

Global Effect Index: A New Approach to Analyzing Allelopathy Survey Data

Author(s): Jose Pedro N. Ribeiro

Source: Weed Science, 59(1):113-118. 2011.

Published By: Weed Science Society of America

DOI: 10.1614/WS-D-10-00062.1

URL: <http://www.bioone.org/doi/full/10.1614/WS-D-10-00062.1>

BioOne (www.bioone.org) is an electronic aggregator of bioscience research content, and the online home to over 160 journals and books published by not-for-profit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

Global Effect Index: A New Approach to Analyzing Allelopathy Survey Data

Jose Pedro N. Ribeiro*

The analysis of allelopathic bioassay data commonly encounters two problems: one is the small number of biological replicates and the other is that the parameters used to infer allelopathic effects such as percentage and average time of seed germination are analyzed individually, and consequently, information on the global effects resulting from the cumulative effects of the tested agent (e.g., leaves extract) may be missed. Therefore, we propose an index to analyze several parameters altogether so as to have a better view of the global influence of a donor plant on a receptor, whose interference is more likely to be detected. The global effect index can help to detect allelopathic interferences of one plant on another, allowing for a more accurate interpretation of the data in the actual biological setting.

Nomenclature: Data analysis, n value, methodology, statistic, weed control.

Experimental designs used to test allelopathic effects of plant extracts commonly have two inherent problems. The first is the small number of biological replicates n , usually five or less (Gatti et al. 2008; Kato-Noguchi et al. 2009; Ribeiro et al. 2009; Yang et al. 2007), that substantially decreases the degrees of freedom and therefore the power of the statistical tests. The second is that the parameters used to infer allelopathic effects (PIAEs), such as percentage and average time of seed germination, size, or biomass of the plant/seedling and so forth, are analyzed individually. Thus, the global effects resulting from the cumulative effect of each PIAE may be underestimated or completely ignored.

Small differences in several PIAEs, such as a decrease in seed germination and an increase in average germination time, and a decrease in seedling size, may pass undetected when analyzed separately (in many cases because of small n). Nevertheless, the PIAEs reflect only the visible effects of the allelochemical on the plant metabolism (Chiapusio et al. 1999), and the cumulative effect of small differences in several PIAEs might result in great global effects.

One possible alternative is to increase the number of biological replicates (n). However, that may not be feasible if a great number of treatments is required (e.g., several extract concentrations) or little biological material is available (e.g., final stages of extract fractioning). On the other hand, analyzing PIAEs altogether may allow the evaluation of the global effect of one plant on another. Combining descriptors (i.e., PIAEs) is often used in the analysis of biological data (Legendre and Legendre 1998). This coding technique is a useful data-reduction tool, as it combines scores arising from different variables in one single value (Babbie 1973). Furthermore, it is unrealistic to expect that isolated PIAEs could be used to explain allelopathy, and this is one of the reasons why in many cases laboratory bioassays fail to predict responses in the field (Inderjit and Weston 2000). However, analyzing all PIAEs together as they occur in nature (i.e., one given allelochemical or a mixture in one extract may affect different processes and these alterations may have an additive impact resulting in reduced growth or survival of the receiving plant) could lead to a more accurate analysis of what happens in the actual biological. Hence, the objective of this work was

to develop an index to analyze several PIAEs together, and so determine the global influence of a donor plant on a receptor.

Determination of the Mann-Whitney Test Efficiency for Allelopathy Survey Data

There is an extensive debate on which statistical approach would be best to analyze data from allelopathic assays. Different authors use different procedures that include data manipulation, and parametric and nonparametric tests (Djurdjevic et al. 2007; Santana and Ranal 2004). Despite all the discussion on the best approach, the index described herein was developed and analyzed by the nonparametric Mann-Whitney test. This test does not make assumptions about the distribution (e.g., normality) and sample size (Zar 1999). Thus, it is certainly not inaccurate.

To test the efficacy of the Mann-Whitney test in detecting differences among groups, a group of five numbers ($n = 5$) that ranged from 50 to 100 (intended to represent the control group germination percentages in an allelopathic assay) was randomly selected and called “control.” Then another group of five numbers, ranging from 0.9 to 1, was randomly selected, and called “multipliers.” After that, to generate groups with clear differences among them, a new group was generated by multiplying the control values by the different multipliers (Table 1). As the multiplier group average is 0.95, the treatment group is about 5% smaller than the control group.

The procedure was repeated by using groups of multipliers ranging from 0.8 to 0.9, 0.7 to 0.8, 0.6 to 0.7, 0.5 to 0.6, 0.4 to 0.5, 0.3 to 0.4, 0.2 to 0.3, 0.1 to 0.2, and 0 to 0.1, so that the differences between the control group (that always ranged from 50 to 100) and the treatment group became respectively 15, 25, 35, 45, 55, 65, 75, 85, and 95%.

Following that, the control and all the treatment groups were compared by using the Mann-Whitney test, and for each analysis, the numbers were resorted and the test was reapplied 4,000 times. The procedure using groups of 6 ($n = 6$), 7 ($n = 7$), and 10 ($n = 10$) numbers (Figure 1) was repeated. From the obtained results, it is possible to point out the incapability of the test to detect statistical differences when the differences between the control and the treatment group are 15% or less, even when using $n = 10$, which is an unusually large n number for an allelopathy assay. Besides, when using the Mann-Whitney test, the adoption of the $n = 6$ can bring accuracy to the statistical analyses without increasing expressively the costs or efforts of the surveys.

DOI: 10.1614/WS-D-10-00062.1

* Ph.D Candidate, Systematic and Chemical Ecology Laboratory, Department of Botany, Federal University of São Carlos, Via Washington Luiz, Km 235, CEP-13565-905, São Carlos, São Paulo, Brazil. Corresponding author's E-mail: jpnr@alelopatia.com.br

Table 1. Example of generated values for both the “Control” and “Multiplier” groups and the resultant “Treatment” group. The values in bold represent the average.

| Control | Multipliers | Treatment |
|--------------|--------------|--------------|
| 62.95 | 0.953 | 59.99 |
| 74.31 | 0.942 | 70.00 |
| 83.25 | 0.951 | 79.18 |
| 76.52 | 0.931 | 71.22 |
| 77.71 | 0.971 | 75.46 |
| 74.95 | 0.950 | 71.17 |

Premise

When a given test plant presents a germination percentage of 100%, of every 100 seeds 100 will become seedlings. If each seedling weighs 100 g, these 100 seedlings will present a final weight of 10,000 g. If this plant has three growing cycles in 1 yr, the total biomass produced after this period will be 30,000 g (100 seedlings by 100 g each by 3 cycles a year). As an example, if these values are reduced in 35% by the application of an allelochemical, of 100 seeds 65 will become seedlings. These seedlings weigh now 65 g each, and they have only 1.95 cycles in 1 yr, producing after this period 8,235 g (65 seedlings by 65 g each by 1.95 a year), which would turn out to be a reduction of about 72%. As isolated reductions of 35% are likely to be detected by the Mann-Whitney test ($n = 5$, $P < 0.05$) only 36.2% of the times, global effect of 72% is unlikely to be detected. Therefore, looking on PIAEs separately might lead to a misinterpretation of the meaning of the global biological results. The development of the index takes this principle into account: to combine the isolated effects on PIAEs into one single value that reflects more accurately the global effects of a donor plant on a receptor plant.

Global Effect Index

The data sets mentioned in this section are hypothetical. To generate trial control data, the germination percentage values of all the control n replicates were divided by the average value of germination percentage of the control. This division aimed at transforming those values into dimensionless values. For biomass/size of the seedlings, the same transformation was

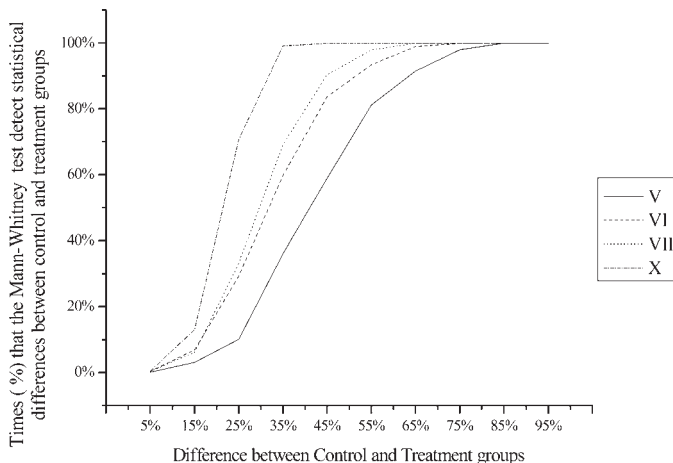


Figure 1. Efficiency of the Mann-Whitney tests in allelopathy survey data. V, VI, VII, and X mean n values equal to 5, 6, 7, and 10, respectively.

Table 2. Hypothetical allelopathy survey data. Values in bold represent the average. The arrow indicates the direction of the allelopathy effect (down means inhibition and up means stimulation). The ns indicates that these effects were statistically insignificant (Mann-Whitney $P < 0.05$).

| | Control | | Treatment | | |
|-------------|-------------|-----------------|------------------|------------------|---------------------|
| | % | AT ^a | B | % | AT |
| 97 | 32 | 0.0100 | 80 | 30 | 0.0079 |
| 94 | 25 | 0.0090 | 96 | 27 | 0.0088 |
| 95 | 28 | 0.0076 | 75 | 43 | 0.0130 |
| 88 | 29 | 0.0103 | 91 | 33 | 0.0082 |
| 92 | 24 | 0.0093 | 86 | 37 | 0.0072 |
| 93.2 | 27.6 | 0.00924 | ↓ 85.6 ns | ↓ 34.0 ns | ↓ 0.00902 ns |

^a Abbreviations: AT, average time of germination; B, biomass.

applied to generate a data set. For the average time of germination, the average value of the control data was divided by each n replicate value (the smaller the germination percentage or seedling biomass/size, the higher the inhibition, and the longer the germination average time, the higher the inhibition) (Equation 1):

$$\text{Index for control} = (\%rC / \%aC) \times (BrC / BaC) \times (ATaC / ATrC) \quad [1]$$

where % is germination percentage, B is biomass/size, AT is average time of germination, r is replicate, C is control, and a is average of.

To generate trial treatment data, the germination percentage values of all the treatment n replicates were divided by the average value of germination percentage of the control. For biomass/size of the seedlings the same thing was done. For the average time of germination, the average value of the control was divided by each treatment n replicate value (as for average time a small value means stimulation, not inhibition). It is important to notice that the reference is always the average value of the control (Equation 2):

$$\text{Index for treatment} = (\%rT / \%aC) \times (BrT / BaC) \times (ATaC / ATrT) \quad [2]$$

where % is germination percentage, B is biomass/size, AT is average time of germination, r is replicate, C is control, T is treatment, and a is average of.

After the index is applied to an assay with n replicates, all the n -sized group values of PIAEs will become one n -sized group of values for control and one for treatment, as demonstrated next.

In this hypothetical example, the following values for percentage and average time of seed germination and for biomass of the seedlings were used (Table 2).

Analyzing all PIAEs separately with the Mann-Whitney test ($P < 0.05$), no significant differences for any of them were found. Subsequently, the following index was applied: control percentage of germination (Demonstration Table 1); control biomass (Demonstration Table 2); control average time (Demonstration Table 3); hence, the final index value for control is in Demonstration Table 4.

For treatment, the same method was used, as demonstrated next: treatment percentage of germination (Demonstration Table 5); treatment biomass (Demonstration Table 6); treatment average time (Demonstration Table 7); hence, the final index value for treatment is in Demonstration Table 8.

Demonstration Table 1. Index application on values of the control percentage of germination. Values in bold represent the averages. Underlined values highlight the used control values.

| Control | | Index for % |
|-------------|-------------------|-------------|
| % | | |
| 97 | = 97/ <u>93.2</u> | 1.040 |
| 94 | = 90/ <u>93.2</u> | 1.008 |
| 95 | = 95/ <u>93.2</u> | 1.019 |
| 88 | = 88/ <u>93.2</u> | 0.944 |
| 92 | = 92/ <u>93.2</u> | 0.987 |
| 93.2 | | |

Demonstration Table 2. Index application on values of the control biomass (B), and control percentage of germination. Values in bold represent the averages. Underlined values highlight the used control values.

| Control | | Index for B |
|----------------|--------------------------|-------------|
| B | | |
| 0.0100 | = 0.0100/ <u>0.00924</u> | 1.082 |
| 0.0090 | = 0.0090/ <u>0.00924</u> | 0.974 |
| 0.0076 | = 0.0076/ <u>0.00924</u> | 0.822 |
| 0.0103 | = 0.0103/ <u>0.00924</u> | 1.110 |
| 0.0093 | = 0.0093/ <u>0.00924</u> | 1.006 |
| 0.00924 | | |

Demonstration Table 3. Index application on values of the control average time (AT), and control percentage of germination. Values in bold represent the averages. Underlined values highlight the used control values.

| Control | | Index for AT |
|-------------|-------------------|--------------|
| AT | | |
| 32 | = <u>27.6</u> /30 | 0.863 |
| 25 | = <u>27.6</u> /25 | 1.104 |
| 28 | = <u>27.6</u> /28 | 0.986 |
| 29 | = <u>27.6</u> /29 | 0.952 |
| 24 | = <u>27.6</u> /24 | 1.150 |
| 27.6 | | |

Demonstration Table 4. Final index values for the control data, and control percentage of germination. Values in bold represent averages. Underlined values highlight the used control values.

| % | Biomass | AT ^a | Final index value |
|----------|----------|-----------------|-------------------|
| 1.040 | 1.082 | 0.863 | 0.972 |
| 1.008 | 0.974 | 1.104 | 1.085 |
| 1.019 | 0.822 | 0.986 | 0.826 |
| 0.944 | 1.110 | 0.952 | 1.002 |
| 0.987 | 1.006 | 1.150 | 1.143 |
| 1 | 1 | 1.010 | 1.005 |

^a Abbreviation: AT, average time.

Demonstration Table 5. Index application on values of the treatment percentage of germination, and control percentage of germination. Values in bold represent the averages. Underlined values highlight the used control values.

| Treatment | | Index for % |
|-------------|-------------------|-------------|
| % | | |
| 80 | = 80/ <u>93.2</u> | 0.858 |
| 96 | = 96/ <u>93.2</u> | 1.030 |
| 75 | = 75/ <u>93.2</u> | 0.804 |
| 91 | = 91/ <u>93.2</u> | 0.976 |
| 86 | = 86/ <u>93.2</u> | 0.922 |
| 85.6 | | |

Demonstration Table 6. Index application on values of the treatment biomass (B), and control percentage of germination. Values in bold represent the averages. Underlined values highlight the used control values.

| Treatment | | Index for B |
|----------------|--------------------------|-------------|
| B | | |
| 0.0079 | = 0.0079/ <u>0.00924</u> | 0.854 |
| 0.0088 | = 0.0088/ <u>0.00924</u> | 0.952 |
| 0.0130 | = 0.0130/ <u>0.00924</u> | 1.406 |
| 0.0082 | = 0.0082/ <u>0.00924</u> | 0.887 |
| 0.0072 | = 0.0072/ <u>0.00924</u> | 0.779 |
| 0.00902 | | |

Demonstration Table 7. Index application on values of the treatment average time (AT), and control percentage of germination. Values in bold represent the averages. Underlined values highlight the used control values.

| Treatment | | Index for AT |
|-------------|-------------------|--------------|
| AT | | |
| 32 | = <u>27.6</u> /32 | 0.920 |
| 27 | = <u>27.6</u> /27 | 1.022 |
| 43 | = <u>27.6</u> /43 | 0.642 |
| 33 | = <u>27.6</u> /33 | 0.836 |
| 37 | = <u>27.6</u> /37 | 0.746 |
| 34.4 | | |

Demonstration Table 8. Final index values for the treatment data, and control percentage of germination. Values in bold represent the averages. Underlined values highlight the used control values.

| % | B ^a | AT | Final index value |
|--------------|----------------|--------------|-------------------|
| 0.858 | 0.854 | 0.920 | 0.675 |
| 1.030 | 0.952 | 1.022 | 1.003 |
| 0.804 | 1.406 | 0.642 | 0.727 |
| 0.976 | 0.887 | 0.836 | 0.725 |
| 0.922 | 0.779 | 0.746 | 0.536 |
| 0.918 | 0.976 | 0.833 | 0.733 |

^a Abbreviations: B, biomass; AT, average time.

Demonstration Table 9. Final index values. Values in bold represent the averages. The arrows indicate the direction of the allelopathy effect (down means inhibition and up means stimulation). The s indicates that these effects were statistically significant (Mann-Whitney P < 0.05).

| Control | Treatment |
|--------------|------------------|
| 0.972 | 0.675 |
| 1.085 | 1.003 |
| 0.826 | 0.727 |
| 1.002 | 0.725 |
| 1.143 | 0.536 |
| 1.005 | ↓ 0.733 s |

After applying the proposed transformations, two $n = 5$ sized groups, which are made of dimensionless values, were obtained. These represent the control and treatment values. Using these new groups, a new Mann-Whitney test analysis was run and significant differences were found between control and treatment, detecting potential biological allelopathic effects that may have otherwise passed undetected when data were analyzed separately (Demonstration Table 9).

The global effect index (GEI) was developed by using percentage and average time of seed germination and biomass/size of seedlings, as they are the most common PIAEs used in

Table 3. Randomly generated values representing control percentage (%) and control average time of germination (AT) and control biomass (B), and their respective multiplier values and the resulting treatment groups. Values in bold represent the averages.

| Control | | | | | Treatment | | | |
|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| % | AT | B | Multiplier | | % | AT | B | |
| 55.92 | 88.53 | 77.19 | 0.937 | 0.954 | 0.914 | 52.39 | 84.45 | 70.55 |
| 74.26 | 73.41 | 76.58 | 0.977 | 0.942 | 0.964 | 72.55 | 69.15 | 73.82 |
| 65.71 | 59.42 | 65.26 | 0.923 | 0.940 | 0.903 | 60.65 | 55.85 | 58.92 |
| 91.15 | 84.62 | 77.47 | 0.925 | 0.965 | 0.998 | 84.31 | 81.65 | 77.31 |
| 77.35 | 80.20 | 75.73 | 0.956 | 0.923 | 0.927 | 73.94 | 74.02 | 70.20 |
| 72.88 | 77.24 | 74.45 | 0.943 | 0.945 | 0.941 | 68.77 | 73.05 | 70.16 |

allelopathic surveys (Chiapusio et al. 1999; Inderjit and Dakshini 1995). Nevertheless, it is possible to replace these PIAEs by other ones. It is of crucial importance not to use related PIAEs, such as germination speed and germination average time or seedling biomass and seedling size in the same analysis, so as not to overestimate the effect of one given factor on the overall index. It is also possible to use the GEI using only two PIAEs, by simply excluding from the equation the values that refer to the nonanalyzed PIAEs.

The nonparametric Mann-Whitney tests for the development of the GEI were used. Nevertheless, after the index is calculated, the data can be analyzed with other tests and each author might use the ones they find more suitable for their own data. The data can also be transformed by a second index (An et al. 2005) and then analyzed.

Using the GEI with Simulated Data

Data were generated to test the index the same way it was done when assessing the efficacy of the Mann-Whitney test. The only difference is that this time two or three groups of numbers (that intend to represent different PIAEs) were used (Table 3).

The GEI was applied to the generated data by using two or three PIAEs. As done previously, The Mann-Whitney test efficiency was tested in allelopathy survey data by using multiplier groups that ranged from 1–0.9 to 0.1–0 and groups of 5, 6, 7, and 10 elements (*n*). For all situations, the numbers were resorted and the GEI reapplied and the analysis reperformed 4,000 times (Figure 2).

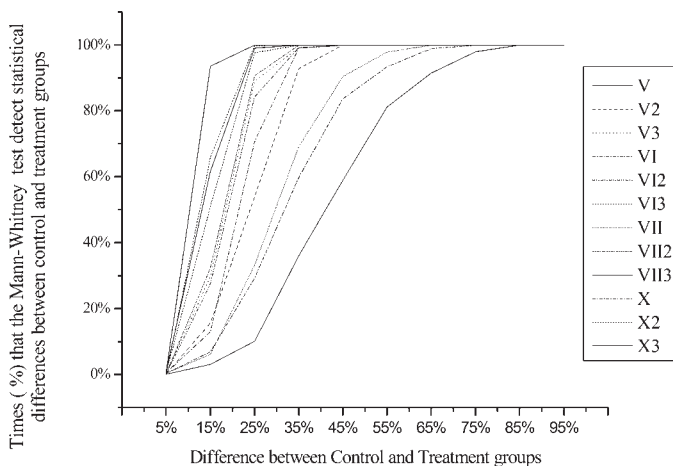


Figure 2. Efficacy of the Mann-Whitney tests in allelopathy survey data after the global effect index is applied. Roman numbers represent the *n* values and the Arabic numbers represent the numbers of PIAEs used.

Table 4a. Example of bioassay data for which statistical analysis of separate parameters used to infer allelopathic effects (PIAEs) found no differences between control and treatment percentage of germination (%) and average time of germination (AT, in hours). After the global effect index (GEI) is applied, the statistical test reveals the differences. Values in bold represent the averages. The arrows indicates the direction of the allelopathic effect (down means inhibition and up means stimulation). The s and the ns indicate whether these effects were statistically significant or not, respectively (Mann-Whitney $P < 0.05$).

| Control | | | Treatment | | |
|--------------|--------------|--------------|-------------------|-------------------|----------------|
| % | AT | GEI | % | AT | GEI |
| 96.67 | 43.20 | 1.043 | 83.33 | 40.55 | 0.958 |
| 76.67 | 46.95 | 0.761 | 86.67 | 54.92 | 0.735 |
| 76.67 | 43.50 | 0.821 | 70.00 | 46.86 | 0.696 |
| 93.33 | 32.57 | 1.316 | 83.33 | 54.72 | 0.710 |
| 86.67 | 34.15 | 1.183 | 83.33 | 54.24 | 0.716 |
| 86.00 | 40.07 | 1.029 | ↓ 81.33 ns | ↓ 50.25 ns | 0.763 s |

The results indicate that the use of the index significantly increases the capacity of the tests to detect differences. For example, in $n = 5$, when differences were of the magnitude of 35%, the Mann-Whitney tests find differences in about 36% of the tests. When applying the index to two PIAEs, this value goes up to 92.9% and with three, the Mann-Whitney test finds significant differences in 99.2% of the cases.

Using the Index with Obtained Data

After testing the index with the simulated data, it was tested with data from the São Carlos University Allelopathy Research Group with two or three PIAEs. They are always referred to as “control” and “treatment” to preserve the unpublished raw data from contributor authors. Several results were obtained, summarized as follows:

- I. Isolated analysis that presents nonsignificant differences for either PIAE. When using the GEI, the differences were detected (Tables 4a and 4b).
- II. Isolated analysis that presents nonsignificant differences for either PIAE. When using the GEI, I still found no significant differences (Table 5).
- III. One PIAE that presents significant differences (usually the germination average time) and the other(s) do not. When using the index, the differences were detected (Tables 6a and 6b).
- IV. PIAEs that point to opposite direction (such as percentage of germination and average time of germination).

Table 4b. Example of bioassay data in which statistical analysis of separate parameters used to infer allelopathic effects (PIAEs) found no significant differences between control and treatment percentage of germination (%), average time of germination (AT, in hours), and biomass (B, in grams). After the global effect index (GEI) is applied, the statistical test reveals the differences. Values in bold represent the average. The arrows indicates the direction of the allelopathy effect (down means inhibition and up means stimulation). The s and the ns indicate whether these effects were statistically significant or not, respectively (Mann-Whitney $P < 0.05$).

| Control | | | | Treatment | | | |
|-------------|-------------|----------------|--------------|------------------|------------------|---------------------|----------------|
| % | AT | B | GEI | % | AT | B | GEI |
| 97 | 32 | 0.0100 | 0.979 | 80 | 31 | 0.0079 | 0.659 |
| 90 | 25 | 0.0090 | 1.047 | 96 | 28 | 0.0088 | 0.975 |
| 95 | 28 | 0.0076 | 1.033 | 75 | 43 | 0.0130 | 0.732 |
| 88 | 29 | 0.0103 | 1.010 | 91 | 33 | 0.0082 | 0.730 |
| 92 | 24 | 0.0093 | 1.152 | 86 | 37 | 0.0072 | 0.540 |
| 92.4 | 27.6 | 0.00924 | 1.005 | ↓ 85.6 ns | ↓ 34.4 ns | ↓ 0.00902 ns | 0.727 s |

Table 5. Example of bioassay data in which statistical analysis of separate parameters used to infer allelopathic effects (PIAEs) found no differences between control and treatment percentage of germination (%) and average time of germination (AT, in hours). After the global effect index (GEI) is applied the statistical test still found no differences. Values in bold represent the average. The arrows indicates the direction of the allelopathy effect (down means inhibition and up means stimulation). The ns indicates that these effects were statistically insignificant (Mann-Whitney $P < 0.05$).

| Control | | | Treatment | | |
|--------------|---------------|--------------|----------------|-------------------|-----------------|
| % | AT | GEI | % | AT | GEI |
| 50.00 | 68.00 | 0.898 | 60.00 | 83.33 | 0.879 |
| 90.00 | 89.78 | 1.225 | 53.33 | 71.25 | 0.914 |
| 56.67 | 79.06 | 0.875 | 53.33 | 83.25 | 0.782 |
| 66.67 | 77.4 | 1.052 | 66.67 | 84.40 | 0.965 |
| 53.33 | 72.75 | 0.895 | 66.67 | 94.20 | 0.864 |
| 63.33 | 77.398 | 0.989 | ↓ 60 ns | ↓ 83.28 ns | 0.881 ns |

tion) are reduced by the same extract. In these cases the GEI tends to neutralize the differences and finds no global effects (Table 7).

Other Applications

As the GEI is dimensionless, it is possible to compare different donor plants with a single target plant, and determine, for example, which donor plants have greater inhibition effects. This can be particularly useful when trying to decide which donor plant to use when each donor plant causes different effects on one specific PIAE (Table 8).

Table 6a. Example of bioassay data in which statistical analysis of separate parameters used to infer allelopathic effects (PIAEs) (percentage of germination [%] and average time of germination [AT, in hours]) found differences between control and treatment in only one PIAE and after the global effect index (GEI) is applied, the statistical test found differences. Values in bold represent the average. The arrows indicates the direction of the allelopathic effect (down means inhibition and up means stimulation). The s and the ns indicate whether these effects were statistically significant or not, respectively (Mann-Whitney $P < 0.05$).

| Control | | | Treatment | | |
|--------------|---------------|--------------|-------------------|-------------------|----------------|
| % | AT | GEI | % | AT | GEI |
| 96.67 | 40.55 | 1.057 | 60 | 45.33 | 0.587 |
| 76.67 | 46.95 | 0.724 | 90 | 49.33 | 0.809 |
| 76.67 | 36.52 | 0.931 | 86.67 | 64.15 | 0.599 |
| 93.33 | 32.57 | 1.271 | 70 | 53.71 | 0.578 |
| 86.67 | 34.15 | 1.125 | 96.67 | 55.86 | 0.767 |
| 86.00 | 38.148 | 1.021 | ↓ 80.66 ns | ↓ 53.676 s | 0.668 s |

Table 6b. Example of bioassay data in which statistical analysis of separate parameters used to infer allelopathic effects (PIAEs) (percentage of germination [%] and average time of germination [AT, in hours]) found differences between control and treatment in only one PIAE, and after the global effect index (GEI) is applied, the statistical test found differences. Values in bold represent the average. The arrows indicates the direction of the allelopathy effect (down means inhibition and up means stimulation). The s and the ns indicate whether these effects were statistically significant or not, respectively (Mann-Whitney $P < 0.05$).

| Control | | | Treatment | | |
|--------------|--------------|--------------|-------------------|------------------|----------------|
| % | AT | GEI | % | AT | GEI |
| 50.00 | 68.00 | 0.898 | 50 | 101.6 | 0.601 |
| 90.00 | 89.78 | 1.225 | 63.33 | 85.26 | 0.908 |
| 56.67 | 79.06 | 0.875 | 60 | 92 | 0.797 |
| 66.67 | 77.40 | 1.052 | 46.67 | 86.57 | 0.659 |
| 53.33 | 72.75 | 0.895 | 63.33 | 114.32 | 0.677 |
| 63.33 | 77.40 | 0.989 | ↓ 56.66 ns | ↓ 95.95 s | 0.728 s |

Table 7. Example of bioassay data in which the parameter used to infer allelopathic effects (PIAE) points to the opposite direction (such as percentage of germination [%] and average time of germination [AT, in hours] are reduced by the same extract) and after the global effect index (GEI) is applied, the statistical test found no differences. Values in bold represent the average. The arrows indicates the direction of the allelopathic effect (down means inhibition and up means stimulation). The s and the ns indicate whether these effects were statistically significant or not, respectively (Mann-Whitney $P < 0.05$).

| Control | | | Treatment | | |
|--------------|--------------|--------------|----------------|------------------|-----------------|
| % | AT | GEI | % | AT | GEI |
| 50 | 68.00 | 0.881 | 76.67 | 81.39 | 1.129 |
| 90 | 82.4 | 1.309 | 70.00 | 81.14 | 1.034 |
| 56.67 | 79.06 | 0.859 | 56.67 | 86.11 | 0.788 |
| 66.67 | 77.4 | 1.032 | 53.33 | 86.25 | 0.741 |
| 53.33 | 72.75 | 0.878 | 73.33 | 84.54 | 1.039 |
| 63.33 | 75.92 | 0.992 | ↑ 66 ns | ↓ 83.88 s | 0.946 ns |

Table 8. Comparing two treatments (T1 and T2) on a single target species using the global effects index (GEI). The control is the same to both treatments, and the reference is still its average values, in this case 63.33 for percentage of germination (%) and 77.4 for germination average time (AT, in hours). Values in bold represent the average. The arrows indicate the direction of the allelopathic effect (down means inhibition and up means stimulation). The s and the ns indicate whether these effects were statistically significant or not, respectively (Mann-Whitney $P < 0.05$).

| Control | | | T1 | | | T2 | | |
|--------------|---------------|--------------|--------------|--------------|--------------|--------------------|-------------------|----------------|
| % | AT | GEI | % | AT | GEI | % | AT | GEI |
| 50.00 | 68.00 | 0.632 | 46.67 | 90.27 | 0.632 | 70.00 | 132.57 | 0.644 |
| 90.00 | 89.78 | 0.661 | 56.67 | 104.70 | 0.661 | 43.33 | 123.69 | 0.427 |
| 56.67 | 79.06 | 0.676 | 56.67 | 102.40 | 0.676 | 60.00 | 147.33 | 0.497 |
| 66.67 | 77.4 | 0.732 | 56.67 | 94.59 | 0.732 | 53.33 | 105.00 | 0.620 |
| 53.33 | 72.75 | 0.708 | 50.00 | 86.29 | 0.708 | 66.67 | 121.30 | 0.671 |
| 63.33 | 77.398 | 0.681 | 53.33 | 95.65 | 0.681 | ↑ 58.669 ns | ↓ 125.98 s | 0.572 s |

Another application of the GEI is that it can be used as a criterion for including or not a plant in the allelopathic list (Ahn et al. 2008). As the index summarizes the allelopathic potential of a plant in one value, its use can be more practical and accurate than choosing one (or more) PIAE. This can be especially useful in surveys that scan large areas for allelopathic plants.

Acknowledgments

I acknowledge the Conselho Nacional de Desenvolvimento Científico (CNPq) for funding this research. I also thank Dr. Maria Inês Salgueiro Lima, Reginaldo Sadao Matsumoto, M.Sc., Leandro Kenji Takao, B.Sc., and Valquiria Mariin Voltarelli, B.Sc. for supplying the raw data used in this paper, and Ph.D candidate Tiago S. Hori for reviewing this manuscript.

Literature Cited

- Ahn, J. K., H. Y. Park, S. J. Hwang, D. S. Kong, S. C. Chun, T. D. Khan, and I. M. Chung. 2008. Screening of aquatic plant extracts for herbicidal, fungicidal and insecticidal activity. *Allelopathy J.* 21:361–372.
- An, M., J. E. Pratley, T. Haig, and D. L. Liu. 2005. Whole-range assessment: a simple method for analysing allelopathic dose–response data. *Nonlinear. Bio. Tox. Med.* 3:245–260.
- Babbie, E. R. 1973. *Survey Research Methods*. Belmont, Canada: Wadsworth. 432 p.
- Chiapusio, G., A. M. Sánchez, M. J. Reigosa, L. González, and F. Pellissier. 1999. Does the knowledge of the relationships of primary and secondary effects improve allelopathy research? Pages 57–62 in F. A. Macías, J.C.G. Galindo, J.M.G. Molinillo, and H. G. Cutler, eds. *Recent Advances in Allelopathy*. Cadiz: Universidad de Cadiz.

- Djurđević, L., M. Mitrović, and P. Pavlović. 2007. Methodology of allelopathy research: 2. Forest ecosystems. *Allelopathy J.* 20:79–102.
- Gatti, A. B., M.I.S. Lima, and S.C.J.G.A. Perez. 2008. Allelopathic potential of *Ocotea odorifera* (Vell) Rohwer on the germination and growth of *Lactuca sativa* L. and *Raphanus sativus* L. *Allelopathy J.* 21:73–82.
- Inderjit and K.M.M. Dakshini. 1995. On laboratory bioassays in allelopathy. *Bot. Rev.* 61:28–44.
- Inderjit and L. A. Weston. 2000. Are laboratory bioassays for allelopathy suitable for prediction of field responses? *J. Chem. Ecol.* 26:2111–2117.
- Kato-Noguchi, H., Y. Fushimi, and H. Shigemori. 2009. An allelopathic substance in red pine needles (*Pinus densiflora*). *J. Plant Physiol.* 199:42–446.
- Legendre, P. and L. Legendre. 1998. *Numerical Ecology*. 2nd ed. Amsterdam: Elsevier.
- Ribeiro, J.P.N., R. S. Matsumoto, L. K. Takao, V. M. Voltarelli, and M.I.S. Lima. 2009. Efeitos alelopáticos de extratos aquosos de *Crinum americanum* L. *Rev. Bras. Bot.* 32:183–188.
- Santana, D. G. d. and M. Ranal. 2004. *Análise da Germinação—Um enfoque estatístico* Brasília: Editora Universidade de Brasília. 247 p.
- Yang, R. Y., L. X. Mei, J. J. Tang, and X. Chen. 2007. Allelopathic effects of invasive *Solidago canadensis* L. on germination and growth of native Chinese plant species. *Allelopathy J.* 19:241–248.
- Zar, J. H. 1999. *Biostatistical Analysis*. 4th ed. Upper Saddle River, New Jersey: Prentice Hall. 663 p.

Received April 28, 2010, and approved August 10, 2010.