AMIGA POWER ANALYSIS USER MANUAL

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1 INTRODUCTION

Welcome to Amiga Power Analysis for environmental risk assessment (ERA) using field trials. With this tool you can calculate the necessary replication for assessing differences and equivalences between a test and a comparator plant variety under different data models for count and continuous data.

This tool builds on EFSA recommendations (Perry et al. 2009, EFSA 2010) and work in the AMIGA project (Goedhart et al. 2013, 2014), It allows to specify the experimental design, additional factors in the experiment, and the method of statistical analysis that will be used. The power of difference tests and equivalence tests (Schuirmann et al. 1987, Perry et al. 2009) is calculated.

This program was developed in the AMIGA project (Assessing and monitoring the impacts of genetically modified plants on agro-ecosystems, http://www.amigaproject.eu/) on the amount of replication needed in field trials for GMO safety assessment.

The program was developed by the Biometris department of Wageningen University and Research centre (http://www.biometris.nl/).

Program developers: Johannes Kruisselbrink, Paul Goedhart, Hilko van der Voet

2 INSTALLATION INSTRUCTIONS

This section will guide you through the installation of Amiga Power Analysis.

2.1 REQUIREMENTS

The software is developed for Windows 7 and requires .NET 4.5 client framework. It has not been tested on earlier or later releases of MS Windows.

This software requires the installation of the statistical software R, version 3.0.0 or higher. If not already installed, it is best to install R before the installation of the this software.

Follow the steps below to install R:

Step 1: Go to the R website for downloading the Windows version on http://cran.rstudio.org.

Step 2: Click on the link "Download *R.x.x.x* for Windows" (or other version). This starts downloading R.*x.x.x*-win.exe file for both 32 and 64 bit.

Step 3: After downloading, double click this file to install R. **Important:** Make sure that you keep the default setting under Additional Tasks: "Save version number in registry" checked.

Step 4: Start R and install the following packages *MASS*, *lsmeans*, *stringr*, *reshape*, *mvtnorm* by typing: *install.packages("package_name_here")*. These packages are used for the analysis.

2.2 INSTALLATION STEPS

Step 1: Double click the installation file (AmigaPowerAnalysis.Installer.msi). This will run a standard installation. Follow the instructions on the screen – the suggested default settings should apply in most situations.

Step 2: Start Amiga Power Analysis using the desktop shortcut, from the start menu, or from the installation directory.

3 GETTING STARTED

Start by opening an existing file or creating a new file. The user interface of Amiga Power Analysis is divided into tabs. In the sections below, the functionality of each tab will be explained separately.

3.1 ENDPOINTS

Enter a list of endpoints. For each endpoint indicate its group (retrieves default settings), and if needed adapt the measurement type and limits of concern (LoC). Endpoint groups can be edited under the Options menu. Note: currently only methods for Measurement type Count have been implemented.

Endpoints can be of different measurement types:

- **Count data:** occurs when the endpoint data is described in terms of the number of organisms found on each experimental unit.
- **Non-negative data:** occurs when the measuring time trend curves.
- **Continuous data:** occurs when there is no limit on the measurement values.

An essential part of ERA is that for each endpoint, it should be decided beforehand which levels of difference between the test-variety and the comparator are still acceptable, and at what level, a difference becomes too high to be ignored. In this software, these limits are defined in terms of limits of concern (LoCs). Limits of Concern are ratios of the expected values for the Test-Variety and the Comparator. Within these limits there is no concern about safety. Provide a lower LoC, an upper LoC, or both. Unspecified (NaN) means no concern for changes in that direction.

3.2 ENDPOINTS DATA

For each endpoint, if needed adapt its distribution type, the binomial total (for fractions), and the power (for Taylor's Power law distribution).

If needed adapt expected values of mean and coefficient of variation (CV) for the comparator variety. Note that the CV will be increased if incompatible with distribution type and mean.

The table below shows the distribution models that are available per measurement type.

Measurement type	Model	Distribution parameters	Restrictions	Recommended
Counts	Poisson	$\lambda = \mu$	$\mu > 0$	
	Overdispersed Poisson	$\lambda = \mu$ $\omega = cv^2 \cdot \mu$	$\mu > 0$ $cv > \sqrt{(1/\mu)}$	*
	Negative Binomial	$\omega = cv^2 - 1/\mu$ shape = $1 / \omega$ scale = $\omega \cdot \mu$	$\mu > 0$ $cv > \sqrt{(1/\mu)}$	
	Poisson-Lognormal	$\mu = \mu$ $\omega = cv^2 - 1/\mu$	$\mu > 0$ $cv > \sqrt{(1/\mu)}$	
	Power model	$\mu = \mu$ $\omega = cv^2 - \mu^{2-p}$	$\mu > 0$ $cv > 1 / \sqrt{\mu}$	
Nonnegative	Log-normal	$\mu = \mu$ $\sigma = \lvert\mu \cdot cv\rvert$	$\mu > 0$	*

Continuous	Normal	$\mu = \mu$ $\sigma = \lvert\mu \cdot cv\rvert$		*

3.3 FACTORS

The main factor in variety-comparative evaluation experiments is always variety, with at least the levels test-variety and comparator. If the design contains more varieties, these can be expressed as additional variety levels.

If the design contains more factors (e.g. spraying treatments), add additional rows in the Factor table, and specify the levels and relative frequencies in the Levels table.

Unequal numbers of plots per variety, or for specific other factor level can be corrected by using (relative) frequencies. If numbers of plots per variety are not equal, change the (relative) frequencies.

3.4 DESIGN

Two design types are supported: completely randomized, and randomized complete blocks. This tab allows you to specify the type of experimental design.

3.5 DEFINE COMPARISONS

When other factors have been specified, the comparisons between Test-Variety and the Comparator can be expected to be the same for all levels of such a factor (no interaction) or different (interaction).

If such interactions are expected, select the factors for which this is the case, and deselect the levels for which there is an interaction between test-variety/comparator.

If the comparisons are different for all/some endpoints, uncheck the box 'Use interactions for all endpoints' will allow you to specify specific endpoints in the next screen. Note: Interactions with Variety will lower the effective replication, because comparisons are now needed at the separate levels of the other factor.

3.6 DEFINE COMPARISONS PER ENDPOINT

This tab allows you to specify/modify the comparisons per endpoint.

3.7 ADDITIONAL MEANS

There are data which are not directly involved in the comparison test-variety to comparator. Such data may be useful for pooling variance estimates, but the usefulness may depend on the expected means. Indicate if you expect less informative data due to low means. If so, specify expected mean values.

3.8 FACTOR MODIFIERS

The power of tests will be lower if data are uninformative or less informative, e.g. if counts are very low (<5). In principle, the already specified Comparator Means and CVs are sufficient to perform the power analysis. However, it should be specified if other factors in the design are expected to make part of the data less informative.

For fixed factors, provide multiplication factors for factor levels where data may become less informative (e.g. counts less than 5).

A restriction for the modifiers is that the joint effect of the modifiers should be neutral:

$$\frac{\sum_{i=1}^{n} \mu_i \cdot w_i}{\sum_{i=1}^{n} w_i} = \mu$$

, where μ_i denotes the modified mean for level i and w_i denotes the frequency of this level.

3.8.1 MODIFIERS FOR COUNTS AND NON-NEGATIVE

For counts and non-negative measurement types, the modifier effect for level i with modifier Δ_i is

$$\mu_i = \Delta_i \cdot \mu$$

. Following the restriction that the joint effect should be neutral, the modifier Δ_i for level i is computed from the other levels as

$$\Delta_i = \frac{\sum_{j=1}^{n} w_j - \sum_{j=1, j\neq i}^{n} \Delta_j \cdot w_j}{w_i}$$

.

A lower bound for the modifier is $\Delta_i \geq \Delta_l > 0.1$ and from this follows an upper bound the following upper bound

$$\Delta_i \leq \frac{\sum_{j=1}^{n} w_j - \Delta_l \sum_{j=1, j\neq i}^{n} w_j}{w_i}$$

.

3.8.2 MODIFIERS FOR FRACTIONS

For fractions, the modifier effect for level i with modifier Δ_i is defined as

$$\mu_i = \left(1 + \frac{1-\mu}{\Delta_i \cdot \mu}\right)^{-1}$$

.

Following the restriction that the joint effect should be neutral, the modifier Δ_i for level i is computed from the other levels as

$$\Delta_i = \left(\frac{1}{\mu} - 1\right)\left(\frac{w_i}{\mu \cdot \sum_{j=1}^{n} w_j - \sum_{j=1, j\neq i}^{n} \mu_j} - 1\right)^{-1}$$

.

A lower bound for the modifier is $\Delta_i \geq \Delta_l > 0.1$ and $\Delta_i \geq \Delta_u > 1000$.

3.8.3 MODIFIERS FOR CONTINUOUS

For continuous measurement types, the modifier effect for level i with modifier Δ_i is defined as

$$\mu_M = \Delta + \mu$$

.

However, for this measurement type, the modifier will have no effect on the power analysis.

3.9 BLOCK MODIFIERS

For randomized complete block designs, it may be that there large differences between blocks, causing part of the data to be less informative. If this is the case, then use this tab to specify the variation between blocks in terms of a CV (%).

3.10 ANALYSIS

Specify how to perform the power analysis and which methods of analysis are to be compared. In simple cases (continuous and non-negative with log(x+m) method) a direct calculation is made. For other cases results can be based on Simulation, but it is advised first to use the Approximate method (Lyles et al. 2007) because it is much faster.

For count data it is suggested to use the log(N+1) method for the difference tests and the Log-linear model with overdispersion for the equivalence tests.

For non-negative data it is suggested to use the log(x+m) method for the difference tests and the Gamma model for the equivalence tests. (Note: Approximate method not yet available for gamma)

3.11 OUTPUT

This panel shows the power analysis outputs that are produced within this project. Select an output and press load to set this output as the default output of the project and to view the results.

3.12 RESULTS PER COMPARISON

Choose endpoint in table. Choose method of analysis if more have been investigated. Power is shown for difference tests or equivalence tests, and as a function of the number of replicates or the Ratio Test/Comp (on a ln scale).

Note: Number of plots in design is Number of replicates times Number of plots per block.

3.13 RESULTS PER COMPARISON

The power analysis is based on the minimum power across the primary comparisons, in terms of Concern Standardized Differences (CSD, equals 1 at the Limit of Concern).

Select primary comparisons. Choose method of analysis if more have been investigated.

Power is shown for difference tests (upper graphs) and equivalence tests (lower graphs), both as a function of the number of replicates (left) and the Concern Standardized Difference (right).

Note: Number of plots in design is Number of replicates times Number of plots per block.

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