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The critical period for weed control (CPWC) is a period in the crop growth cycle during which weeds must be controlled to prevent yield losses. Knowing the CPWC is useful in making decisions on the need for and timing of weed control and in achieving efficient herbicide use from both biological and economic perspectives. An increase in the use of herbicide-tolerant crops, especially soybean resistant to glyphosate, has stimulated interest in the concept of CPWC. Recently, several studies examined this concept in glyphosate-resistant corn and soybean across the midwestern United States. However, these studies presented various methods for data analysis and reported CPWC on the basis of a variety of crop- or weed-related parameters. The objectives of this study are (1) to provide a review of the concept and studies of the CPWC, (2) to suggest a common method to standardize the process of data analysis, and (3) to invite additional discussions for further debate on the subject. Wide adoption of the suggested method of data analysis will allow easier comparison of the results among sites and between researchers.

Nomenclature: Glyphosate; corn, *Zea mays* L.; soybean, *Glycine max* (L.) Merr.

Key words: Crop–weed interactions, duration of interference, critical timing of weed removal, critical weed-free period, nonlinear regression, experimental design.

Integrated weed management (IWM) involves a combination of cultural, mechanical, biological, genetic, and chemical methods for effective and economical weed control (Swanton and Weise 1991). The principles of IWM should provide the foundation for developing optimum weed control systems and efficient use of herbicides. The critical period for weed control (CPWC) is a key component of an IWM program. It is a period in the crop growth cycle during which weeds must be controlled to prevent yield losses. The CPWC is useful for making decisions on the need for and timing of weed control. Since the introduction of the concept of CPWC by Nieto et al. (1968), studies have been conducted involving a number of crops (see summary by Zimdahl 1980) but no practical use of the concept was reported. In the United States, movement toward the use of postemergence (POST) herbicides with little or no residual activity has resulted in renewed interest in determining the most appropriate timing and periodicity for weed control, especially in systems involving the use of herbicide-tolerant crops (HTCs). The popularity of crops resistant to glyphosate has generated many studies to determine the optimum timing of weed control across the corn and soybean production areas of the United States (Dalley et al. 1999; Evans and Knezevic 2000; Gower et al. 1999; Kalaher et al. 2000; Mulugeta and Boerboom 2000; VanGessel et al. 2000). Optimal timing of weed removal has been reported on the basis of weed height (Dalley et al. 1999; Gower et al. 1999; Kalaher et al. 2000), weeks after crop emergence (Sellers and Smeda 1999), and crop growth stage (Evans and Knezevic 2000; Mulugeta and Boerboom 2000).

Analysis of the CPWC data varied. Some studies compared the effects of weed duration on crop yield using multiple comparison techniques (Dalley et al. 1999; Gower et al. 1999; Kalaher et al. 2000; VanGessel et al. 2000; Vizantinopoulos and Katranis 1994), whereas others suggested various nonlinear regression models (Evans and Knezevic 2000; Hall et al. 1992; Martin et al. 2001; Mulugeta and Boerboom 2000; Van Acker et al. 1993). Berti et al. (1996) referred to the multiple comparison techniques and nonlinear regression models as the classical and functional approaches, respectively. Several authors have criticized the use of the classical approach, suggesting that regression is a more appropriate method for the analysis of structured data, such as the time of weed removal in stepwise increments (Cousens 1988, 1991; Hall et al. 1992; Knezevic and Lindquist 1999; Van Acker et al. 1993).

To provide insight on the subject, the objectives of this study were to (1) provide a review of the pros and cons of the concept and studies of the CPWC, (2) suggest a common method to standardize the process of data analysis, and (3) invite additional discussion for further debate on the subject. Wide adoption of the suggested method of data analysis will allow easier comparison of the results among sites and between researchers.

The Critical Period Concept

The CPWC has been defined in several ways. Zimdahl (1988) defined it as a “span of time between that period after seeding or emergence when weed competition does not reduce crop yield and the time after which weed competition will no longer reduce crop yield.” Swanton and Weise (1991) defined the CPWC as the time interval when it is essential to maintain a weed-free environment to prevent crop yield loss. In recent years, university extension weed specialists (Knezevic 2000) and agricultural consultants (J. Mulliken, personal communication), have commonly described the CPWC as a "window" in the crop growth cycle during which weeds must be controlled to prevent unacceptable yield losses.

Examples of historical reasons for studying this concept include (1) the potential to reduce the amount of herbicide used by achieving optimal application timing (Hall et al. 1992; Van Acker et al. 1993; Zimdahl 1980), (2) the potential to reduce environmental and ecological degradation associated with the prophylactic use of herbicides (Swanton and Weise 1991; Weaver 1984; Weaver and Tan 1983), and (3) to provide a test to determine whether the methods of weed control are based on biological necessity (Weaver 1984). Most recently, there is a need for the economic optimization of weed control tactics in HTCs through timely application of POST herbicides. We contend that knowing the CPWC in major crops can aid in making decisions on the need for and timing of weed removal in cropping systems that use both HTCs and conventional crop cultivars.

In essence, the CPWC represents the time interval between two separately measured crop–weed competition components: (1) the critical timing of weed removal (CTWR) or the maximum amount of time early-season weed competition can be tolerated by the crop before the crop suffers irrevocable yield reduction, and (2) the critical weed-free period (CWFP) or the minimum weed-free period required from the time of planting to prevent unacceptable yield reductions. The former component is estimated to determine the beginning of the CPWC, whereas the latter determines its end. Results from both components are combined to determine the CPWC. Theoretically, weed control before and after the CPWC may not contribute to the conservation of the crop yield potential.

The beginning and end of the CPWC determined using the functional approach will depend on the level of acceptable yield loss (AYL) used to predict its beginning and end (Figure 1). Many studies report anywhere from 2 to 5% as the maximum AYL (Hall et al. 1992; Van Acker et al. 1993). But the AYL can be adjusted depending on the cost of weed control and the anticipated financial gain. For example, depending on the market price of the crop and the cost of weed control, the AYL can be adjusted for a particular field. This is easily achieved by selecting AYL from the regression curves according to the economic risk one is willing to take.

Assumptions and Limitations of the CPWC Concept

As with most theoretical concepts, there are certain limitations with the practical application of the concept of CPWC. Some have suggested that the variability inherent with this concept may appear to negate its usefulness (Gunsolus and Buhler 1999) and that its utility should be carefully judged (Mohler 2001). Others indicated that the concept is useful to practitioners (Evans et al. 2002; Knezevic 2000; J. Mulliken, personal communication) because there is a time during which weed interference affects crop yield (Hall et al. 1992; Martin et al. 2001; Van Acker et al. 1993). In order to document the causes of variability one should collect additional data (see Typical Variables) to provide

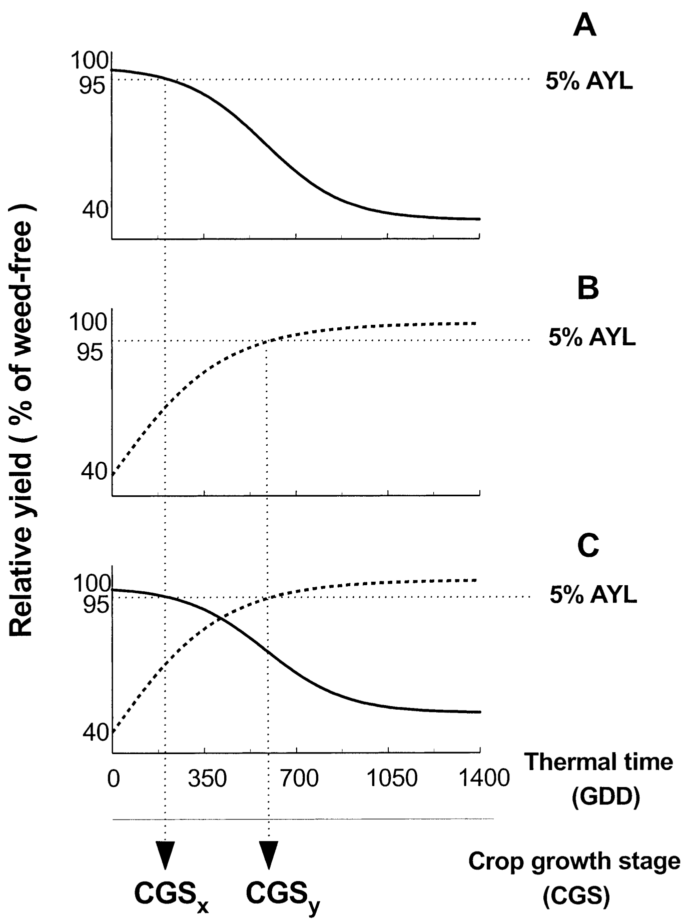

FIGURE 1. Functional approach used for the determination of the critical period for weed control (CPWC). (A) The critical timing for weed removal (CTWR) is determined from the logistic model, or the weedy curve (——), fit to data representing an increasing duration of weed interference. (B) The critical weed-free period (CWFP) is determined from the Gompertz model, or the weed-free curve (- - -), fit to data representing an increasing duration of weed-free period. (C) The value of the *x* axis that corresponds to 95% relative yield or an acceptable yield loss (AYL) of 5% is determined for both curves and related to the crop growth stage (CGS). The CPWC is then defined as the time period between the two crop growth stages (CGS_x to CGS_y) and represents the length of weed control required to protect crop yield from more than a 5% yield loss.

background information that quantifies the competitive environment under which experiments are conducted. This additional knowledge is critical in extrapolating the results to other conditions and can serve as the basis for re-evaluating the CPWC both spatially and temporally. It is well known that many relationships studied in agroecosystems are variable and dependent on the environmental conditions, but this should not hinder the development of the guidelines that can be useful to practitioners. The beginning and duration of the CPWC can vary depending on several factors, including the characteristics of the crop and the weeds, the environmental variables (Hall et al. 1992), the cultural practices, and the assumptions made regarding the methods employed to determine the CPWC.

Crop and Weed Characteristics

Because of species-specific differences in morphology, physiology, and development, the CPWC is likely to be unique for every crop. By similar reasoning, one would expect the CPWC for a given crop to vary with the composition and density of the weed population as well as with the time of emergence relative to the crop. For example, it is likely that large differences in the CPWC within the same crop may be observed when the crop is in competition with annual vs. perennial weed species. Most reported studies involving the determination of CPWC have been based on the outcome of the competition between annual weed species and the respective crop (Halford et al. 2001; Hall et al. 1992; Van Acker et al. 1993; Zimdahl 1980, 1988). The effect of the duration of competition with perennial weed species is still unclear as their control can be complicated by the persistence of both seeds and vegetative propagules (Lemieux et al. 1993; Schimming and Messersmith 1988). Vegetative propagules may provide an advantage to weeds in terms of early-season growth rate and resource acquisition. It is plausible that the control of perennials is required regardless of the crop developmental stage to prevent weed populations from reaching unmanageable levels. Therefore, application of the CPWC concept may not be appropriate for all weed species. This is an area that needs further investigation, especially in no-till systems that can contain more perennial weeds (Wrucke and Arnold 1985).

Emergence patterns of the competing weed species and size of the weed seed bank are both important factors influencing the timing and length of the CPWC (Martin et al. 2001; Van Acker et al. 1993). The periodicity of weed emergence is a function of both the weed species and its interaction with the microenvironment (Forcella et al. 1997). A larger seed bank may result in greater weed density but has little effect on the time of weed emergence. Weed density appears to be more important in the determination of the beginning of the CPWC, whereas it has a less notable effect on its end (Martin et al. 2001). At very low weed densities there may be no CPWC. This was observed by Martin et al. (2001) in canola (*Brassica napus* L.) and Van Acker et al. (1993) in soybean. This should not be construed as to indicate that crop yield was not vulnerable but rather that the competitive environment was inadequate to pose a threat to crop yield. Selection of fields with moderate to high weed infestations can help avoid such problems in experiments. In addition, results from numerous weed threshold studies indicated that the relationship between density and yield loss is important but not consistent. In fact, most of the times the relative weed emergence time was more important than the actual weed density (Knezevic et al. 1994, 1997).

Environment

Studies illustrate that the competitive ability of crops and weeds is heavily dependent on the environmental conditions (Lindquist et al. 1999). In addition to influencing the emergence patterns, the environment can play a large part in regulating the crop–weed competitive relationships. For example, weeds and crops respond differently to the variation in temperature, water availability, and soil fertility (Black et al. 1969). Hence, plant response to variations in each of these factors needs to be predicted.

Cultural Practices

The CPWC can vary depending on the management strategies that are imposed on the system. Growers can influence the biological system they work with through various management practices. One of the most critical factors affecting the CPWC is the time of planting. For example, early seeding of canola resulted in the need for a somewhat longer CWFP because field operations were conducted relatively early in the emergence period of the weeds resulting in higher weed infestation levels for a longer time (Martin et al. 2001). In addition, the manipulation of edaphic factors, including the alteration of soil nutrient supply and soil available water are believed to influence the crop–weed interference relationships, especially in determining the CTWR (Weaver et al. 1992). For example, the competitive ability of crops and weeds can be significantly influenced by the amount of fertilizer applied, its location, and the time of application (Di Tomaso 1995; Evans 2001; Tollenaar et al. 1994). Therefore, the understanding of the influence of the cultural practices on CPWC is an area that warrants further investigation.

Assumptions of Methodology

The methodology for determining the CPWC has assumptions, some of which affect weed control recommendations. These assumptions are generally associated with (1) the dynamics of weed species composition and the relative time of emergence, and (2) the way in which the CPWC is determined from two separately measured components.

It is typically assumed that weed emergence, species composition, and density are spatially and temporally uniform across the experiment. Previous experience dictates that emergence and growth of a weed are less consistent than those of a crop, and weed species composition is spatially variable. Therefore, it is not uncommon for experimental data to exhibit a high degree of variability, thus contributing to the inadequate description of the beginning and end of the CPWC. For example, a "late-beginning" of the CPWC is possible when crop yield losses and weed competitiveness are lower than anticipated because of late weed emergence. Similarly, an "early-end" of the CPWC can occur when there is a lower than anticipated crop loss caused by a lack of later-season weed emergence in some of the experimental units (S. Z. Knezevic, unpublished data). In both situations the outcomes are circumstantial and do not adequately identify the time when crop yield is most vulnerable. However, although these scenarios occur in many studies they do not necessarily reflect typical conditions and cannot be well explained by commonly measured environmental data.

The CPWC is a single measure of time, which is inferred rather than verified from two separately measured, distinctly different competition components. Therefore, the likelihood of substantial error increases (Weaver 1984) and reduces the accuracy of the estimate. Both the beginning and the end of the CPWC are determined independent of the other. The yield loss caused by the delay in the beginning of weed control is not accounted for in determining the end of the CPWC. Therefore, a CPWC based on AYL of 5% may in reality result in a yield loss that is slightly greater than 5% of the weed-free condition. In addition, because two separately measured components are used to determine the CPWC, it is possible that these two components will not overlap in such a way that a single discrete weed control period can be defined (Martin et al. 2001; Roberts 1976; Van Acker et al. 1993). This generally occurs when a yield loss of more than 10 to 20% is used to calculate the CPWC but has been observed at yield loss levels as low as 2% (Hall et al. 1992). This can create the potential to determine an end of the CPWC before its beginning, which indicates that a single weed removal is sufficient to prevent yield loss.

These errors and outcomes may be reduced through careful selection of the study location and the experimental methods. But some of these outcomes are at least partially unavoidable.

We believe that the CPWC can be used as a general decision aid as long as its limitations are recognized. We recommend its use as a general rule of thumb to help practitioners decide on the need for and timing of weed control. Producers desire general guidelines for timing POST weed control to verify their own intuition (J. Mulliken, personal communication). Currently, there is no such tool available. In all fairness to other decision support approaches (e.g., weed thresholds models) and despite the extensive research conducted in the past 30 yr, weed scientists have not improved the potential for producers to use herbicides in conjunction with alternative weed management strategies (Owen 1998). The CPWC has the potential for practical use because most producers and practitioners can understand its meaning and utility (J. Mulliken, personal communication; M. Pavlik, personal communication).

Adoption of the CPWC is currently underway in some parts of the Midwest (J. Mulliken, personal communication; M. Pavlik, personal communication; S. Keck, personal communication) because it is believed that it can help producers and the herbicide industry and is biologically and environmentally sound. Information on the general timing of weed control provides an economic incentive to producers through the optimization of weed control operations and to industry through the marketing of herbicide programs (J. Mulliken, personal communication; M. Pavlik, personal communication; S. Keck, personal communication).

Experimental Approach to Determine the CPWC

Two approaches are commonly used to determine the timing of weed control. Many authors have suggested that for practical purposes the timing of weed control should be based on the weed height (Dalley et al. 1999; Gower et al. 1999; Kalaher et al. 2000), although a few prefer to use crop growth stage (Evans and Knezevic 2000; Knezevic and Lindquist 1999; Martin et al. 2001). For example, Dalley et al. (1999) suggested that yield losses started occurring when the weeds were 30 cm tall in 1998 and 15 cm tall in 1999, without mentioning the respective size or growth stage of the crop. Evans (2001) observed that weed height varied considerably among years and locations because of the relative time of emergence, the variable mixture of weed species, and environmental and soil variables. We argue that weed height does not provide sufficient information for timing weed control unless it is coupled with the crop growth stage. In addition, the height of a weed canopy can be quite variable, particularly in mixed communities. The practical value of weed height is primarily for adjusting the dose of the herbicide (e.g., smaller weeds may require less than the label rate). Therefore, we suggest that from a practical standpoint, the CPWC should be based primarily on the crop growth stage. Weeds are controlled to protect the crop. Therefore, the crop should be the focus of the program. Depending on whether weeds emerge before or after the crop, the CPWC can be adjusted on the basis of differences in their relative emergence time (Evans et al. 2002). Similarly, Kasaian and Seeyave (1969) have suggested that the first 25 to 33% of the crop life cycle should be considered a critical time for weed control. Weed interference should be kept at a minimum for both field and vegetable crops, regardless of the weed spectrum or density.

Reporting the CPWC primarily on the basis of the crop growth stage can be easily adopted by crop producers and agricultural professionals (J. Mulliken, personal communication). Currently, crop consultants in some parts of Nebraska are already making recommendations for the timing of glyphosate application based on the growth stage of glyphosate-resistant soybean (J. Mulliken, personal communication; M. Pavlik, personal communication; S. Keck, personal communication). Because crop–weed interference relationships are complex and dynamic, the adoption of the suggested approach may at least account for the phenological development of the crop. Similarly, others suggested that CPWC should be related to the crop growth stage to account for the environmental variation (Hall et al. 1992; Martin et al. 2001; Van Acker et al. 1993). By linking the CPWC to the crop growth stage, a better understanding of the mechanistic causes for the timing and length of the CPWC can be achieved. For example, Van Acker et al. (1993) found that the beginning of the CPWC coincided with the beginning of the reproductive development for soybean. Such an approach would also make a weed control decision a function of biological necessity, rather than the availability of a herbicide, especially in cropping systems using HTCs.

Typical Variables

Crop–weed interference relationships can vary greatly. Therefore, there is a minimum amount of data that should be collected in CPWC studies. Important variables include crop yield at physiological maturity, weed species composition, weed density, and the date of weed and crop emergence in every experimental unit. Additionally, weekly staging and height measurements of the crop and weeds, maximum weed biomass, and environmental variables such as daily rainfall, average daily temperature (soil and air), soil moisture, and nutrient status should also be acquired. These data provide background information that quantifies the competitive environment in which the experiment was conducted. This knowledge is critical in extrapolating the results to other conditions and may help producers adjust the CPWC spatially and temporally.

Number of Data Points

The regression models we suggest below are based on three regression coefficients. Therefore, at least four data points are needed for fitting the curves that relate crop yield to the time of weed removal. We suggest that six to seven data points are ideal. The additional data points facilitate the testing of differences in curves among years and locations.

Appropriate selection of the times of weed removal is critical for determining the shape of the curves. On the basis of our experience and the summaries of previous studies (Zimdahl 1980), the following can be used as general guidelines for selecting the appropriate times for weed removal: (1) select two to three points clustered around the early part of the season to best determine the suspected beginning of the CPWC, and (2) select two to three points clustered around the time of crop canopy closure to best determine the end of the CPWC. One data point can be in the general area where one assumes that the two curves cross one another.

General Approach for the Suggested Statistical Analysis

We suggest the following steps in the analysis:

(1) Basic analysis of variance (ANOVA): The statistical analysis should start with ANOVA to evaluate the treatment effects on a crop's actual and relative yield. The mixed models procedure (PROC MIXED) in the SAS[1] statistical package is preferred over the use of the general linear models procedure (PROC GLM) for the following reasons—(a) The general linear models procedure was designed primarily to conduct ANOVA using a model encompassing only fixed effects (Littell et al. 1996). In the past, PROC GLM has been modified by the use of optional statements to calculate pertinent statistics. However, in some cases, fixed effect models cannot be modified easily. (b) In addition, missing or unbalanced data are not appropriately handled in PROC GLM. Therefore, even when only fixed effects are involved, PROC MIXED may be the best procedure for ANOVA.

(2) Nonlinear regression analysis: Treatment comparisons should be made using regression analysis. It is a more appropriate method for analyzing structured data than multiple comparison tests (MLCT). The use of MLCT such as the least significant difference, Duncan's multiple range, and Student–Newman–Keuls test are not appropriate for structured data because a 10 to 20% yield difference may occur among treatments before these tests are able to separate them. This type of analysis often results in the calculation of a CPWC that is a statistical entity with little biological or economic significance (Cousens 1988). Time of weed removal (*x*-axis) should be related to actual or relative crop yield (*y*-axis) using regression analysis. We favor the use of growing degree days (GDD) accumulated from crop emergence or planting as the unit for time to quantify the duration of weed presence and length of the weed-free period (*x*-axis). The use of GDD can also help in determining the CPWC on the basis of the respective crop growth stage for practical purpose because the rate of crop development is well correlated with the thermal time. Overall, the use of GDD in the regression is preferred because it (a) is a more biologically meaningful measure of time needed for plant growth and development than some other indicators (e.g., days or weeks after crop emergence) (Gilmore and Rogers 1958), (b) can be a useful means for comparing data from different locations, years, and planting dates, and (c) provides a continuous and precise scale for the *x*-axis. For example, an *x*-axis based solely on the crop growth stage is a continuous scale only during the stages of vegetative growth (e.g., leaf stages 1 to 15), but it becomes uncertain during reproductive growth (e.g., beginning or midpoint of grain filling). The use of GDD also allows for the comparison of the CPWC among crops, which allows for the investigation of noncrop–related reasons for the timing of the CPWC. For example, some authors have noted that the end of the CPWC (i.e., CWFP) is strongly related to the emergence periodicity of the weed complex (Martin et al. 2001; Van Acker et al. 1993), which may be independent of the crop species and more a function of the time of crop seeding.

To conduct the nonlinear regression, we suggest using PROC NLMIXED (SAS 1999), which was utilized in the steps outlined subsequently. This mixed models procedure is useful for the nonlinear regression of data acquired from studies where the experimental design introduces greater than one source of random error. However, if only one source of random error is inherent to the experimental design, then there are many software programs available that are well equipped to conduct nonlinear regression. Examples of such nonlinear regression packages include the PROC NLIN procedure (SAS 1999) in the SAS package, S-PLUS,[2] Axum,[3] and FIG-P.[4]

We suggest the use of a form of the logistic equation modified slightly from that proposed by Hall et al. (1992) to describe the increasing duration of weed interference on relative yield (Figure 1A, weedy curve):

$$Y = [(1/\{\exp[c \times (T - d)] + f\}) + [(f - 1)/f]] \times 100 \quad [1]$$

where Y is the yield (% of season-long weed-free yield), T is the time (*x*-axis expressed in GDD or days after emergence [DAE]), d is the point of inflection (GDD), c and f are constants.

The Gompertz model has been shown to provide a good fit to yield as it is influenced by increasing length of the weed-free period (Figure 1B, weed-free curve) (Hall et al. 1992):

$$Y = a \exp(-b \exp(-kT)) \quad [2]$$

where Y is the yield (% of season-long weed-free yield), a is the yield asymptote, b and k are constants, and T is the time (*x*-axis expressed in GDD or DAE).

Many variations in the logistic and Gompertz models exist (Ratkowsky 1990). From a statistical standpoint, the model that is chosen should provide the best fit to the data. Although this article focuses on an analysis with the above models, we do offer a suggestion for determining goodness of fit in the sections that follow.

The aforementioned nonlinear regressions are conducted using an iterative procedure that requires initial estimates of the regression parameters as input. For models with parameters that have biological significance, estimates can be obtained from visual evaluation of the data plot. For example, the parameter a of the Gompertz model (Equation 2) defines the upper asymptote of actual or relative yield. Therefore, logical estimates would be the mean of the observed values for the season-long weed-free yield, or if the relative yield is the response variable, then 100 would be the theoretical maximum value. For more complex models such as the logistic equation (Equation 1), starting values may be obtained using of a range of values obtained from the literature or by the derivative technique similar to that described by Bates and Watts (1988).

Most studies report the use of an arbitrary AYL of 2 to

10% to signify the beginning and the end of the CPWC. This range allows a producer or crop consultant to adjust the CPWC to the risk he or she is willing to take. Such decisions should consider the economics of weed control including the cost of the herbicide and its application, crop price, and anticipated gain from control. In all the following examples, an AYL of 5% was used as a threshold to determine the beginning and the end of the critical period, as presented in Figure 1C.

Example 1: Analysis of a Replicated Split-Plot Design with Fixed Block Effects

The split-plot design (SPD) is well suited for experiments that examine the effects of multiple levels of two-treatment factors. One factor is assigned as the whole-plot factor, which is then divided into split plots of the second factor. This creates an experiment where each whole plot becomes a block for the levels of the split-plot factors. In a split-plot model, there are two sources of variation: the variation among whole plots (whole-plot error) and the variation among the split plots (split-plot error). The observations within whole plots are correlated with each other. Standard nonlinear regression programs make the assumption that all observations are uncorrelated and that only one source of random error is inherent to the experimental design (Gumpertz and Rawlings 1992). If these techniques are used to analyze a SPD, the comparisons made between the effects of whole-plot factors cannot be validly constructed and estimates of the model parameters and their standard errors may not be correct. We present a method for the nonlinear regression analysis of data from a SPD that accounts for the multiple error terms encountered in such a design.

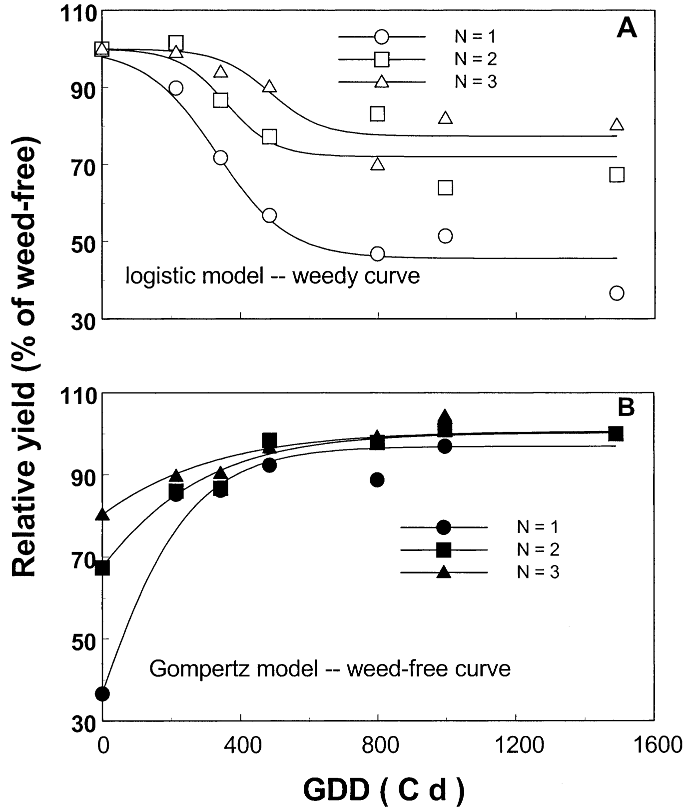

FIGURE 2. Graphical representation of least squares means of corn relative yields from a split-plot experimental design investigating the influence of nitrogen level on the critical period for weed control. Data are presented separately for each nitrogen (N) level as characterized by the fitted regression lines for (A) the logistic model (weedy curve) fit to data representing an increasing duration of weed interference and (B) the Gompertz model (weed-free curve) fit to data representing an increase in the length of weed-free period. Lines represent the predicted regression lines as determined from the mixed models regression procedure.

Case Study: Effect of Three Nitrogen Rates on the CPWC in Corn

This experiment was laid out in a standard SPD. Because there was greater interest in the effects of the timing of weed removal or duration, nitrogen (N) application rates were assigned to the whole plots and the split plots consisted of five treatments of increasing weed duration used to determine the CTWR and five treatments of increasing length of the weed-free period used to determine the CWFP. Three N application levels were utilized, hereafter referred to as N level 1, 2, and 3. The levels of the split-plot factor were randomized within each N level, and there were four replicates. The treatments of increasing the duration of weed interference and the length of the weed-free period up to the predetermined corn growth stages (V3, V6, V9, V15, R1) were compared with season-long weed-free and season-long weedy controls established for each N level. Because the experiments were conducted at locations not randomly selected, effects of blocks (i.e., replicates) were assumed fixed. Specific details regarding the experimental site and the procedures are not provided because the focus of this study is not to discuss the biological interpretation of the results.

Figures 2A and 2B are the graphical representations of the N rate by timing of weed control (N × trt) least square means (LSMs) for the observed data used to fit the logistic and Gompertz models. The LSM values were obtained from the ANOVA (Table 1, Step 2). Differences in curves among N levels were tested to determine if they are in phase with one another (i.e., are the curves parallel). Significant differences in any of the model parameters between N levels would indicate that the curves are out of phase (i.e., not parallel). This test is easily performed using the NLMIXED procedure (Table 1). The procedure is run separately for each of the nonlinear models, once for the Gompertz model (Table 1, Step 5) and once for the logistic model (Table 1, Step 6).

The first step in the procedure is to classify the experimental units (Table 1, Step 1, Lines 003 to 008). Lines 003 to 005 create identifiers for the fixed effects of each of the four replicates. The fourth block is not specified; it can be estimated because of the constraint that the sum of all the block effects is zero. This is an acceptable approach only if blocks are considered as fixed effects. Lines 006 to 008 establish identifiers for each of the three N levels (whole plots), which are in1, in2, and in3 to identify N levels 1, 2, and 3, respectively. The second step is to conduct an ANOVA using the mixed linear models procedure (Table 1, Step 2, Lines 014 to 020). The output from the ANOVA (Lines 022 to 023) provides estimates of the random error effects where the whole-plot error is equivalent to N × blk and the split-plot error is equivalent to Residual. These estimates of the random error provide good starting values for s2w and s2e in the nonlinear procedures to follow. In this example, variance estimates are calculated with all experimental data. In some cases, the variance estimates for data used to determine the beginning of the CPWC might be considerably different when compared with that used to determine the end of the CPWC. In such circumstances, better starting values may be obtained by subjecting each set of data to ANOVA separately.

In addition to providing estimates for the random error terms, the ANOVA gives some indication as to the influence of the treatment effects on relative yield. The treatments (i.e., timing of weed removal and length of weed-free period) have a significant effect on the relative yield (Line 026) $P < 0.0001$, indicating that regression may be appropriate, and upon investigation of the data it is apparent that the relationships are nonlinear (Figure 2). The N level also has a significant effect on the relative yield (Line 25) $P = 0.0186$. Therefore, a regression analysis will likely reveal significant differences in the model parameters for N levels. Similarly, if ANOVA showed a significant interaction between the N rate and the timing of weed control ($N \times$ trt), the analysis would need to segregate the data for the three N levels. Single degree of freedom contrasts are constructed by the "lsmeans . . . /diff" statements (Lines 018 to 020). The output from these comparisons has been omitted from Table 1 but can be used to compare similar treatments across N levels. For example, one could compare the extent of yield loss for corn in competition with weeds for the entire season (season-long weedy) and determine if it is higher under any specific N regime.

The data are then sorted into two subsets depending on whether the data corresponds to the weed-free periods or duration of weediness (Table 1, Step 3, Lines 028 to 041 and 043 to 056). Observations from the season-long weedy and season-long weed-free controls are included in both subsets. Lines 042 and 057 create the variable "whole," which is the combined fixed effect of replications (blk) and N levels. Therefore, the variable whole identifies each whole-plot experimental unit. The numerical value of 10 is simply a multiplication factor that provides a unique identifier for each whole plot ($N \times$ blk). This variable is defined for the analysis of both the models.

In Step 4, the data are plotted (data not shown). Plotting all data allows a visual evaluation to spot outliers and possible heterogeneity of variances. In most cases, variances are not expected to be independent of the timing of weed control, especially in studies utilizing naturally occurring weed populations. Perhaps, more critical is that such heterogeneity is independent of the N level. Accounting for the heterogeneity of variances in a complicated experimental design is possible (Carroll and Ruppert 1988) but rather difficult and beyond the scope of this study.

In Steps 5 and 6, a mixed nonlinear model procedure (PROC NLMIXED) is used to perform the regression on the Gompertz and logistic models, respectively. The general framework for the mixed regression models in the procedure outlined is that the response variable (y) can be expressed by the following relationship:

$$y = \text{mean model} + \text{variability model} \qquad [3]$$

In Lines 069 to 073 and 120 to 124, the parameters to be used in the mixed model are identified and starting values are specified. Starting values for the Gompertz and logistic models were obtained from reported values in the literature (Hall et al. 1992) and refined using a parameter grid search (data not shown) in the NLIN procedure (SAS 1999). The parameters s2w and s2e represent the main-plot ($N \times$ blk) and split-plot (Residual) random error terms, respectively. These starting values were obtained from the ANOVA output (see output for Step 2). In Lines 075 and 126, the Gompertz and logistic models are expanded to contain all fixed effects with the exception of the effects of blocking. This model defines the treatment mean (muij) without adjustments for blocking or random error. In Lines 076 and 127, blocking effects and random whole-plot errors are added to the treatment in the creation of the variable "temp." In Lines 077 and 128 the distribution of the response variable relative yield (ryld) is defined. We assume a normal distribution with a mean of temp (i.e., the treatment mean as it is influenced by all fixed effects and the whole-plot error) and a variance of s2e. The distribution of the whole-plot error (wpe) is specified in Lines 078 and 129. The random effect associated with whole plots is assumed to follow a normal distribution with a mean of 0 and a variance of s2w.

Estimate statements are constructed (Table 1, Lines 079 to 087 and 130 to 138) to compare the model parameters among whole plots. These contrasts are used to test the differences between effects of N levels on relative yield. The denominator degrees of freedom (df) used for these tests are calculated as follows:

$$\text{df} = (N_i - 1) - P_i \qquad [4]$$

where N_i is the total number of nonmissing observations used and P_i is the number of parameters to be estimated (excluding parameters for the random effects) for the ith model. A total of 84 nonmissing data points were used to fit both the Gompertz and logistic models. In addition, the expanded forms of both models contain 12 fixed-effect parameters. Therefore, for each model, $\text{df} = (84 - 1) - (12) = 71$ for the denominator.

Major results of the regression analysis are presented for the Gompertz and logistic models in Table 1, Lines 089 to 117 and 140 to 168, respectively. Some of the output such as the iteration record has been omitted for brevity. The first set of output is the information regarding the fit statistics of the nonlinear model used (Lines 089 to 094 and 140 to 145). The Akaike's information criterion provided in the output is a statistic that can be used to compare the goodness of fit of two similar models. As previously stated, many forms of Equations 1 and 2 could be used to describe the classical nonlinear response of yield to the timing of weed removal. In this case, the best-fit statistics were produced with the logistic equation presented in Equation 1. Lines 095 to 108 and 146 to 159 give the parameter estimates, their associated standard errors, and 95% confidence limits. These estimates are used to plot the estimated regression relationships in Figure 2.

The results of the contrasts (Lines 109 to 117 and 160 to 168) are used to determine differences between the N levels. For the Gompertz model, most parameters (e.g., a, k) were not significantly different, except the b parameter when contrasted across all N levels. For example, parameter b was significantly different ($P = 0.0017$ and $P = 0.0015$) for the $n = 1$ response curve when compared with the response curves of $n = 2$ and $n = 3$ levels, respectively (Lines 112 and 113),

Table 1. Analysis of a split-plot design with fixed block effects to determine the critical period for weed control.

SAS program and output	Comments
Step 1: create SAS data set and classification variables	
`Data all;`	Names the input data set.
`input loc$ N trt blk ryld;`	Assigns variable names to the data.

`ib1=(blk=1)-(blk=4);`
`ib2=(blk=2)-(blk=4);`
`ib3=(blk=3)-(blk=4);`
	Defines classification variables for the four replications of the experiment. Replication #4 is set at zero by default so that the effects of the other replications are estimable. This step is required for the nonlinear regression procedure.

`in1=(n=1);`
`in2=(n=2);`
`in3=(n=3);`
	Defines classification variables for the three nitrogen(N) levels of the experiment. This step is required for the nonlinear regression procedure.

`cards;`	Indicates the data lines will follow.

`M1 1 1 1 44.31`
`...`
`M1 3 12 4 101.49;`
	The raw data set (142 data points not shown).

`run;`	Initiates above command sequence.
Step 2: ANOVA	
`Proc mixed data=all;`	ANOVA using a mixed linear model.
`class N trt blk;`	Classifies the variables used in the model.
`model ryld = blk N trt N*trt/ddfm=satterth;`	Partitions fixed effects [blocks (blk), nitrogen levels (N), and duration of weedy or weed-free period (trt)] and relevant interactions.
`random N*blk;`	Defines whole-plot random error term.
`Lsmeans N/diff;`	Calculates N means and paired *t* tests.
`Lsmeans trt/diff;`	Calculates trt means and paired *t* tests.
`Lsmeans N*trt/diff slice=N slice=trt;`	Calculates N*trt means and paired *t* tests.
`run;`	
Output from step 2	
Covariance parameter estimates Cov parm Estimate N*blk 15.1632 Residual 225.48	N*blk is the estimate of whole-plot error (s2w). Residual is the estimate of the split-plot error (s2e).
Type 3 Tests of fixed effects Effect Num DF Den DF F Value Pr > F Blk 3 6 1.79 0.2482 N 2 6 8.33 0.0186 trt 11 99 10.67 <.0001 N*trt 22 99 1.22 0.2523 Output for LSMand *t* tests not shown	Effect of blocks (replications) is NS (0.05 level). Effect of N on response is SIG (0.05 level). Effect of timing of weed control is SIG (0.05 level) Interaction is NS (0.05 level).
Step 3: sort data into two data sets to be used to fit the Gompertz and logistic models separately	
`data gompertz;` `set all;`	Creates new data set from the initial data set to identify data to be used in fitting the Gompertz equation.

`if trt=1 then GDD=0;`
`if trt=2 then delete;`
`if trt=3 then delete;`
`if trt=4 then delete;`
`if trt=5 then delete;`
`if trt=6 then delete;`
`if trt=7 then GDD=1490;`
`if trt=8 then GDD=214;`
`if trt=9 then GDD=342;`
`if trt=10 then GDD=485;`
`if trt=11 then GDD=799;`
`if trt=12 then GDD=996;`
	Defines values of the independent variable (GDD) to be used to fit the Gompertz model and removes data associated with other treatments used to fit the logistic model. In this case, the value of GDD refers to the duration that the crop was maintained weed-free from the time of planting (length of weed-free period).

`whole=10*blk+N;`	Combined effects of blocks and N level to be imposed on the overall mean.

data logistic; set all;	Creates new data set from the initial data set to identify data to be used in fitting the logistic model.

if trt=1 then GDD=1490;
if trt=2 then GDD=214;
if trt=3 then GDD=342;
if trt=4 then GDD=485;
if trt=5 then GDD=799;
if trt=6 then GDD=996;
if trt=7 then GDD=0;
if trt=8 then delete;
if trt=9 then delete;
if trt=10 then delete;
if trt=11 then delete;
if trt=12 then delete;
	Defines GDD to be used to fit the logistic model and removes data associated with other treatments used to fit the Gompertz model. In this case, the value of GDD associated with each treatment corresponds to the duration of weed competition from planting (duration of weed interference).

whole=10*blk+n;	Previously defined.
run;	
Step 4: plot the data for each data set	
proc gplot data=gompertz; by N; plot ryld*GDD;	Plots relative yield as a function of GDD for data used to fit the Gompertz model separately for each N level.
run;	
proc gplot data=logistic; by N; plot ryld*GDD;	Plots relative yield as a function of GDD for data used to fit the logistic model separately for each N level.
run;	
Step 5: nonlinear regression analysis: Gompertz model	
proc nlmixed data=gompertz;	Applies mixed-model nonlinear regression procedure to data used to fit the Gompertz model.
Parms blk1=0 blk2=0 blk3=0 a1=100 a2=100 a3=100 b1=1.1 b2=1.3 b3=1.2 k1=0.0001 k2=0.0001 k3=0.0001 s2w=15.1632 s2e=225.48;	Starting values for parameters of Gompertz model. Effect of replication (assumes ∑blk=0). Starting values for parameter 'a' for each N level. Starting values for parameter 'b' for each N level. Starting values for parameter 'k' for each N level. Starting values for parameters 's2w' (whole-plot error) and 's2e' (split-plot error) from ANOVA output.
bloc=blk1*ib1 + blk2*ib2 + blk3*ib3;	Defines the combined fixed effect of blocks (replicates) where the effect of blk4 can be estimated under the constraint that ∑blk=0.
muij=(a1*in1+a2*in2+a3*in3)*exp(-(b1*in1+b2*in2+b3*in3)* exp(-(k1*in1+k2*in2+k3*in3)*(GDD));	Expanded form of the Gompertz model that takes into account the effect of N level and duration of weed-free period on the treatment mean, i.e., the treatment mean before effects of blocking or random effects are added. This is the mean effect model.
Temp=bloc+muij+wpe	The mean effects model after adding fixed effects of blocks and random effects associated with the whole-plot experimental units.
model ryld~normal(temp,s2e);	Describes the distribution of the response variables. A normal distribution is assumed with a mean of 'temp' and variance of 's2e'.
Random wpe~normal(0,s2w) subject=whole;	Describes the distribution of the whole-plot error (wpe), assumed to be normally distributed with a mean of '0' and a variance of 's2w'. The subject statement identifies the experimental units to which the error corresponds.

estimate 'a1 vs a2' a1-a2 df=71;
estimate 'a1 vs a3' a1-a3 df=71;
estimate 'a2 vs a3' a2-a3 df=71;
estimate 'b1 vs b2' b1-b2 df=71;
estimate 'b1 vs b3' b1-b3 df=71;
estimate 'b2 vs b3' b2-b3 df=71;
estimate 'k1 vs k2' k1-k2 df=71;
estimate 'k1 vs k3' k1-k3 df=71;
estimate 'k2 vs k3' k2-k3 df=71;
	Estimate statements to test differences in parameters between the three N levels. The degrees of freedom (df) for the tests are computed by the difference between the total df (N-1) and the number of fixed effect parameters estimated by the expanded form of the model. In this case, the total df = 84 - 1 = 83, where N is the total number of observations used to fit the model. The number of parameters used to fit the model is 12. Therefore, the df utilized for the estimates is 83 - 12 = 71 for the denominator.

run;	

Output from step 5

```
        Note: GCONV convergence criterion satisfied.
                   The NLMIXED procedure
                      fit statistics
          Description                           Value
89        -2 Log likelihood                     674.7
90        AIC (smaller is better)               696.7
91        BIC (smaller is better)               723.5
92        Log likelihood                       -337.4
93        AIC (larger is better)               -348.4
94        BIC (larger is better)               -361.7
                    Parameter estimates
                   Std                         95%      95%
     Parm     Est     error  DF  Pr>|t|     Lower    Upper
95   blk1   -0.4651  2.9293  83  0.8742   -6.2914   5.3612
96   blk2   -4.3664  2.9318  83  0.1402  -10.1977   1.4648
97   blk3    6.7702  2.9268  83  0.0232    0.9489  12.5916
98   a1     96.0600  4.3867  83  <.0001   87.3339 104.7800
99   a2    100.3600  5.3412  83  <.0001   93.7391 110.9900
00   a3    101.0900  5.9295  83  <.0001   94.7891 111.8547
01   b1      0.9647  0.2053  83  <.0001    0.5564   1.3731
02   b2      0.3956  0.1116  83  0.0007    0.1735   0.6176
03   b3      0.2259  0.0912  83  0.0154    0.0443   0.4074
04   k1      0.0060  0.0019  83  0.0010    0.0027   0.0104
05   k2      0.0041  0.0017  83  0.0194    0.0006   0.0076
06   k3      0.0034  0.0020  83  0.1014   -0.0006   0.0075
07   s2w    14.7530 28.4372  83  0.6053   -41.807   71.3134
08   s2e    225.07  28.4372  83  <.0001    168.51   281.630
                   Additional estimates

     Label        Est    Std error DF  Pr>|t|   Lower      Upper
09   a1 vs a2  -4.3037    5.9173  71  0.5358  -14.096    6.4889
10   a1 vs a3  -5.0291    6.3972  71  0.4988  -16.778    4.2345
11   a2 vs a3  -0.7253    5.9767  71  0.9278  -12.546    5.7899
12   b1 vs b2   0.5692    0.2337  71  0.0017    0.0035   1.0348
13   b1 vs b3   0.7389    0.2247  71  0.0015    0.0012   1.1865
14   b2 vs b3   0.1697    0.1442  71  0.0024    0.0016   0.4570
15   k1 vs k2   0.0019    0.0026  71  0.3449   -0.0027   0.0076
16   k1 vs k3   0.0026    0.0028  71  0.2612   -0.0024   0.0088
17   k2 vs k3   0.0007    0.0026  71  0.7876   -0.0046   0.0061
```

Fit statistics may be used to compare adequacy of different nonlinear models used to describe the data.

Estimates used to parameterize the model separately for each N level. Graphical depiction in Figure 2B.

's2e' is equivalent to the residual mean square error of the regression.

Estimates provide means to test differences in model parameters between N levels. Significant differences in any of the parameters would justify fitting regression separately for each N level. In this example, the parameter 'b' is significantly different between all three N levels, indicating nonparallel curves.

Step 6: nonlinear regression analysis: logistic model

18	`Proc nlmixed data=logistic;`	Applies mixed nonlinear regression procedure to data used to fit the logistic model.

19
 	
20
21
22
23
24
	`parms`
	
	`blk1=0 blk2=0 blk3=0`
	`c1=0.0100 c2=0.0165 c3=0.0120`
	`d1=270 d2=270 d3=375`
	`f1=1.8112 f2=3.5630 f3=4.4812`
	`s2w=15.1632 s2e=225.48;`
		Starting values for parameters of logistic equation.
		Effect of replication (assumes $\sum$blk=0).
		Starting values for parameter 'c' for each N level.
		Starting values for parameter 'd' for each N level.
		Starting values for parameter 'f' for each N level.
		Starting values for parameters 's2w' (whole-plot error) and 's2e' (subplot error) from ANOVA output.

25	`bloc=blk1*ib1+blk2*ib2+ blk3*ib3;`	Previously defined.

26	`muij=((1/(exp((c1*in1+c2*in2+c3*`
	`in3) *(GDD-`
	`(d1*in1+d2*in2+d3*in3)))`
	`+(f1*in1+f2*in2+f3*in3)))`
	`+((f1*in1+f2*in2+f3*in3)-1)`
	`/(f1*in1+f2*in2+f3*in3))*100;`
		Expanded form of the logistic model that takes into account the effect of N level and duration of weed-free period on the treatment mean, i.e. ,the treatment mean before effects of blocking or random effects are added. This is the mean effects model.

27	`temp=bloc+muij+wpe;`	Previously defined.
28	`model ryld~normal(temp,s2e);`	Previously defined.
29	`random wpe~normal(0,s2w)` `subject=whole;`	Previously defined.

as well as the contrast of $n = 2$ vs. $n = 3$ (P = 0.0024, Line 114). Because the parameter b is significantly different among all three response curves, we can reject the null hypothesis that the curves are parallel. Therefore, a separate curve should be used to describe the influence of the CWFP on the yield for each of the three N levels.

For the logistic model (Lines 160 to 168), the f parameter is significantly different for all curves when compared across

Table 1. Continued.

```
estimate 'd1 vs d2' d1-d2 df=71;
estimate 'd1 vs d3' d1-d3 df=71;
estimate 'd2 vs d3' d2-d3 df=71;
estimate 'c1 vs c2' c1-c2 df=71;
estimate 'c1 vs c3' c1-c3 df=71;
estimate 'c2 vs c3' c2-c3 df=71;
estimate 'f1 vs f2' f1-f2 df=71;
estimate 'f1 vs f3' f1-f3 df=71;
estimate 'f2 vs f3' f2-f3 df=71;
```

Estimate statements to test differences in parameters between the three N levesl. The df for the tests are computed by the difference between the total df (N-1) and the number of parameters estimated by the expanded form of the model. In this case, the total df = 84 - 1 = 83, where N is the total number of observations used to fit the model. The number of parameters used to fit the model is 12. Therefore, the df utilized for the estimates is 83 -12=71 in the denominator.

```
run;
```

Output for step 6

```
          NOTE: GCONV convergence criterion satisfied.
                    The NLMIXED procedure
                       fit statistics
             Description                      Value
             -2 Log likelihood                 692.9
             AIC (smaller is better)           720.9
             BIC (smaller is better)           755.0
             Log likelihood                   -346.5
             AIC (larger is better)           -360.5
             BIC (larger is better)           -377.5
                     Parameter estimates
```

Same as above.

```
Parm     Est      Std error  DF   Pr>|t|   Lower     Upper
blk1   1.2033     2.9340     83   0.6828   -4.63     7.03
blk2  -3.3526     2.9340     83   0.2565   -9.18     2.48
blk3   3.8352     2.9340     83   0.1948   -2.00     9.67
c1     0.00968    0.004632   83   0.0396   -0.01    -0.0004
c2     0.01444    0.01777    83   0.4188   -0.04     0.020
c3     0.01332    0.01404    83   0.3454   -0.04     0.014
d1     270.01     55.3037    83   <.0001    160.01  380.00
d2     270.04     94.4157    83   0.0054    82.25   457.83
d3     374.99     167.42     83   0.0278    42.00   707.97
f1     1.8374     0.1572     83   <.0001    1.52    2.15
f2     3.5799     0.6117     83   <.0001    2.36    4.79
f3     4.4481     0.9004     83   <.0001    2.65    6.23
S2w    15.3566    20.2106    83   0.4495   -24.84   55.55
S2e    225.67     20.2106    83   <.0001    185.48  265.87
                    Additional estimates
```

Estimates used to parameterize the model separately for each N level. Graphical depiction in Figure 2A.

's2e' is equivalent to the residual mean square error of the regression.

```
Label       Est      Std error DF   Pr>|t|   Lower      Upper
d1 vs d2  0.07725    109.28    71   0.991   -217.94    217.87
d1 vs d3 -104.98     176.40    71   0.552   -456.71    246.74
d2 vs d3 -104.95     192.28    71   0.583   -488.34    278.45
c1 vs c2 -0.0047     0.0183    71   0.794   -0.0318    0.0413
c1 vs c3 -0.0036     0.0148    71   0.801   -0.0258    0.0331
c2 vs c3  0.00117    0.0227    71   0.961   -0.0462    0.0440
f1 vs f2 -1.7426     0.6315    71   0.001   -1.0018   -0.4833
f1 vs f3 -2.6107     0.9140    71   0.001   -2.4332   -0.7882
f2 vs f3 -0.8682     0.0885    71   0.001   -1.0386    1.3023
```

Estimates provide means to test differences in model parameters between N levels. Significant differences in any of the parameters would justify fitting regression separately for each N level. In this example, the parameter 'f' is significantly different between all N levels. Therefore, separate regression curves should be used for all N levels.

all three N levels. There were no significant differences among N levels for the *d* and *c* parameters. Because the *f* parameter is different for all N levels, we can reject the null hypothesis that the curves are parallel. Therefore, a separate curve should be used to describe the influence of the duration of weed interference on yield for each of the N levels.

Theoretically, if the results of the aforementioned contrasts for parameter estimation were not significantly different among the N levels, then combining the data from these particular levels and fitting a curve through the combine data would be statistically appropriate and scientifically acceptable. However, this test may be conservative with variable data (Blankenship et al. 2002). Therefore, a more useful comparison is a *t* test comparing the point estimates of the beginning and end of the CPWC (Table 2) (Blankenship et al. 2002).

The final step in the analysis is to determine the CPWC on the basis of some level of AYL, traditionally 5% of the weed-free yield. Therefore, the value of the explanatory variable (T) that corresponds to a value of 95% for the response variable (Y) for each model must be determined. Estimation of an unknown value of an explanatory variable corresponding to a fixed value of the response variable poses exceptional problems because there is no estimate of the variance associated with the explanatory variable. Therefore, difficulty arises in the determination of a confidence interval of the point estimate and in testing the equality between two different point estimates. Fortunately, this type of calibration problem has been dealt with elsewhere (Schwenke and Milliken 1991). For the SPD analysis, the procedure proposed by Schwenke and Milliken (1991) can be modified to accommodate the variance structure of mixed models

TABLE 2. Point estimates and confidence limits (CI_{95}) corresponding to a 5% acceptable yield loss level for the logistic (weedy curve) and Gompertz (weed-free curve) models used to determine the beginning and end of the critical period for weed control in corn under three levels of nitrogen (N) fertilization. The point estimates are then related to and reported as the crop growth stage (CGS).

Model	N level	GDD[a]	CI_{95} lower[b]	CI_{95} upper[c]	CGS
Logistic (weedy curve)	1	96	94	98	1
	2	253	251	255	4
	3	315	313	319	6
Gompertz (weed-free curve)	1	537	531	544	11
	2	490	487	493	10
	3	394	390	397	8

[a] Growing degree days corresponding to 95% relative yield.
[b] 95% confidence interval—lower limit.
[c] 95% confidence interval—upper limit.

(Blankenship et al. 2002). This was the method used to calculate the point estimates for the beginning and end of the CPWC in this example (Table 2). An objective may be to determine the differences in the components of the CPWC between N levels. It is evident that both the beginning and the end of the CPWC are different for each N level. Ultimately, data analyzed in this manner could provide insight into the influence of different experimental factors on the CPWC components. Quantifying these effects would identify the explanatory mechanisms that control the variability between multilocation and multiyear experiments.

Example 2: Analysis of a Randomized Complete Block Design with Random Block Effects

The randomized complete block design (RCBD) is one of the most commonly used designs in weed research. The primary feature of the RCBD is the presence of complete blocks (replications) arranged in a way that reduces experimental error within the block. Frequently, more complicated experimental designs include components that are RCBDs in themselves. In Example 1 levels of the main plot factor were assigned to experimental units that were arranged as randomized complete blocks. However, the SPD is more complicated in that there are two sizes of experimental units. In the RCBD there is only one size of experimental unit and, therefore, potentially fewer sources of random error.

Case Study: CPWC in Glyphosate-Tolerant Corn

For the purpose of demonstrating the analysis for the RCBD, a portion of the study described in Example 1 was used. A data set representing only a single N level (e.g., $N = 1$) was selected. The treatments of weed removal or duration were the same as those in Example 1.

Table 3 contains a similar but less complex procedure than that in Table 1. The major difference is that the random error associated with the whole-plot factor is removed from the model, whereas the random error associated with blocks is incorporated into the model and the variance structure is appropriately modified. As in Example 1, the first step in the analysis is to conduct ANOVA using PROC MIXED (Table 3, Step 2). The treatment effect of duration of the weedy and weed-free periods on crop yield is the only fixed effect pertinent to the model. The only source of random error in addition to the experimental error variance comes from random blocks. This is taken into account in Line 04. In Lines 07 to 09, estimates of the random effect of blocks (blk) as well as the experimental error variance (Residual) are given. The significance of the effect of treatments in Line 09 suggests that relative yield (ryld) is significantly influenced by the length of weedy and weed-free periods. Steps 3 and 4 are the same as those in Example 1 (Table 1). Parts of Steps 5 and 6 that are the same as those in Example 1 (Table 1) include (1) providing starting values for the parameters for the Gompertz and logistic models, and (2) the parameter s2e is common for both models and is the estimate of the experimental error variance, respectively. Parts of Steps 5 and 6 that differ from Example 1 (Table 1) are presented in Table 3 and they include that (1) because blocks are considered random, independence must be assumed therefore unlike Example 1 where the blocks were fixed, effects of individual blocks cannot be appropriately estimated from the effects of the other blocks. The contribution of each block is specified in Line 10; (2) Line 11 is the nonlinear function that accounts for only the fixed effects of treatments; (3) the random effects of blocks (bloc) are added in Line 12; (4) the response variable (ryld) is assumed to be normally distributed with a mean of temp and a variance equal to s2e (Line 13); and (5) the distribution and variance structure of the random block effect is specified in Line 14, which makes the individual block effects (blk1, blk2, blk3, and blk4) random effects. These effects are normally distributed, all have a mean of 0, are uncorrelated (indicated by 0's in the variance matrix), and all have the variance s2b. The aforementioned Lines 10, 11, 12, 13, and 14 are applicable for the Gompertz model (Step 5). The same lines must be copied in the same order for the logistic model (Step 6). However, Line 11 should be replaced with Line 15 (Table 3). The output results for the RCBD analysis from Step 5 are not presented because they are very similar to the output of the same step in Table 1. The aforementioned examples show that the CPWC data collected from either type of experimental design can be analyzed in a way that is both biologically meaningful and statistically rigorous. The complexity of the experimental design should be dictated by the objectives of the study and the arrangement of the treatment factors.

Potential Value to Weed Scientists and Weed Control Practitioners

In this article, a simple method for determining the CPWC was illustrated. The statistical analyses of two experimental designs were demonstrated. If widely adopted, this method will allow for a much easier comparison of the results between experiments. The additional value of this data analysis approach is that it can be easily adopted for other types of studies using nonlinear regression.

Furthermore, if adopted by practitioners, the concept of CPWC has the potential to influence decision making on the timing of POST herbicide application on millions of hectares. For example, currently more than 60% of the 25 million ha of soybean grown in the United States annually are glyphosate-resistant cultivars (USDA/NASS

Table 3. Partial analysis of randomized complete block design with random block effects to determine the critical period for weed control.

	SAS program and output	Comments
	Step 1: create SAS data set and classification variables: same as in Table 1	
	Step 2: ANOVA	
1	`Proc mixed data=all;`	ANOVA using a mixed linear model.
2	`Class trt blk;`	Classifies the variables used in the analysis.
3	`Model ryld=trt/ddfm=satterth;`	Relative yield as influenced by timing of weed control.
4	`Random blk;`	Accounts for randomly assigned blocks.
5	`Lsmeans trt;`	Calculates trt means.
6	`Run;`	
	Output from step 2	
	Covariance parameter estimates Cov parm Estimate	
7	blk 3.6864	"blk' is estimate of the random effect of blocks (s2b).
8	Residual 281.28	'Residual' is the estimate of the experimental error variance (s2e).
	Type 3 tests of fixed effects Effect / Num DF / Den DF / F Value / Pr > F	
9	trt 11 33 6.76 <.0001	Timing and duration of weed control significantly influences relative yield.

	Step 3: sort data into two data sets to be used to fit the Gompertz and logistic models separately: same as in Table 1	
	Step 4: plot the data for each data set: same as in Table 1	
	Step 5: nonlinear regression analysis (Gompertz equation): presented only parts that differ from Table 1	
0	`bloc=blk1*ib1 + blk2*ib2 + blk3*ib3 + blk4*ib4;`	Defines the total random effect of blocks. Each block must be expressed separately under the assumption of independence.
1	`muij= a*exp(-b*(exp(-k*GDD)));`	The Gompertz nonlinear function accounting for fixed effects of length of weed-free period.
2	`temp=bloc+muij`	Treatment mean after the fixed and random effects are added.
3	`model ryld~normal(temp,s2e);`	Describes the distribution of the response variables. A normal distribution is assumed with a mean of 'temp' and variance of 's2e'.
4	`random blk1 blk2 blk3 blk4 ~ normal ([0,0,0,0],[s2b,0,s2b,0,0,s2b,0,0,0, s2b]) subject=blk;`	Describes the distribution of the random error associated with blocks. The errors are normally distributed have a mean of '0', and are uncorrelated (independent). The lower triangle of the variance matrix must be specified in row order for SAS to compute.
	Output for step 5: similar to output in Table 1	
	Step 6: nonlinear regression analysis (logistic model): presented only parts that differ from Table 1	
5	`muij=((1/(exp(c*(GDD-d))+f))+((f-1)/f))*100;`	The logistic nonlinear function accounting for fixed effects of length of weedy period.
	Output for step 6: similar to the output in Table 1	

2000). Some states or counties may have as high as 90% of the fields planted with glyphosate-resistant soybeans. The most common dilemma on these hectares is how to time POST weed control. We suggest that the concept of CPWC can guide producers in resolving the dilemma. Finally, we hope that this article will achieve a small improvement in statistical usage, as advocated by Cousens (1988, 1991).

Sources of Materials

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

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

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

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

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