TEACHING CORNER

Spreadsheet tools for biometry classes in crop science programmes

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ABSTRACT

Teaching biometry to undergraduate students in crop science or similar programmes may be a challenging task, as these students show a small background in mathematics and computer programming and are mainly interested in practical applications. It is therefore fundamental to use 'realistic' case-studies and provide the students with statistical software that does not overload them with a steep learning curve. This paper wishes to bring to the attention of readers two new EXCEL macro add-ins (DSAASTAT and BIOASSAY97), which have been extensively tested, validated and used as teaching aids in biometry courses. They only require a basic knowledge of spreadsheet environments and may also be useful for technicians and practitioners for general data analyses of 'routine' field experiments.

Key Words: *ANOVA; nonlinear regression; statistical teaching software; spreadsheet.*

INTRODUCTION

Starting a biometry course for undergraduate students in crop science and related disciplines is always a reason of concern for teachers. First of all, those students have a very wide scientific background in applied biology, but their previous knowledge of mathematics and related disciplines (including probability and statistics) is rather small. Secondly, in contrast to PhD students, undergraduate students have very rarely been involved in advanced research projects and/or scientific publications. Therefore, they have not yet become fully aware of the role played by experimental errors and related uncertainties in the outcome of scientific experiments. One further reason is that several students in crop science do not show any particular love for mathematics (although this is just our personal opinion and we have no literature reference to support this!).

For the above reasons, when undergraduate students in crop science are exposed to biometry classes, their first reaction is often negative, as they tend to perceive this discipline as very 'abstract'. "Why should we study such an odd and complex subject?" is their frequent question. It might either be that our students are not a representative sample or that

we are not such good teachers, but we are fairly confident that the above anxieties are shared by many biometry teachers, in various countries worldwide.

There are two key issues that might (hopefully) help ensure success in a biometry course: (1) start early with meaningful case studies, which the students can easily relate to; (2) do not overload the students.

The importance of 'real life' case studies may be easily understated. We noted, however, that certain overly simplified or 'artificial' examples, as often found in classic biometry books, may help deliver the message to the most motivated students, but make the great majority of them even more skeptical about the real usefulness of biometry. There are indeed several types of field or laboratory experiments that may be successfully used. For instance, genotype/herbicide evaluation trials are particularly suitable to introduce students to linear (ANOVA-like) models, as well as other types of experiments aimed at optimising cropping techniques (fertilisation, sowing rates, tillage...). On the other hand, bioassays may play a similar role in nonlinear regression models. Though all these types of 'routine' experiments may not be highly advanced and innovative, their relevance can be immediately grasped by students, creating a good attitude in their minds. In any case, some sort of statistical software is always necessary, which leads us to the second issue above, i.e. the risk of overloading students.

Nowadays, the scientific community seems to suggest the universal use of R as an all-purposes statistical software (R Core Team, 2014). This very complete and freeware environment offers not only various classic statistical analyses, but also specific packages for agricultural experiments, for both genotype evaluation (e.g. the *agricolae* package; de Mendiburu 2014) and bioassay (e.g. the *drc* package; Ritz and Streibig 2005). Even though we normally use R for our statistical analyses and recommend its usage to all PhD students, we argue that the same recommendation should not hold for undergraduate students in crop science and related disciplines. Indeed, this software requires some computer skills that are normally beyond the abilities of crop science undergraduate students, who need some specific initial training. According to our experience, such an overload makes the student lose focus on biometrics and should be avoided by all means.

We have found that the use of a generic spreadsheet programme, such as Microsoft Excel, can be very beneficial at the beginning. Indeed, most students are already well experienced with it and thus do not need any specific training. Therefore, they can concentrate on case studies being presented, not worrying about computer coding.

One relevant problem may be that Microsoft Excel in itself is very little suitable to cope with all the peculiarities of field research in agriculture, particularly in crop science and plant protection. We thought therefore that it might be relevant to develop some user-friendly add-ins to be used as teaching aids in biometry courses and, in a rather long time span, we ended up with a wide collection of macros, piled up under two workbooks (DSAASTAT.xls and BIOASSAY97xp.xls). We have been successfully using these workbooks for teaching purposes (but not only for this) and, therefore, we would like to bring them to the attention of readers, either teachers, students, technicians or practitioners.

GENERAL FEATURES

All code was initially written in Quick Basic, lately ported to Visual Basic for Applications (VBA) and implemented in EXCEL 97. The two collections are named DSAASTAT and BIOASSAY97 and they are contained in two workbooks (DSAASTAT.xls and BIOASSAY97xp.xls), which are compatible with all most recent Excel versions. These workbooks are virus-free and can be freely downloaded as supplementary files ([LINK](#)) or from the first author's blog ([stats4biology.blogspot.it](#)). Installation instructions, examples and detailed explanation can be found there too. BIOASSAY97 is based on the solver add-in, which is normally available when you install Microsoft Office or Excel. Should you get

prompted that the Solver add-in is not currently installed on your computer, you may install it simply by clicking on the appropriate button in the dialog form or by running a custom Excel setup process.

DSAASTAT

The first set of macros (DSAASTAT.xls) is now at version 1.512 (June 2014) and an earlier version (1.018) was presented in Onofri (2007). Basically, DSAASTAT performs the ANOVA for more than 20 different types of designs, covering the main part of teaching needs for undergraduate courses.

When dealing with case-studies relating to genotype evaluation or other similar experiments with qualitative experimental factors, DSAASTAT may be useful under the following 'cookbook':

•   *Step one*: students should make sure that they have interpreted the case study with care. Experimental design and data analyses must match closely to each other and this is perhaps the most important lesson for students.

•   *Step two: preliminary analyses*. One important task that students must learn caring for is a good preliminary evaluation of data, to identify and remove possible problems relating to the presence of outliers, heteroscedasticity or removable non-additivity. Such an inspection is possible in DSAASTAT for one-factor completely randomised and randomised block designs (for multi-factor experiments, the combinations of factor levels should be considered as levels of one factor). A graph of 'residuals vs expected values' is provided. The highest residuals are iteratively inspected and regarded as outliers when they exceed a certain threshold level. This level is calculated by the macro, depending on the number of data, on the number of degrees of freedom for the residual mean square and on a sort of 'protection' rule against the risk of erroneously removing observations (see Anscombe & Tukey, 1963 for information). Furthermore, the homogeneity of variances is tested by using Bartlett's and Levene's tests, while non-additivity for completely randomised blocks designs is tested by using Tukey's test (Snedecor and Cochran 1991). Based on the results of the above tests, the students should be able to answer the following question: „Does our dataset need some sort of stabilising transformation prior to ANOVA/regression?". The transformation proposed by Box and Cox (1964) has been implemented, so that students can find a support to select the most appropriate type of data transformation, according to the dataset at hand. We are aware that this step should logically come after the ANOVA, but, at least at the beginning, we have experienced that students tend to better understand its role if we anticipate it here.

•   *Step three: perform the ANOVA and get appropriate estimates of error terms.* Following the classical scheme in Le Clerg et al. (1962), DSAASTAT can handle experiments with up to four explanatory variables (five, including the block effect). For one-way ANOVAs, completely randomised, randomised complete block and latin square designs are handled. For two- and three-way ANOVAs, fully factorial, split-plot and split-split plot designs are handled, both with completely randomised and randomised complete block arrangements for the main factor. Split-block designs for two- and three-way ANOVAs as well as fully factorial or split-split-split-plot designs for four-ways ANOVAs are handled only for randomised complete block designs.

Students can also practice with repeated (multi-year and/or multi-location) one-way and two-ways designs and they are asked to make the choice whether the repeating factor (years or locations) should be regarded as random or fixed. For multi-year experiments, both annual and perennial crops may be considered. Likewise, possible subsampling schemes (where multiple measures are taken on each observational units) should be recognised and analysed appropriately, by using the appropriate option in DSAASTAT.

Designs must be naturally balanced, which is fairly normal with agricultural field experiments and does not represent a limitation, at least for teaching purposes. However, one-level completely randomised unbalanced designs may be also analysed appropriately.

 • *Step four: Multiple Comparison Procedures (MCP)*. Fisher's LSD, Duncan's MRT, Newman-Keuls's test, Tukey's multiple range test, Tukey's HSD, Sheffe's method and Dunnet's test for a two sided comparison with a control' have all been implemented for balanced experiments. The correct selection is left up to the students, who must be aware of multiplicity problems and must be able to select the appropriate error term from the ANOVA table. Ensuring that students are aware of the limitations of MCPs is our full responsibility! Useful guidance is provided in Onofri et al. (2010), while an exhaustive discussion of this subject is given in Hsu (1996).

Those listed above represent the most useful features of DSAASTAT for teaching purposes. However, the macro provides also some other functions to obtain correlation matrices, perform simple linear and multiple regression, and to compare regression lines. These features are already implemented in Excel, but DSAASTAT provides a more user-friendly interface.

BIOASSAY97

The second set of macros (BIOASSAY97) is now at version 2.651 (June 2014) and an earlier version (1.815) was presented in Onofri (2005). Basically, BIOASSAY97 performs non-linear regression analyses on bioassay data and returns model parameter estimates, ED-levels (Effective Doses) and other tools to assess the goodness of fit for the selected response models. All the rationale behind this macro has been taken by Streibig et al. (1993).

According to the case-study at hand, students can either fit a single dose-response curve to estimate ED-levels for a single preparation or simultaneously fit several curves to compare preparations and estimate their relative potencies.

A possible 'cookbook' for using BIOASSAY97 with bioassay experiments might be:

 • *Step one: preliminary actions*. We normally use replicated bioassays as case-studies and, therefore, students will have already worked with DSAASTAT and they will already be aware of possible problems with basic assumptions.

 • *Step two: select a response model*. Currently, three choices are available (only two for multiple curves fitting): a traditional log-logistic equation for sygmoidal symmetric responses on the logarithm of dose, a Weibull model (i.e. a Gompertz equation based on the logarithm of dose) for clearly asymmetric responses, and a peaked model for the case of hormesis (stimulation of responses at low herbicide doses; Brain and Cousens 1989). Model parameterisations are as reported in Streibig et al. (1993).

Constraints can be put on the lower and/or upper asymptotes, which might be necessary with some datasets. Likewise, for the case of multiple curve analyses, the different curves may be forced to assume the same lower and/or higher asymptotes and/or slope (parallel curves). At this stage students should already know from the step 1 whether a stabilising transformation is needed. BIOASSAY97 provides the Box and Cox (1964) transformation family and students should select the most appropriate value for λ. In order not to modify the scale of estimated parameters, a Transform Both Sides technique is used (Streibig et al. 1993) and students should be aware of this.

 • *Step three: provide starting values*. With this type of models we do not use any self-starting algorithm. Basically, students need to enter, for each response curve, a reasonable guess for the upper/lower asymptotes, slope and ED50 (dose giving a response half-way between the lower and upper asymptotes, corresponding to the inflection point for the log-logistic equation). Obtaining these guesses is quite easy by visually inspecting the observed responses, which we find a good exercise for students!

- *Step four: testing model adequacy*. Students must learn that this is a fundamental step. BIOASSAY97 provides graphs of (1) observed/predicted values against doses, and (2) residuals against predicted values. Students are encouraged to study both these graphs with great care! They will immediately reveal any problems with respectively (1) lack of fit and (2) heteroscedasticity. In case of problems, students should go back to step 2 and select another model and/or another stabilising transformation.

EXAMPLE 1: A GENOTYPE EVALUATION EXPERIMENT

DSAASTAT.XLS can be opened as a normal Excel workbook; on loading (make sure macros are enabled in Excel), some new entries are added to the ribbon, under the 'Add-ins' menu (e.g. Diagnostic tools, ANOVA, Multiple comparison tests, Correlation matrices, Regression analyses). As an example, we will use a dataset referring to a genotype experiment with 8 winter wheat genotypes and 3 blocks (a randomised complete block design). This experiment was carried out in 2004 in central Italy as a part of the national genotype testing programme and represents the typical genotype evaluation experiment for all field crops.

Note that the dataset should be organised as a normal database, with observations in rows and variables in columns; the first row is reserved for variable names. Basically, one column variable (numeric) plus one or more explanatory variables (string or numeric) are required, coding for all effects to be included in ANOVA (Fig. 1).
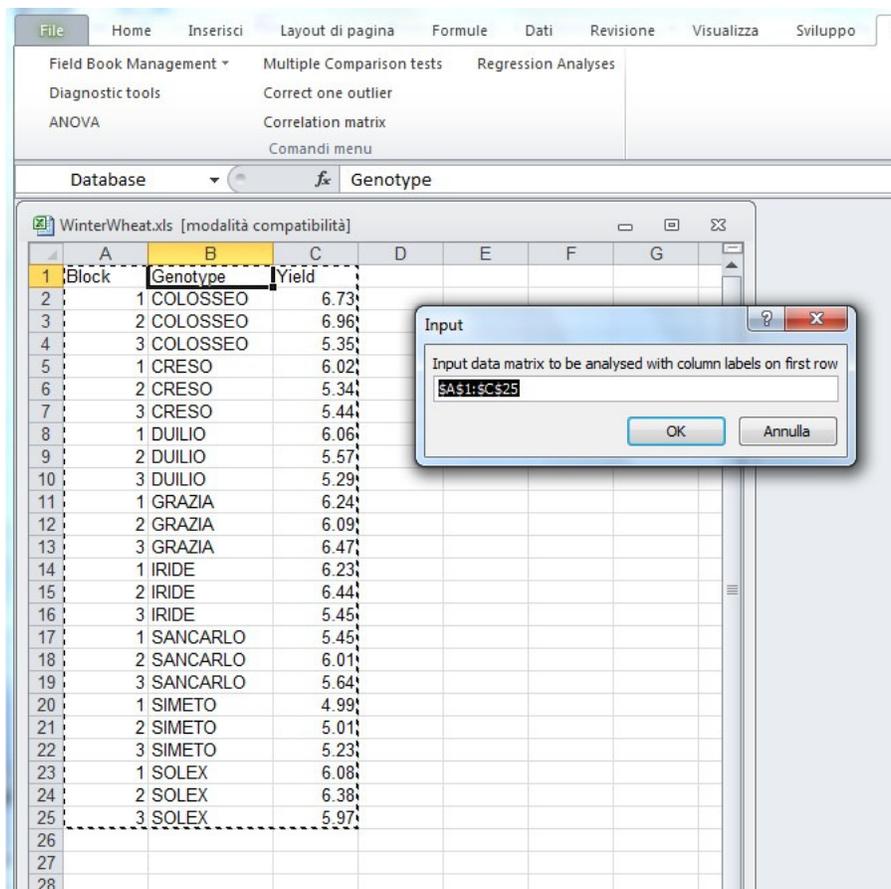
Figure 1: Selection of a dataset in DSAASTAT and BIOASSAY97

To preliminarily inspect this dataset we hit the 'Diagnostic tools' entry from the ribbon and we are prompted for database selection (Fig. 1). A second form permits the selection of design and variables, by means of drop-down list controls (Fig. 2). Results are displayed in a new worksheet (Fig. 3) and give us a few signs of warning, including a significant Levene's test (P = 0.016; not shown).
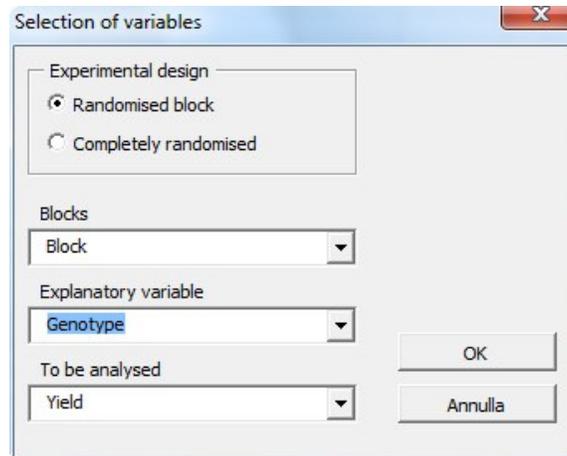
Figure 2. Selection of variables for one-way designs
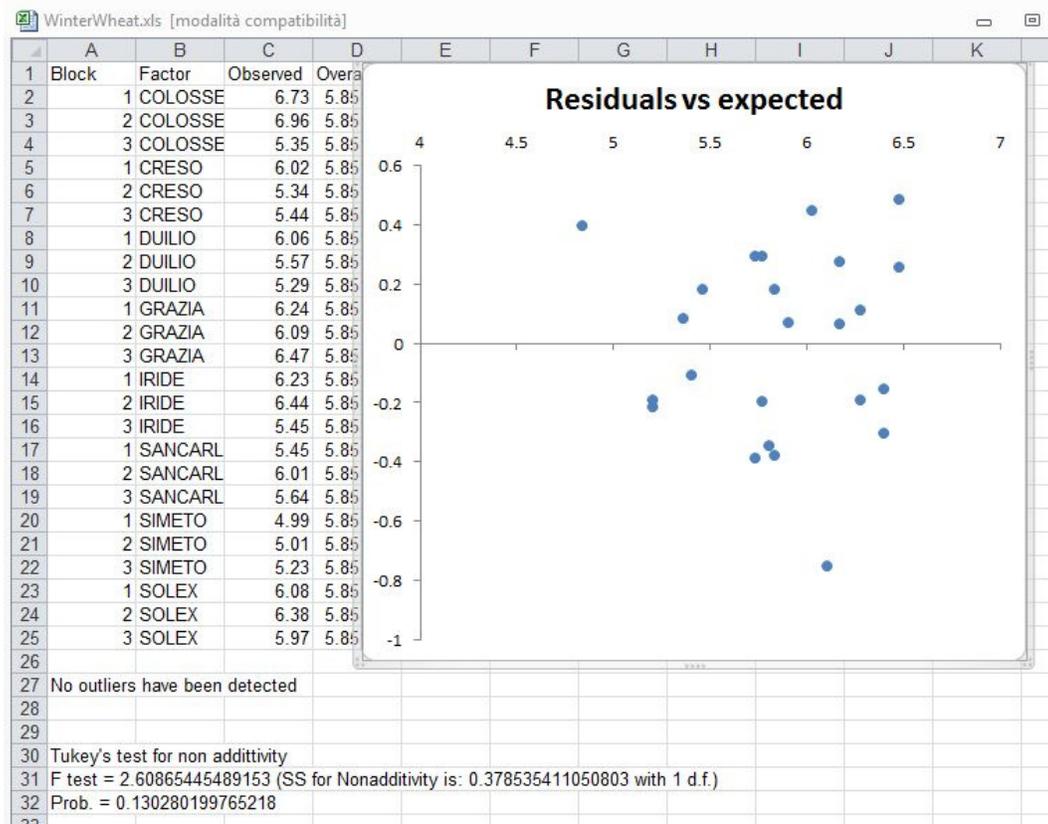
Figure 3: Analyses of residuals in DSAASTAT for the winter wheat experiment (see data in Fig. 1)

Indeed, the selected dataset shows a couple of suspect outliers, but, according to Anscombe and Tukey's procedure, these data do not deviate enough from their expectations to be considered as such. This is the typical drawback of working with real datasets: the situation is never as clear as teachers would like it to be! In our opinion, students have much to gain from these 'odd' case-studies. A closer look at the results of the Box and Cox procedure (not shown) suggests that there is no real need for a stabilising transformation and thus we can proceed to ANOVA.

Consequently, we hit the ANOVA entry from the ribbon, select the appropriate design and variables, and look at the results in a new worksheet (Fig. 4). We clearly see that the F test for the genotype effect is significant (P = 0.025), the coefficient of variation is 6.9% and the Standard Error of a Difference (SED) is 0.328 t ha^{-1}.

Figure 4: Results of ANOVA for the winter wheat genotype trial.

Figure 5: Example of the form for multiple comparison testing, following the ANOVA in Fig. 4.

We can use the above information for multiple comparison testing. We hit the appropriate entry from the ribbon, input the required information on the form (Fig. 5), and obtain the means augmented with a letter display. We should point out that such a letter display works only with balanced experiments (where the SED is unique), while for unbalanced experiments more advanced solutions are needed (see for example Piepho 2012). Results of MCP are shown in a new worksheet or elsewhere (as shown in Fig. 5).

EXAMPLE 2: DETERMINATION OF THE ED50 FOR ONE HERBICIDE/WEED SPECIES COMBINATION

BIOASSAY97xp.XLS can be opened as a normal Excel workbook; on loading (make sure that macros are enabled in Excel and the SOLVER add-in is installed and enabled. See the instructions on the author's website), a new entry is added to the ribbon, under the Add-ins menu (e.g. Biologic Assay).

As an example, we will use a dataset referring to an experiment carried out at the Department of Integrated Pest Management, University of Aarhus, Denmark (Pannacci et al. 2010). Plants of *Tripleuspermum inodorum* were treated with a sulphonylurea herbicide (tribenuron-methyl) at increasing doses and the fresh weight of treated plants per pot was recorded 3 weeks after treatment (Fig. 6).
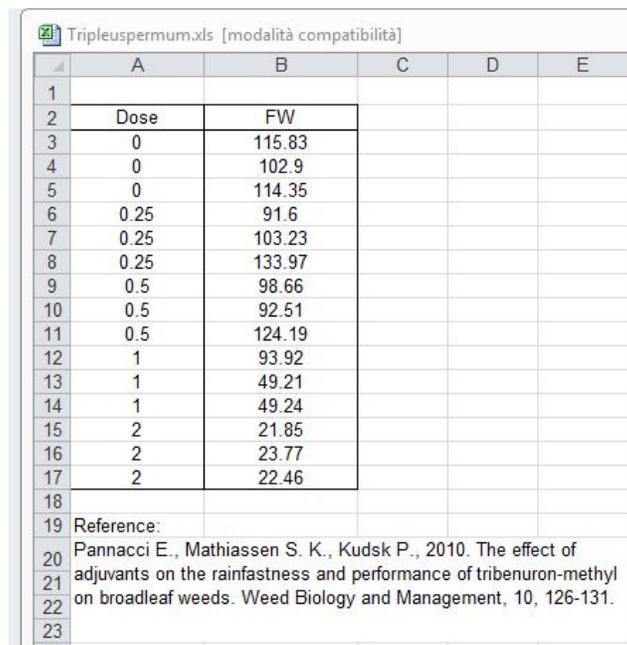
	A	B	C	D	E
	Tripleuspermum.xls [modalità compatibilità]				
1					
2	Dose	FW			
3	0	115.83			
4	0	102.9			
5	0	114.35			
6	0.25	91.6			
7	0.25	103.23			
8	0.25	133.97			
9	0.5	98.66			
10	0.5	92.51			
11	0.5	124.19			
12	1	93.92			
13	1	49.21			
14	1	49.24			
15	2	21.85			
16	2	23.77			
17	2	22.46			
18					
19	Reference:				
20	Pannacci E., Mathiassen S. K., Kudsk P., 2010. The effect of				
21	adjuvants on the rainfastness and performance of tribenuron-methyl				
22	on broadleaf weeds. Weed Biology and Management, 10, 126-131.				
23					

Figure 6: Dataset relating to a biological assay with *Triplospermum inodorum*.

A preliminary inspection by using DSAASTAT (not shown) reveals that this dataset is clearly heteroscedastic and a square root transformation is necessary to meet the basic assumptions for regression analysis. Keeping this in mind, we proceed to nonlinear regression analysis, by hitting the appropriate entry on the ribbon and select „Single curve: ED estimation" on the first form. Afterwards, a new form appears where we are prompted to select a model. We opt for a log-logistic model with four parameters and specify our dose and response variables. Looking at the observed data, we can provide 115, 1, 1 and 20, as starting values, respectively for higher asymptote, slope, inflection point and lower asymptote. These initial values will suffice to obtain quick convergence.

A successive form enables us to enter a λ value for the Box-Cox transformation (we enter 0.5, as mentioned above) and specify the response levels for which we would like to get an

estimate of the ED (10%, 30%, 50%, 70% and 90% growth reduction are set by default). When we hit OK, the SOLVER starts to iterate and finally converges.

The results are displayed on a new worksheet. We do not observe any evident lack of fit or deviations from basic assumptions (Fig. 7) and, therefore, we can look at the parameters' estimates. With this reference, we should note that, in spite of the Box-Cox transformation, estimates are obtained on the original scale, thanks to the 'transform-both-sides' approach.

We observe that the estimated value for the lower asymptote is 17.7 (Fig. 8), with a very large standard error (16.07) and confidence limits including 0. Indeed, we should better erase this parameter and refit the model, by including a constraint on the lower asymptote. In BIOASSAY97, this could be done by entering 0 in the corresponding field of the initial guess form. In general, we would leave this step to the students and take the chance to introduce the F test for comparing nested models.
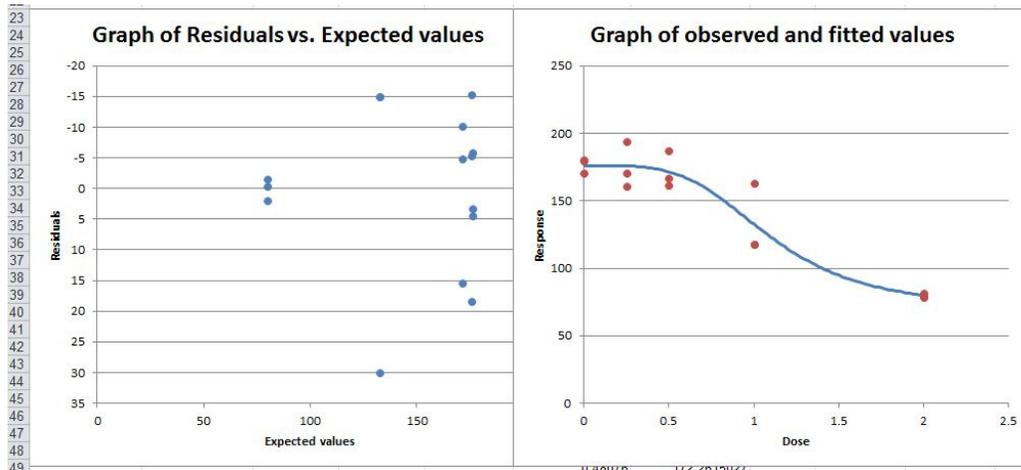

Figure 7: Graphical inspection of residuals from nonlinear regression analysis.

	A	B	C	D	E	F	G
1	Biological assay - non-linear regression analyses						
2	DataFile: Tripleuspermum.xls - Replicated trial / Response: FW - Dose: Dose						
3	Fitted function: Log-logistic model (4 parameters)						
4	Number of estimated parameters: 4						
5	Lambda value for Box & Cox Transformation: 0.5 ; -2*log likelyhood: 7.77507007914187						
6	Number of observations: 15						
7	Residual Sum of Squares: 2380.51011835413						
8	Degrees of freedom: 11						
9							
10							
11	Parameter	Estimated value	St. err.	t value	Prob(t)	Upper limit (0.95)	Lower limit (0.95)
12	Higher asymptote	110.1559532	8.031426368	13.71561516	2.91301E-08	127.8330035	92.47890298
13	Lower asymptote	17.69172279	16.06750556	1.10108708	0.294369377	53.05606409	-17.67261851
14	Stimulation	0	not included				
15	Slope	4.044326075	3.770263365	1.072690601	0.306379329	12.34261979	-4.253967642
16	Inflection point	0.9853226	0.121855532	8.085989888	5.89996E-06	1.253524817	0.717120382
17	ED10	0.572311479	0.264867882				
18	ED30	0.799084138	0.14119087				
19	ED50	0.9853226	0.121855532				
20	ED70	1.214966709	0.334188837				
21	ED90	1.696385031	0.973650407				
22							

Figure 8: Results of nonlinear regression analysis for a case-study with *Triplospermum inodorum*, as analysed by using BIOASSAY97

DISCUSSION AND CONCLUSIONS

Work carried out during previous years and the intensive use of DSAASTAT and BIOASSAY97 by undergraduate students with all kinds of statistics and computer backgrounds have shown that these tools have the flexibility and the simplicity to accomplish the main needs of biometry teachers. When giving practicals in a computer room, it takes just a few minutes to distribute the files, have the students load them and we are ready to talk about biometry with real data at hand. BIOASSAY97 may require slightly more time to help students enable the SOLVER, but this needs to be done only once and it is always within an acceptable time interval.

There has been over time a debate on using generic spreadsheets for statistics. Indeed, Clayton (2007) reports that the American Statistical Association, with reference to Programmes in Undergraduate Mathematical Sciences, stated that „... *Generic packages as Excel are not sufficient even for the teaching of statistics, let alone for research and consulting...*”. Several other authors pointed out the inaccuracies of statistical procedures of Excel (Knuesel 2005, Mc Cullough and Wilson 2005). We note that all those authors are mentioning different applications of Excel, in support to either students in mathematically oriented disciplines or to scientists in advanced research programmes. Very often we have to give talks, lectures and show some practical aspects of data analyses to audiences composed by people with limited knowledge of statistics and computer programming. This is not only the case with undergraduate students in crop science, but also with technicians, practitioners and applied biologists in general. In these conditions, using Excel to perform the analyses may be a great contribution to clarity and help those people to build a better scientific approach to biological problems. They may be able to move on to a more advanced software later on, if they wish to do so.

We also note that several people published research paper wherein they acknowledged the use of DSAASTAT or BIOASSAY97 (among the most recent, see for example Bishi et al. 2013 or Collavo and Sattin 2014). Intense validation work has shown that the results are reliable enough to be used for data analyses in the case of 'routine' field experiments.

It may be argued that DSAASTAT and BIOASSY97 are free software, while Excel is not; this is true, though we note that this spreadsheet is very widely available. However, porting the code to other free cross-platform spreadsheets may be worth the effort in the future.

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