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SHORT COMMUNICATION

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Recurrent inconsistencies in publications that involve Maguire's germination rate formula

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Abstract

Aim of study: The objective of this study was to present statistical information pertaining to recurrent inconsistencies found in the literature of the last 25 years that involve calculation of the GR and the errors that this may entail when interpreting the seed vigor of different botanical materials.

Materials and methods: After filtering articles that did not present numerical results or in which the application of Maguire's formula was not clearly indicated, a total of 124 papers were ultimately used in this study.

Main results: Germination rate (GR) represents the potential of seeds to germinate and, along with germination percentage (GP), are the two most important measurements when evaluating seed quality. The correct calculation of GR allows the seeds classification according to their vigor and capacity to produce healthy seedlings, so it is very important to rely on the adequate mathematical formula for this purpose. According to the formula developed by Maguire, this parameter is calculated by the sum of values obtained when dividing the GP at different times (i.e. daily) by the total time elapsed since the start of the test. We have found from the relevant literature that different authors have been using different ways of applying the formula, which makes it difficult to directly compare the results.

Research highlights: A total of 54.8 % of the papers reviewed, belonging mainly to the theme of forestry, presented objectionable application of the formula. Publication of this warning may help to reduce the occurrence of this situation in the future.

Additional keywords: Germination percentage, germination speed, Maguire's formula, seed vigor.

Authors´ contributions: RP conceived the study, performed the statistical analysis and drafted the manuscript. GV conducted the literature review and helped to draft the manuscript.

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Supplementary material: Tables S1 to S3 accompany the paper on FS's website.

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Introduction

Determination of germination potential is one of the most important measurement when the quality of seeds is evaluated, and it represents the germination when seeds are exposed to appropriate conditions, expressed as the speed rate with which the germination process occurs (germination rate GR). For international seed trade, it is very useful to have a common standard for evaluating germination potential. González & Orozco (1996) and Ranal & García (2006) presented reviews in which they accurately describe the different methods that have been used for the calculation of seed germination parameters, where GR and germination percentage (GP) represent the most commonly used.

The reliability of calculation of a germination parameter is necessarily linked to the number of seeds evaluated. Increased numbers produce more accurate results; however, if the number is too high, there may be an unnecessary increase in the cost of the research and the time required to complete it.

Based on the above, the International Seed Testing Association (ISTA) developed criteria to define minimum sample sizes in the germination tests of different species, mainly from the temperate zone. According to this organization, the evaluation of commercial seeds should be based on a quantity of 400 seeds divided into four replicates of 100 units each, although less seeds could be used depending on their size and collection difficulties (ISTA, 2012).

While GP represents seed viability, GR is a parameter that reflects the vigor of the seed and varies depending on the time required for the germination process to occur. Groups of seeds with similar GP may differ significantly in their GR.

A formula widely used for the calculation of GR in seed studies was developed by Maguire (1962), who adapted it from the original of Throneberry & Smith (1955). Another valuable equation, developed by Czabator (1962), and known as germination value, is an index that varies directly and proportionally with the GR, GP, or both, although this index has been much less used than Maguire's formula.

Despite the rationale presented by Brown & Mayer (1988) against Maguire's formula, the method has been shown to be suitable for seeds of woody perennials, a group of plants characterized by slow germination rates (Hartmann *et al.*, 2002). The method is a popular manner of assessing germination rates of different plant species and has been cited 4709 times according to Google Scholar, with 9895 downloads up to March 2019, according to the ACSESS Digital Library (https://dl.sciencesocieties.org/publications/cs/abstracts/2/2/CS0020020176).

Germination rate, as proposed by Maguire (1962), must be calculated as the sum of the values obtained when dividing the percentage of partial germination in each count by the time elapsed since the beginning of the test. It is literally expressed as follows:

$$GR = \frac{Number\ of\ normal\ seedlings}{Days\ to\ final\ count} + \cdots + \frac{Number\ of\ normal\ seedlings}{Days\ to\ final\ count}$$

In the tests, it is inferred that Maguire used 100 seeds, but in the numerator of his formula he placed "number of seedlings" instead of "percentage of seedlings", as it should be according to the concept correctly indicated by him. However, in the text, he made clear that his calculations referred to 100 seeds. Obviously, in his case, the "number of seedlings" was exactly the same as the percentage of seedlings.

The use of number of seedlings instead of percentage of seedlings in many of the subsequent seed studies where the sample size differed from 100 seeds has led to serious inconsistencies in the reported results and the impossibility of direct comparison with those of other studies.

In a review of the pertinent bibliography of articles of recent decades referring to seed germination, it can be observed that different formulas have been used to calculate the seed germination rate. Since in several cases errors have been detected in the application of these formulas, the present study reviewed an important number of publications related to seed germination in order to determine the frequency of occurrence of inaccuracies in the application of the aforementioned formula. The aim of this study is to present statistical information regarding the inconsistencies found in these publications and the errors that these imply when interpreting the vigor of the seeds of many botanical materials.

Materials and methods

A search was conducted through Google Scholar and the databases of the Web of Science and Scopus, utilizing a filter that included only articles from the last 25 years (1994 to 2018). This produced a total of 216 articles that involved the word germination in the title and, of these, 152 articles were selected that cited the article by Maguire (1962) in their references. This literature included parameters described as germination speed, germination rate index or germination rate. The papers were obtained in PDF format from Proquest, Springer, Scielo, or directly from open access journals.

Articles that did not present numerical results or in which the methodology was not clearly indicated were excluded, along with those that, despite having determined germination parameters, used different representations of the germination rate or had incorrectly transcribed Maguire's formula. This filtering process left a total of 124 articles, which were those ultimately used in this study.

We evaluated the articles in which there would be some form of imprecision when applying the formula developed by Maguire or that had not followed the recommendation of ISTA (2012) in relation to the number of seeds to be used in the tests. Special attention was paid to articles in which the sample size was lower than 100 seeds without conversion to percentages, and that clearly indicated that the number of seeds was used for the purposes of the calculations. In order to determine whether the number of cases has varied over time, the results were classified into periods of the year and, to determine whether they occur more frequently in certain agricultural species or certain geographical areas, they were classified by plant species or country of origin of the paper. The results are presented comparatively in graphs showing the total number of papers and the number of these in which Maguire's formula would have been incorrectly applied.

A linear regression analysis was carried out between the number of seeds used in each paper and the mean value reported for the germination rate. When a paper stated that measurements had been converted to percentages, the independent variable was considered

as 100, regardless of the actual number of seeds tested. A total of 107 papers were used for this analysis, with 17 papers discarded because their results were unclear or highly variable. The regression was developed by means of the SAS v.9.1 program (Cary, NC, USA).

Results and Discussion

The regression analysis detected a highly significant effect ($P \leq 0.0001$) of the number of seeds tested on the germination rate result (Fig. 1). The coefficient of determination was not high ($R^2 = 0.3983$), but can be attributed to the high and predictable natural variability that existed between the germination rates of the different plant species considered.

It is notable that the values reported for the germination rate tended to diminish with lower numbers of seeds evaluated in the tests, which corresponds to the magnitude of the error in the application of Maguire´s formula. Long time ago, Bouton *et al.* (1976) recommended the use of the percentage rather than the number of newly germinated seedlings in order to avoid this problem associated with sample size.

The number of studies that met the requirements for this study increased as we examined more recent publications (Fig. 2). This can be attributed to the greater number of articles currently published online by the different scientific journals due to the worldwide advance of the digitized information. With the exception of the five-year period 2004-2008, the objectionable articles comprised more than 50% of the total articles evaluated.

The papers that featured objectionable application of the formula came mainly from the field of forestry (Fig. 3) and wide variation was observed in the reported GR values. For the original data see Table S1 [suppl.].

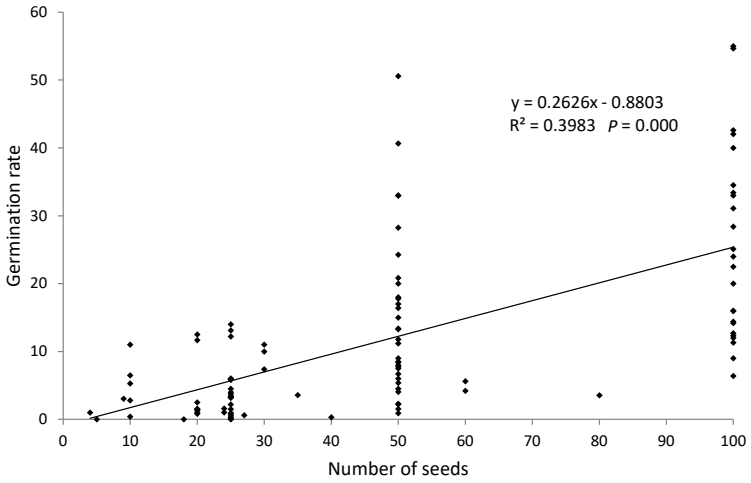

Figure 1. Regression of the effect of number of seeds tested on the resulting germination rate.

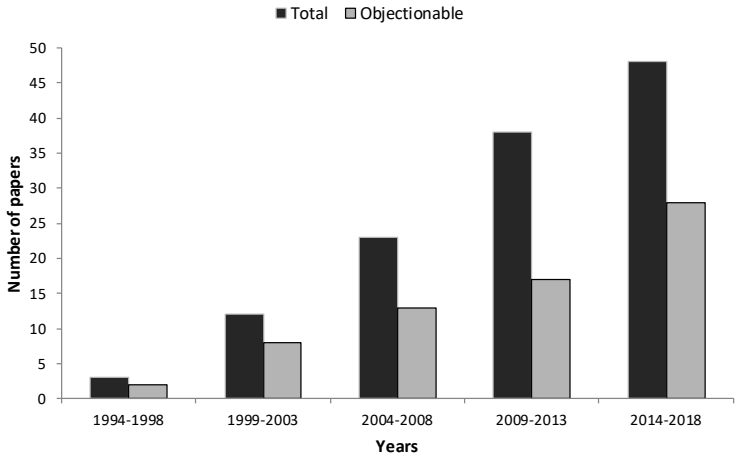

Figure 2. Number of total and objectionable articles published within five-year periods.

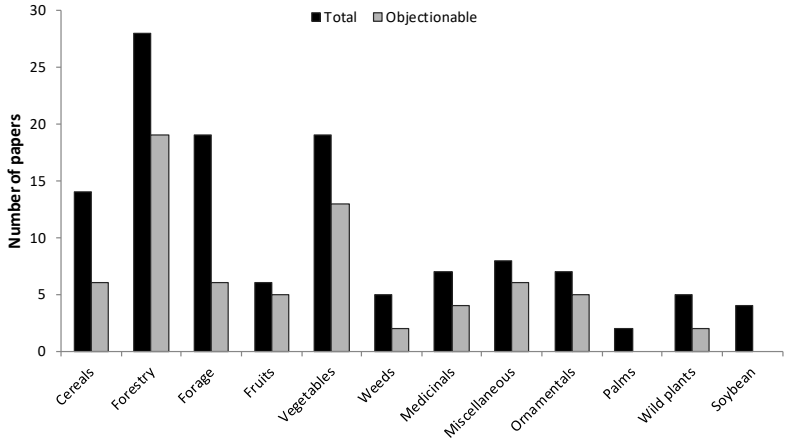

Figure 3. Number of total and objectionable articles according to the plant types studied.

The geographical distribution of the origin of the articles evaluated has predominance in Brazil (57 papers), country where a large number of papers on seed germination are produced. It was followed by China, Iran, USA, and México, with 13, 12, 8 and 5 papers, respectively. Other countries contributed only with three or less articles: Argentina, Colombia, and Peru (for America), France, Greece, Poland, and Sweden (for Europe), Arab Emirates, Bangladesh, Japan, Jordan, Pakistan, Saudi Arabia, and Turkey (for Asia), and Nigeria, South Africa, and Zimbabwe (for Africa). Objectionable papers among countries varied from 31 to 75 %.

A significantly smaller number of articles used other units of measurement, catalogued as indices or rates. In others, the authors used the term "germination rate" to refer to "germination percentage". Likewise, it was observed in a moderate number of articles that germination rate was expressed as "mean germination time" (Ranal *et al.*, 2009), also referred to as "germination rate index" (IVG) by Hartmann *et al.* (2002). None of the above cases were considered for our study.

In the study, some articles claimed to have used Maguire's formula, despite having mentioned the term IVE (speed of emergence index), or its translated equivalent, in the article. There were also articles that used the formula but cited it through other published references.

It should be emphasized that only a few of the evaluated papers presented sufficient details of the application of the formula that would allow us to precisely verify its correct use. The majority of papers judged as "objectionable" were therefore those that reported calculations based on the "number of germinated seeds". In contrast, some papers that did not mention the "number of seeds" but, instead, mentioned "the counts", were given the benefit of the doubt and were classified as not objectionable.

A second identified source of error was the use of the total percentage of seeds germinated in each evaluation, when the appropriate value for use would have been that of the difference between the current and previous measurements. For this reason, it is good practice in trials to discard all newly germinated seedlings immediately after measurement, so that there is no possibility of confusion when carrying out the subsequent measurement. While Maguire (1962) does not emphasize that measurements should include only the new seedlings that emerged in the period of each count, the example that he presented allows us to infer that detail.

The results of our study show that, in terms of the application of Maguire's formula, 68 out of 124 papers (54.8 %) were considered objectionable. This represents a considerable number of articles that we can assume to have calculated the GR erroneously. These errors would have occurred for one of the following two reasons:

a) Incorrect use in the formula of number of seeds instead of percentage of seeds in cases where the tests were conducted using less than 100 seeds (in this case, between 10 and 50 seeds per replicate).

b) Failure to eliminate germinated seeds in each count in order to avoid readings of seedlings that had already been recorded in previous readings.

The inconsistencies found in these publications indicate that errors of considerable magnitude exist when interpreting the seed vigor of certain botanical material, and the incorrect application of the formula for calculating the GR makes it impossible to compare the results of different studies.

Examples of two cases in which there were inaccuracies in relation to the number of seeds used or the erroneous counting of seedlings already recorded in previous readings are shown in Tables 2 and 3 [suppl.]. For both cases, the published and corrected results are presented, detailing the expected procedure.

Conclusions

For many years, numerous papers have featured an incorrect application of Maguire´s formula to estimate seed vigor by calculating the rate or speed of germination. This has caused inconsistencies when comparing the values of this index among different plant species, leading to erroneous results. With respect to the application of Maguire's formula, 54.8 % of the papers examined in this study, mainly from the field of forestry, were considered objectionable.

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