results and appropriate inferences.

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Ignoring such problems can result in imprecise or biased estimation and potentially lead to misinformed conclusions. Alternatively, the use of statistical methodology that incorporates mixed-effects models, appropriate data distributions, or traditional data transformation techniques can counter these conditions and provide technically correct analyses that lead to more-precise model estimates and, subsequently, more-reliable statistical inference. Advancements in computer and software technologies have improved the ability of researchers to use and apply these techniques, particularly in cases of discrete data types. In this article, several examples, encompassing both continuous and discrete data, were demonstrated with relevant, specific details provided for illustrating the corrective measures. Application of these techniques is intended to provide research professionals a foundation for applying suitable dose–response methods in their future research.

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Appendix 1

A. SAS Codes

```
proc nlin data=wheat; /** Least-squares estimation log-logistic **/
    parms d=.94 b=1 I=2.748;
    if Dose=0 then mu=D; /** Steps to avoid log(0) **/
    else mu = (D)/(1 + exp(B*(log(Dose)−log(I))));
    model percent = mu;
    output out = LSpredict p=pred student = LSresid;
  title1 'Winter Wheat: Continuous Response Least-Squares Method';
  run;
  proc nlin data=wheat; /** Least-squares estimation Weibul**/
    parms d=.95 b=.66 I=5;
    if Dose=0 then mu=D; /** Steps to avoid log(0) **/
    else mu = (D)*exp(−exp(b*(log(Dose) − log(I))));
    model percent = mu;
    output out = LSWpredict p=pred student = LSWresid;
  title1 'Winter Wheat: Continuous Response Least-Squares Method';
  run;
  proc nlmixed data=wheat corr; /** Maximum-likelihood Weibull with Box-Cox ~TBS equivalent **/
    parms d=.95 b=.66 I=5 lsig = −2 lamda=1;
    sig2 = exp(2*lsig);
    if Dose=0 then mu=D; /** Steps to avoid log(0) **/
    else mu = (D)*exp(−exp(b*(log(dose) − log(I))));
    V = mu**(2*(1-lamda)); /** Davidian and Giltinan - 1998, pg 61) **/
    model percent ~ normal(mu, V*sig2);
```


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```
    predict mu out = BXpredict;
    predict percent - mu out = BXresid;
  title1 'Winter Wheat: Continuous Box-Cox Transformation';
  run;
  proc sort data=wheat;
    by rep dose;
  proc nlmixed data=wheat corr; /** Maximum-likelihood
Weibull mixed-model estimation **/
    parms d=.95 b=.66 I=5 lsig = −2 lsig2=0.05;
    sig2 = exp(2*lsig);
    sigU2 = exp(2*lsig2);
    D1 = D + U;
    if Dose=0 then mu=D1; /** Steps to avoid log(0) **/
    else mu = (D1)*exp(−exp(b*(log(Dose) − log(I))));
    model percent ~ normal(mu, sig2);
    random U ~ normal(0, sigU2) subject=plate;
    predict (D)*exp(−exp(b*(ldose − log(I)))) out =
Rpredict;
    predict percent - mu out = Rresid;
  title1 'Winter Wheat: Continuous Mixed-Model—Ran-
dom D';
  run;
```

B. R Codes

```
  WW<-read.table("C:/ …. /Wheat.txt",header=T)
  attach(WW)
  WW$PERCENT <- WW$PERCENT/100
  ############################## Fits using
drc package
  ############################## Fit log-lo-
gistic model
  Wheat.dr<-drm(WW$PERCENT~WW$TRMT, data = WW,
  fct = LL.3(), type = "continuous")
  summary(Wheat.dr)
  modelFit(Wheat.dr)
  ############################## Fit Wei-
bull model
  Wheat.W.dr<-drm(WW$PERCENT~WW$TRMT, da-
ta = WW,
  fct = W1.3(), type = "continuous")
  summary(Wheat.W.dr)
  modelFit(Wheat.W.dr)
  ############################## Compare
log-logistic and Weibull models
  mselect(Wheat.dr, list(LL.3(), W1.3()))
  ############################## Fit Box-
Cox Weibull model
  Wheat.BC.W.dr<-boxcox(Wheat.W.dr, lambda = seq(−2, 2,
by = 0.05))
  summary(Wheat.BC.W.dr)
  modelFit(Wheat.BC.W.dr)
  ############################## Fit Mixed
Effects Weibull model
  ############################## using nlme
package
  WWData <- groupedData(PERCENT~TRMT | Rep,
data=WW,
    labels=list(x='Dose', y='ALS Percent'),
    units=list(x="(microM/L)", y="(%)"))
  attach(WWData)
  model.func <- function(TRMT, D, B, I) (D)*exp(−exp
(B*(log(TRMT) − log(I))))
  Wheat.R.W.dr <- nlme(model = PERCENT ~ mod-
el.func(TRMT, D, B, I), data = WWData,
    fixed = D + B + I ~ 1,
    random = D ~ 1 | Rep,
    start = c(.95, .66, 5))
  summary(Wheat.R.W.dr)
```

Appendix 2

A. SAS Codes

```
  proc nlmixed data=peas corr; /** Fit Poisson likelihood
log-logistic model**/
    parms D=70 I=1 B=2 C=10;
    if Dose=0 then mu=C; /** Steps to avoid log(0) **/
    else mu = C + (D-C)/(1 + exp(−B*(log(Dose)−"
log(I))));
    quan = 63.4;
    EDx = exp((log(((D-C)/(quan-C)) − 1)/−B) + log(I));
    model branches~poisson(mu);
    predict C + (D-C)/(1 + exp(−B*(log(Dose)−log(I))))
out=Ppred;
    predict branches - mu out=Presid;
    estimate 'EDx' EDx/100; /** Bring dose back to original scale **/
  title1 'Poisson Likelihood Function;
  run;
  proc nlmixed data=peas2 corr; /** Fit negative binomial
likelihood log-logistic model**/
    parms D=70 I=1 B=2 C=12 k=8;
    if Dose=0 then mu=C; /** Steps to avoid log(0) **/
    else mu = C + (D-C)/(1 + exp(−B*(log(Dose)−"
log(I))));
    quan = 63.4;
    EDx = exp((log(((D-C)/(quan-C)) − 1)/−B) + log(I));
    p = k/(k+mu);
    model branches ~ negbin(k,p);
    predict C + (D-C)/(1 + exp(−B*(log(Dose)−log(I))))
out=Npred;
    predict branches - mu out=Nresid;
    estimate 'EDx' EDx/100; /** Bring dose back to original
scale **/
  title1 'Negative Binomial Likelihood';
  run;
```

B. R Codes

```
  Peas<-read.table("C:/ …. /peas.txt",header=T)
  attach(Peas)
  ############################## Fits using
drc package
  ############################## Fit Pois-
son likelihood and log-logistic model
  Peas.dr<-drm(Branches~Dose, data = Peas,
  fct = LL.4(), type = "Poisson")
```


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```
summary(Peas.dr)
############################## Fit Box-Cox to Poisson and Log-Logistic Model
Peas.BC.dr<-boxcox(Peas.dr, lambda = seq(−2, 2, by = 0.05))
summary(Peas.BC.dr)
############################## Fit Poisson and negative binomial using gnlm package
Branches<-Peas$Branches
ln_Dose<-log(Peas$Dose)
mu1<-function(p) (p[1] + (p[2] − p[1])/(1 + exp(−p[3]*(ln_Dose-log(p[4])))))
Variance <- function(C, D, B, I) (C + (D-C)/(1 + exp(−B*(ln_Dose-log(I)))))
############################## Fit Negative Binomial and Log-Logistic Model
peas1.fit<-gnlr(Peas$Branches, dist="Poisson", mu=mu1,
pmu=c(11.68,69.15,1.99, 1.07), pshape=c(11.68,69.15,1.99, 1.07))
############################## Fit Negative Bionomial and Log-Logistic Model
peas2.fit<-gnlr(Peas$Branches, dist="negative binomial", mu=mu1,
    pmu=c(11.68,69.15,1.99, 1.07), pshape=1)
```

Appendix 3

A. SAS Codes

```
proc nlmixed data=peast corr;
   PARMS Dg=30 Ig=.8 Bg=4.6 Dc=70 Ic=2 Bc=2 Cc=12 kg=8 kc=8;
/*****************************************************/
/********* Set up full dummy variable model **************/
/*****************************************************/
   if variety = 'G' then do;
   Cg=0.000001; D=Dg; B=Bg; I=Ig; k=kg; /** C here is set to very small value **/
   If Dose=0 then mu = Cg;
   else mu = Cg + (D-Cg)/(1 + exp(−B*(log(Dose)−‴ log(I))));
   end;
   else do;
   C=Cc; D=Dc; B=Bc; I=Ic; k=kc;
   if Dose=0 then mu = Cc;
   else mu = C + (D-C)/(1 + exp(−B*(log(Dose)−‴ log(I))));
   end;
/*****************************************************/
/********** Compute some ED values *********************/
/*****************************************************/
   Cg=0;
   Q1= 15;
   EDc1 = exp((log(((Dc-Cc)/(Q1-Cc)) − 1)/−Bc) + log(Ic));
   EDg1 = exp((log(((Dg-Cg)/(Q1-Cg)) − 1)/−Bg) + log(Ig));
   EDg90 = exp((log(((Dg-Cg)/(26.2-Cg)) − 1)/−Bg) + log(Ig));
   Q2= 18;
   EDc2 = exp((log(((Dc-Cc)/(Q2-Cc)) − 1)/−Bc) + log(Ic));
   EDg2 = exp((log(((Dg-Cg)/(Q2-Cg)) − 1)/−Bg) + log(Ig));
   Q3= 21;
   EDc3 = exp((log(((Dc-Cc)/(Q3-Cc)) − 1)/−Bc) + log(Ic));
   EDg3 = exp((log(((Dg-Cg)/(Q3-Cg)) − 1)/−Bg) + log(Ig));
   Q4= 24;
   EDc4 = exp((log(((Dc-Cc)/(Q4-Cc)) − 1)/−Bc) + log(Ic));
   EDg4 = exp((log(((Dg-Cg)/(Q4-Cg)) − 1)/−Bg) + log(Ig));
   Q5= 27;
   EDc5 = exp((log(((Dc-Cc)/(Q5-Cc)) − 1)/−Bc) + log(Ic));
   EDg5 = exp((log(((Dg-Cg)/(Q5-Cg)) − 1)/−Bg) + log(Ig));
   p = 1/(1+mu/k);
   model branches ~ negbin(k,p);
/*****************************************************/
/************* Compare varieties ********************/
/*****************************************************/
   contrast 'Lines between Varieties' Cg-Cc, Dg-Dc, Ig-Ic, Bg-Bc;
   contrast 'D between Varieties' Dg-Dc;
   contrast 'I between Varieties' Ig-Ic;
   contrast 'B between Varieties' Bg-Bc;
/*****************************************************/
/************ Estimate some relative potencies ***********/
/*****************************************************/
   estimate 'Relative potency' Ic/Ig; /*** direct comparison of inflection points => wrong ***/
   estimate 'RP1' EDc1/EDg1; /*** Comparison only in range supported by the data *****/
   estimate 'RP2' EDc2/EDg2;
   estimate 'RP3' EDc3/EDg3;
   estimate 'RP4' EDc4/EDg4;
   estimate 'RP5' EDc5/EDg5;
title1 'Negative Binomial Likelihood: Unadjusted';
run;
```

Appendix 4

A. SAS Codes

```
/*****************************************************/
/********* Binomial Data: Log-Logistic Model *************/
/*****************************************************/
proc nlmixed data=lettuce corr;
   parms D=.9283 B=4 I=0.44;
   if Dose=0 then mu=D; /** Steps to avoid log(0) **/
   else mu = (D)/(1 + exp(B*(log(dose) − log(I))));
   model yes ~ binomial(total, mu);
```


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```
    predict (D)/(1 + exp(B*(log(dose2) − log(I)))) out=predU;
    predict yes/total - mu out=resid;
  title1 'Binomial log-logistic;
  run;
  /*****************************************************/
  /******* Binomial Data: Brain-Cousens Hormesis Model *****/
  /*****************************************************/
  proc nlmixed data=lettuce corr;
    parms D=.90 B=2.1 I=0.305 f=1.6;
    if Dose=0 then mu=D; /** Steps to avoid log(0) **/
    else mu = (D + f*dose)/(1 + exp(B*(log(Dose) −
log(I))));
    model yes ~ binomial(total, mu);
    predict (D + f*dose)/(1 + exp(B*(log(dose) − log(I))))
out=predH;
    predict yes/total - mu out=residH;
    predict ((Yes - total*mu)**2)/(total*mu) out=pearsonH;
  title1 'Binomial Hormesis Model';
  run;
```

B. R Codes

```
  Lett<-read.table("C:/ …. /Lettuce.txt",header=T)
  ########################## Fit binomial
and log-logistic using drc package
  Lett.dr<-drm(Yes/total~dose, weights = total, data =
Lett,
  fct = LL.3(), type = "binomial")
  ############## Fit binomial and Brain Cousens
hormesis model using drc package
  Lett.dr2<-drm(Yes/total~dose, weights = total, data =
Lett,
  fct = BC.4(), type = "binomial")
  summary(Lett.dr)
  summary(Lett.dr2)
  modelFit(Lett.dr)
  modelFit(Lett.dr2)
  ################ Compare Log-Logistic and
Brain-Cousens Hormesis Models
  mselect(Lett.dr, list(BC.4()))
```


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