**Additive Design: The Concept and Data Analysis**

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**Running title:** Additive design for weed competition

**Summary**

Crop-weed competition is extensively studied in weed science. The additive design, in which weed density varies, and the crop density is kept constant, is the most commonly utilized design in plant competition studies. The additive design is important to calculate economic weed thresholds and improve weed control decision making. The crop-weed competition studies are usually conducted by weed scientists, who sometimes report misleading conclusions because of lack of statistical knowledge needed for proper data analysis. Therefore, the objective of this manuscript is to provide the basics about the concept of additive design and demonstrate the model selection approach for describing crop-weed density relationship to non-statisticians. We evaluated three models routinely used in the literature to interpret data from additive designs, including polynomial quadratic, sigmoid, and rectangular hyperbola curves. Based on the described statistical criteria, we demonstrated the rectangular hyperbola to be the most appropriate model to describe data from an additive design study looking at *Richardia brasiliensis* and *Commelina benghalensis* competition with corn (*Zea mays* L.). We propose the use of the rectangular hyperbola as a standardized model for crop-weed competition in additive design. Moreover, we describe step-by-step how to perform the statistical analysis in R software and interpret the results of crop-weed competition studies.

**Keywords**: crop-weed competition, model selection, rectangular hyperbola, weed interference, weed threshold.

**Introduction**

One of the most common dilemmas that farmers and practitioners face is how decide on the timing of weed control operation, or just said: “when to spray an herbicide.” Before initiating weed control procedures, the following are some general guidelines to consider: field scouting and mapping weed patches and utilizing the concepts of the critical period of weed control, weed threshold, and decision support computer models. Field scouting typically involves assessing the type and number of weeds to determine if a spray operation is necessary. Mapping and monitoring weed patches over time will also help to determine the effectiveness of the control program.

Studies of crop-weed competition showed that yield loss is sensitive to small differences in the period between crop and weed emergence. It brings to light the importance of the concepts of a critical period of weed control (Knezevic et al., 2002; Knezevic & Datta, 2015) and economic thresholds (Coble & Mortensen, 1992; Wilkerson et al., 2002). Threshold is described as ‘a point at which weed density causes important crop losses’ (Knezevic et al., 2017). Knowledge of thresholds can help agriculturists make decisions on the need for herbicide applications, in deciding whether remedial weed control efforts are necessary or economically justified.

Economic threshold has been defined as ‘the weed density at which the cost of weed control equals the increased return on yield in the current year’ (Knezevic et al., 2017). Because they account for crop losses only in the current cropping season, economic thresholds are single-year measures of weed effects. Also, economic thresholds are based on factors such as the price of the crop at harvest, herbicide, and application cost, anticipated crop yield, and the yield loss - weed density relationships which are a function of environmental factors (e.g., soil types and climate). Since the primary cause of yield reductions by weeds is through competition for growth-limiting resources (light, water, and nutrients), the economic threshold is not therefore constant for particular weed-crop combinations and can differ within the same geographic region.

In crop-weed competition, the additive design study is a preliminary step for calculating thresholds. In additive design, the weed density varies, while crop density is kept constant (Swanton et al., 2015). Several review papers are recommending the use of rectangular hyperbola in the weed science literature (Knezevic & Horak, 1998; Ritz et al., 2015; Swanton et al., 2015). However, there is still a distinct number of empirical models fitted for additive design studies (Silva et al., 2015; Strieder et al., 2007; Trezzi et al., 2015; Voll et al., 2002). Four significant regressions curves are frequently used: linear (Figure 1a), polynomial quadratic (Figure 1b), sigmoid (Figure 1c), and rectangular hyperbola (Figure 1d). The commonly used criteria for selection of linear and nonlinear regression models is the equation with highest R-squared (R2). The R2 tests the goodness of fit for linear models however it is statistically inadequate for nonlinear model selection (Archontoulis & Miguez, 2015; Zuur et al., 2007). There are several appropriate statistical criteria for selecting a nonlinear model for datasets: Akaike’s information criterion (AIC), Bayesian information criterion (BIC), F-test, and likelihood ratio (Anderson, 2007; Lewis et al., 2011; Zucchini, 2000). These statistical criteria are used in non-nested and nested models. Non-nested models are models with different structure and parameters, such as an exponential decay and a rectangular hyperbola models. In contrast, nested are models that are a particular case of each other and have identical terms, whereas one must have one or more additional terms, an example are three or four parameter log-logistic models. Therefore, the AIC and BIC are indicated for model selection in non-nested models and F-test, AIC, BIC, and likelihood ratio are appropriate for nested models.

From a practical standpoint, the top model should be selected upon a balance between statistics and biological relevance, which will help scientists answer their research questions (Onofri et al., 2010; Archontoulis & Miguez, 2015). In additive design studies, the model that provides a good fit and essential biological parameters is considered a strong candidate model to describe the dataset. The advances in statistical software have facilitated the use of standardized nonlinear regression analysis that can be performed by non-statisticians (Knezevic et al., 2007). Therefore, the objectives of this manuscript were to:

1. Provide the basics about the concept of crop-weed competition and additive designs.
2. Test the suitability of three non-nested candidate models (polynomial quadratic, sigmoid, and a rectangular hyperbola) for describing the crop-weed density relationship.
3. Test the null hypothesis that the weed species *R. brasiliensis* and *C. benghalensis* compete similarly in corn. This hypothesis was tested after top model selection (objective 2) using the F-test.

Data from an experiment under greenhouse conditions looking at *Richardia brasiliensis* and *Commelina benghalensis* competition with corn (*Zea mays* L.) was used for the model selection exercise. The data analysis concept presented here would work with other weed species and crops either in greenhouse or field conditions.

**Material and Methods**

*Plant material and growth conditions*

Seed heads of *R. brasiliensis* were harvested along roadsides near Diamantina, Minas Gerais (MG), Brazil in March of 2011 and dried at room temperature (25 °C), cleaned, and stored at 5 °C until the onset of the experiment. Ten days before the experiment began (September 2011), stolon (vegetative propagules) of *C. benghalensis* were collected in wetlands, near Diamantina, MG. Seeds of *R. brasiliensis* and stolon of *C. benghalensis* were seeded and transplanted to separate trays (1210 cm3) filled with red latosol (pH 6.1 and 1% organic matter). A single seed of glyphosate-resistant (GR) corn (AG8088) was sown in 8 dm3 plastic pots filled with the aforementioned soil source. This procedure was performed to maximize the competition between species. The soil was fertilized following the local recommendations, and N was applied at 15 and 30 DAE (days after corn emergence) at a rate of 55 mg dm-3 of ammonium sulfate. Greenhouse conditions were 28/19 °C day/night, and pots were watered daily.

*Experimental procedures*

The experiment was conducted under greenhouse conditions over a period of 60 days at the Federal University of Jequitinhonha and Mucuri, Diamantina, MG. In this study, the additive design was used; therefore, weed densities varied, and corn density was kept constant (Swanton et al., 2015). The treatment design was a factorial with two weed species, *R. brasiliensis* and *C. benghalensis*, and five weed densities (0, 1, 2, 3, and 4 plants pot-1), in a completely randomized design with four replications.

Corn dry matter was harvested at 60 DAE from each experimental unit. Shoot biomass was oven-dried at 65 °C until reaching constant weight, and dry weight recorded. The corn dry matter (g) data (shoot) were converted into yield loss (%) compared with the control treatment (no weeds):

Equation 1

where *Μ* is the mean dry mass (g) of the control treatment, and is the dry mass (g) of individual corn plants competing with weed(s).

*Statistical Analysis*

Three models were fitted to yield loss data (%) in response to weed density (plants pot-1):

*Rectangular hyperbola model* proposed by Cousens (1985):

Equation 2

where *I* represent YL (yield loss) per unit weed density as *D* (density) approaches 0, and *A* represents YL as D approaches ∞ (or maximum expected yield loss). The rectangular hyperbola model was fitted using the *nls* function of R version 3.3.1 (R Core Team, 2016).

*Sigmoid model* (four parameters log-logistic curve):

Equation 3

where *c* is the (lower limit or YL at low weed density), *d* is the asymptote (upper limit or YL at high weed density), and *e* represents the weed density (weeds pot-2) that causes 50% yield loss (inflection point). The parameter *b* is the relative slope around the parameter *e*, and *D* is the number of weeds pot-1. Parameters for the sigmoidal model (four-parameter logistic) were estimated using the *drm* function of drc package in R software (Ritz & Streibig 2005).

*Polynomial quadratic model* (second order):

Equation 4

where *α* is the intercept in the y-axis (no yield loss by weed competition), a represents the slope of the model. The parameter b is the quadratic term of the model, and *D* is the number of weeds plot-1. The parameters for the polynomial quadratic equation were estimated using the *lm* function of R software.

*Top model selection to describe crop-weed competition*

The AICc (corrected AIC for finite sample size) criterion, which is indicated for non-nested model selection (Sugiura, 1978; Hurvich & Tsai, 1991), was calculated as:

Equation 5

where is the likelihood function and is the number of estimated parameters in the model, and *n* is the sample size of the model. According to the AICc criterion, the top model has the lowest AICc value. The AICc values for each model were estimated using the *AICc* command of package *AICcmodavg* in R software

*Model selection to evaluate weed competitiveness with the crop*

Assuming that rectangular hyperbola is the top model, the impact of *R. brasiliensis* and *C. benghalensis* on corn YL is accessed through the variance-ratio or F-test performed using equation [2] (Lindquist et al., 1996). This statistical procedure evaluates the difference of residual sum squares (RSS) of nested models. The F-test is calculated as:

Equation 6

Where RSSRED and RSSFULL represent the minimized residual sum squares of the parameters estimated for the full (step 1) and reduced model (step 2, 3, or 4; steps 1 through 4 are described next), respectively; dfRED and dfFULL represent the degrees of freedom of the full and reduced models, respectively. In practical terms, when P-value >0.05, we fail to reject the null hypothesis and a reduced model should be used (no difference between *I* and/or *A* parameter values between weed species). However, if P-value <0.05, the null hypothesis is rejected and the full model should be used (different *I* and/or *A* parameter values for each weed species). The F-test principle for nonlinear regression analysis was calculated for each model using *nls* *ANOVA* command in R software (Ritz & Streibig, 2008).

Four significant steps need to be completed to compare the parameters using this method (see supplemental file for statistical codes to perform these steps in R software):

Step 1) Fit Equation [2] to the data of each species individually ; it is a single fit which a model having separated parameters for each species ; this represents the Full model, where four parameter values (*I* and *A* for each weed species) will be estimated.

Step 2) Pool the data for both species (*R. brasiliensis* and *C. benghalensis*) and fit Equation [2]. This represents the reduced model (Red. I), where two parameter values (*I* and *A* for both weed species combined) are estimated for the pooled data. This step will allow testing the hypothesis that *I* and *A* do not vary between species, which means that both species compete similarly with corn. If the hypothesis is accepted (P-value>0.05), stop here. Otherwise, there are two additional hypothesis to be tested (steps 3 and 4).

Step 3) Fit equation [2] setting a single parameter *I*, but different *A* parameter for each species (Red. II). This is a reduced model, and three parameters will be estimated. This step tests the second hypothesis, that weed species compete similarly at low densities (*I*), but different at higher densities (*A*).

Step 4) Fit equation [2] setting a single parameter *A*, but different *I* parameters for each species. This is a reduced model (Red. III), and three parameters will be estimated. This step tests the third hypothesis, that weed species compete similarly at higher densities (*A*), but different at low densities (*I*). Additional AICc was also performed for the nested model selection for confirming the F-test model selection.

*Model Goodness-of-Fit*

Root mean squared error (RMSE), model efficiency (ME), and R2 (for the polynomial quadratic model only) were calculated and used to test the goodness-of-fit of non-nested and nested models (Mayer & Butler, 1993; Roman et al., 2000):

Equation 7

Equation 8

*R2* Equation 9

where RSS and RSTare the sums of squares for the residual and total, respectively; is the number of data points; is the number of model parameters; is the observed, is the predicted, and is the mean observed value. The ME values range from -∞ and 1, with values closer to 1 indicating better predictions (Werle et al., 2014c). R2 values range from 0 to 1, and it was used only for the polynomial quadratic model, which is a form of linear regression.

**Results**

*Top model selection to describe crop-weed competition*

The rectangular hyperbola model resulted in the lowest AICc (332.2), followed by a sigmoid model (337.6) and a polynomial quadratic model (343.1). The RMSE and ME resulted in a similar trend for the models tested, except *R. brasiliensis* in the polynomial quadratic model (Table 1).

In the rectangular hyperbola model (top model selected), four parameters were estimated, *I* and *A* for *R. brasiliensis* and *C. benghalensis*. The parameters *I* and *A* for *R. brasiliensis* was estimated at 50.3% and 82.1%, respectively (Table 2). In contrast, for *C. benghalensis,* parameter estimations were 210.2% (*I*) and 108.6% (*A*). The *P*-value showed no lack of fit (P<0.05) for the estimated parameters.

According to AICc, the sigmoid model was the second-best model to describe the data (Table 1). The maximum corn yield loss caused by the competition of *R. brasiliensis* and *C. benghalensis* (*d*) was 67.2% and 93.4%, respectively. The 50% corn yield loss (%) was 1.2 and 0.7 plants pot-1 of *R. brasiliensis* and *C. benghalensis*, respectively. However, parameters for the sigmoid model showed lack of fit (*P*-value > 0.05) for both weed species, including slope (*b*), the lower limit (*c*), and inflection point [(*e*) *R. brasiliensis* only] (Table 3). Also, the standard error in *b* and *c* parameters is bigger than the estimated values (Table 3). The ME was 0.58 and 0.85 for *R. brasiliensis* and *C. benghalensis*, respectively. Moreover, RMSE for the sigmoid model was 13.2.

The polynomial quadratic model had the highest AICc (Table 1). A similar trend was observed for RMSE. However, ME of *R. brasiliensis* was highest (0.71) across the three models tested (Table 1). In addition, as a linear model, R2 was included for the goodness of fit. The R2 was 0.71 and 0.89 for *R. brasiliensis* and *C. benghalensis*, respectively (Table 1). There was a lack of fit (P>0.05) of the intercept (α) for both weed species. But not for the slope (a) and quadratic parameter (b). Slope was 35.5% and 65.5%, and quadratic parameter -5.4 and -11.1 for *R. brasiliensis* and *C. benghalensis*, respectively (Table 4)

*Model selection to evaluate weed competitiveness with the crop*

Based on AICc, the rectangular hyperbola was the top model to describe the data (Table 1). The F-test of the rectangular hyperbola (Full model) indicated that a reduced model with different parameter *I* (corn yield at low weed densities) and similar parameter *A* (corn yield at higher densities) was the top model (Red. III) to describe corn competition to *R. brasiliensis* and *C. benghalensis* with corn (Table 5 and Figure 6). As demonstrated in the steps in the supplemental file, the Red. I and II models were different from the Full model (*P*-value<0.05), thus the hypothesis tested in those models were rejected (Table 5). According to the parameter estimates in the rectangular hyperbola Red. III model, at weed low densities (*I*), corn yield loss was 37.0 and >100% in competition to *R. brasiliensis* and *C. benghalensis*, respectively. However, at higher densities, *R. brasiliensis* and *C. benghalensis* compete similarly, and corn yield loss was 106.1% (Figure 5 and Table 6). AICc corroborates to F-test (Table 5). The model selected by the F-test (different I, but similar A) resulted in the lowest AICc of 330.4. The RMSE was similar in Red. III and Full model, but the highest ME (≥0.95) for *R. brasiliensis* and *C. benghalensis* demonstrated the goodness of fit of the top model selected.

**Discussion**

*Top model selection to describe crop-weed competition*

Rectangular hyperbola model was the top model to describe the corn yield loss (%) in response to both *R. brasiliensis* and *C. benghalensis* competition (Figure 2 and Table 1). The model with the smallest value of AICc was considered the top model or the best descriptor of the full reality given the set of candidate models and the data (Anderson, 2007). This model was also the best for describing corn leaf area, height, and stem diameter reduction in response to *R. brasiliensis* and *C. benghalensis* densities (data not shown).

In the rectangular hyperbola model, four parameters were estimated, which are *I* and *A* for *R. brasiliensis* and *C. benghalensis* (Table 2). However, the parameter *I* and *A* of *C. benghalensis* were estimated over 100% (Table 2). The parameter *I* of *C. benghalensis* curve had a steep inclination, which is likely due to the relatively small pot size used in this study, indicating that *C. benghalensis* is very competitive with corn. Therefore, bigger pots and lower *C. benghalensis* densities would have been necessary for such a study. The parameter *A* for *C. benghalensis* was also over 100%, which is likely due to the pot sized used. Thus, final constant yield (CFY) been reached at low densities for *C. benghalensis*. Parameters *I* and *A* was also overestimated (>100%) in a field study of *Amaranthus retroflexus* in competition with *Sorghum bicolor* (Knezevic & Horak, 1998). Nonetheless, in the present study, there is no lack of fit of parameters *I* and *A* estimated for *R. brasiliensis* and *C. benghalensis* (P<0.05).

According to AICc, the sigmoid was the second best model to describe the data (Table 1). The sigmoid model does not seem to be appropriate to describe the data from additive designs (Figure 3). The problem with using the sigmoid model to describe additive designs is that these models have an inflection point (e), which is usually at 50% of the asymptotic (Figure 1C). The symmetric shape of sigmoid models is related to the rate of change. One of the assumptions when using the sigmoid model is that the inflection point (*e*) is always at 50% of the asymptotic size (Knezevic et al., 2007; Ritz et al., 2015). Therefore, sigmoid curves have no biological meaning for competition studies in additive design. Though the sigmoid model is not recommended for additive design, it is one of the most commonly used and appropriate models in other weed research topics. Sigmoid curves are extensively utilized for predicting weed emergence, herbicide dose-response, and critical time for weed removal (Knezevic & Datta 2015; Ritz et al., 2015; Werle et al., 2014b; 2014a). For example, in herbicide dose-response studies, the parameter *e* is meaningful and important for comparison of herbicides doses that control 50% of a weed population (Oliveira et al., 2017).

The polynomial quadratic model was statistically the least appropriate for describing the data. The α (intercept) and a (slope) parameters estimated from a polynomial quadratic model possibly have biological meaning. However, the b (quadratic) parameter does not. Nonetheless, this model does not provide biological parameters that would improve the discussion, test hypothesis, and help researchers understand the results from crop-weed competition studies. Also, the polynomial quadratic curve is symmetric around the x-axis, which makes such response biologically unlikely in an additive design study (Figure 1B). For example, the maximum corn yield loss (%) is lower at four plants pot-1 than in three plants pot-1 (Figure 2). The highest ME or R2 for *R. brasiliensis* could potentially mislead model selection; however, ME and R2 test only model goodness of fit to the data. Therefore, a polynomial quadratic curve should not be encouraged to fit regression in additive designs.

To understand the nature of crop-weed competition modeling, one needs to comprehend the concept of CFY. The CFY is described from low to high densities, whereas the relationship between total biomass per unit area and density is initially linear, but eventually, reaches a plateau (e.g., biomass remains constant despite the increase in density; Weiner & Freckleton, 2010). Therefore, to use the rectangular hyperbola, CFY needs to be reached; otherwise, parameter estimates will not be statistically and biologically meaningful (Weiner & Freckleton 2010). For example, in our study, the CFY was reached at low density of *C. benghalensis*. As a result, the estimation of parameters I and *A* from *C. benghalensis* was over 100% (Table 2). In contrast, for *R. brasiliensis*, CFY was reached without parameter overestimation. Thus, the weed density for attaining CFY can vary amongst species. Consequently, for proper additive design studies, different weed densities based on the competitive potential of each species might be necessary. Other studies showed that CFY was reached and estimation of *I* and *A* was under 100%, indicating that some weed species may not lead to total crop yield loss (Knezevic et al., 1997, Knezevic & Horak, 1998). Additionally, a competition study that reports a linear relationship trend between crop yield loss and weed density has not reached CFY (Figure 1A). It is likely that either the appropriated weed density for the study was not selected or plants were harvested before significant competition occurred (Weiner & Freckleton, 2010). Therefore, the crop-weed competition experiments need to be properly designed so CFY is achieved and model parameter estimates are statistically accurate and biologically meaningful.

In additive design studies, because of misleading model selection (usually R2), it is common to find multiple equations describing response variables (Ferreira et al., 2015, Silva et al., 2015). For example, over six models were used to describe the competition of two weed species (*Urochloa decumbens* and *Ipomoea grandifolia*) with three neotropical trees (*Senegalia polyphylla, Ceiba speciosa, and Luehea divaricata*; Monquero et al., 2015). It becomes difficult to evaluate and compare weed competitiveness when different equations with non-related parameters are used.

*Model selection to evaluate weed competitiveness with the crop*

It was statistically demonstrated the rectangular hyperbola model was the top model to describe crop weed competition in additive design. The F-test showed that at high densities (*A*) competition of *R. brasiliensis* and *C. benghalensis* in corn yield loss is similar, but at low densities (*I*) is different (Table 5). Therefore, the hypothesis that competition of *R. brasiliensis* is similar to *C. benghalensis* in corn was partially rejected.

A complete review of model parameter *I* and *A* of the rectangular hyperbola is provided by Cousens (1985). Many authors have used this model to answer their research questions and improve weed control decision-making (Cathcart & Swanton, 2003; Fischer et al., 2004; Lindquist et al., 1999; Werle et al., 2014c). For example, using parameters *I* and *A,* it was demonstrated that organic cropping systems have the potential to tolerate great abundance of weeds compared to conventional system (Ryan et al., 2009). Additionally, using the rectangular hyperbola model, it was concluded the higher competitive potential of *Amaranthus palmeri* in corn and *Kochia scoparia* in sunflower (Lewis & Gulden, 2014; Massinga et al., 2001) and interference of *Ambrosia artemisiifolia* in soybeans (Barnes et al., 2018). Parameters *I* and *A* are also useful for estimating weed competition across different locations and appropriate for calculating economic weed thresholds (Lindquist et al., 1996; Lindquist & Mortensen 1998). Thus, the rectangular hyperbola proposed by Cousens (1985) and the F-test nested model selection are important and useful tools of crop-weed competition in additive design.

Here we demonstrate that the rectangular hyperbola was statistically and biologically the best model to describe crop-weed competition data from an additive design study. Potential issues, including parameter overestimation (>100%) were also addressed. Nonetheless, rectangular hyperbola model has an asymptote curve shape that fits well with the expected results from additive design studies. The parameters *I* and *A* are easily interpreted and biologically meaningful. We propose rectangular hyperbola as a standardized model for crop-weed competition studies in additive design. Sigmoid models are adequate to another set of studies in weed research (e.g., herbicide dose-response), and polynomial quadratic curves are not recommended in weed research. Also, a step-by-step statistical analysis with R codes was performed for a competition studies in additive design; we believe that this review would improve and aid to standardize the data analysis performed by non-statisticians in weed competition research. Thus, improving the prediction of weed thresholds in cropping-systems.

**Acknowledgments**

The authors thank CAPES (Brazilian Government Foundation) - Proc. no 9112-13-8, for financial support first author of this manuscript.

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