REPORT ON THE INTERNATIONAL INTERLABORATORY COMPARISON ON THE PHYTOTOXKIT

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EXECUTIVE SUMMARY

The Phytotoxkit microbiotest is a very simple and practical assay, which is more rapid and easy than the conventional assays in pots, for the assessment of the effects of pollutants on higher plants. This assay in now in use in many laboratories in many countries, and the interest for this microbiotest is growing further continuously.

Yet, and contrary to other Toxkit microbiotests, neither validity criteria nor a reference test with a chemical compound have so far been worked out for this attractive assay.

Extensive research performed in the company MicroBioTests Inc. lead to the suggestion of taking germination success of the seeds in the negative controls as a first validity criterion, and the length of the roots in the negative controls as the second validity criterion. In addition, and in analogy to other toxicity tests on higher plants, boric acid was proposed as a suitable compound for a reference test with the Phytotoxkit.

Eventually a procedure for a reference test was worked out, for application “on one concentration” of boric acid.

In order to evaluate the applicability of the proposed two validity criteria and the precision of the reference test with boric acid, an initiative was taken by the Istituto per lo Studio degli Ecosistemi (I.S.E) of the Consiglio Nazionale delle Richerche in Pallanza (Italy), to organize an International Interlaboratory Comparison on the Phytotoxkit microbiotest. In case the outcome of the ring test is considered satisfactory, this microbiotest with higher plants will be proposed to ISO, as a new standard.

An invitation to participate in the International Interlaboratory Comparison was sent out in March 2011 to laboratories, organisations, institutes and companies in different countries, known to regularly apply the Phytotoxkit assay. It was indicated that participation would be free of charge, and that those who subscribed to the ring test would receive a kit containing all the materials needed for performing one assay on one concentration of boric acid, according to the test protocol included in the testkit. The condition for participation was that that the results of the assay had to be sent to the organiser within the stipulated deadline (basically two months after reception of the test kit).

The company MicroBioTests Inc. was asked to assemble the test kit and to send it to the participants. Besides the detailed test protocol, the test kit contained a vial with a solution of boric acid, for performance of the assay with 250 mg boric acid per kg OECD artificial soil.

Thirty positive answers for participation were received, and each subscriber was subsequently sent a kit in May 2011. Results sheet were also sent by email to the participants, with a specific Excel programme which automatically processes the data and calculates the % inhibitions.

From the 30 participants, 28 laboratories, institutes, organisations and companies from 12 countries eventually performed the assay and sent their results to the organiser in May, June and early July 2011.

After reception of all the results, a questionnaire was sent to each participant, asking for replies on a number of technical aspects of the assay.

Three endpoints were taken into consideration for this International Interlaboratory Comparison, for the three plant test species:

1. Seed germination: the number of seeds in each of the three replicates for which a root was observed at the end of the test

2. Mean root length: the mean length of all the roots, in mm, for each of the three replicates, at the end of the test
3. Longest root length: the length of the longest root, in mm, in each of the three replicates at the end of the test.

The statistical analysis of all the data of the Interlaboratory Comparison has been made by the I.S.E, and besides the calculations on the repeatability and the reproducibility according to ISO 5725-2 (2002), the % Germination Index was also determined. It may be underlined that these analyses eventually encompassed more than 10.000 individual data.

The outcome of the results submitted by the individual laboratories, and the subsequent statistical calculations by the organiser are addressed in detail in this Final Report and in the Annexes, with a substantial number of Figures and Tables in both the Report and the Annexes.

The report also includes at the end of each section a discussion on the results, with formulation of conclusions and suggestions for improvement of the test procedure.

The substantial international participation in the International Interlaboratory Comparison was already a clear sign for the interest in a simple and practical microbiotest for rapid assessment of the hazard of pollutants on higher plants.

A first analysis of the Result Sheets already revealed that all the participants had managed to perform the reference test properly and had provided satisfactory data.

The selection of 250 mg boric acid per kg OECD artificial soil was eventually also a good choice since it had lead to “meaningful” % inhibition of root growth for the three plant species (around 40-45%), with the majority of the individual results being within the 95% confidence limits. It may be underlined, however, that at that concentration of the reference chemical, the germination success of the test plants is not affected.

The exercise has furthermore shown that measurement of only the longest root in each test plate, instead of the measurement of all the roots of the germinated seeds, is an interesting alternative which basically gives the same outcome, but which is a substantial time saving.

In comparison to other ringtests, the intralaboratory variability for the mean % inhibition of both the mean root length and the mean length of the longest root are below 20% and hence quite satisfactory. The interlaboratory variability figures are 20% to 35% for the mean % inhibition of the root length, but less than 20% for the mean % inhibition of the longest root, and hence also satisfactory.

The outcome of the International Interlaboratory Comparison can hence be considered as being very successful, and from the data and their statistical analysis, the following validity criteria are proposed for the Phytotoxkit assay:

1. Seventy % of the seeds of the three test species must have germinated in the negative controls at the end of the exposure period
2. The mean root length in the negative controls must be at least 30 mm for the three test species, and 40 mm in case the measurements are based on the mean length of the longest root.

The following criteria are also proposed for the improvement of the Phytotoxkit assay:

1. Seeds are considered as germinated when an emergence of at least 1 mm protrudes from the seed at the end of the exposure period.
2. Length measurements of the roots must be made on the roots of the germinated seeds as of a length of 1 mm emergence of the root from the seed
3. Calculation of the % inhibition can also be based on the % Germination Index which integrates the data on seed germination and root length.

4. A detailed description of a reference test with boric acid at a test concentration of 250 mg boric acid per kg of OECD artificial soil, will be included in the Standard Operational Procedure of the Phytotoxkit microbiotest
5. Acceptance limits for a reference test with boric acid will be prescribed in the Standard Operational Procedure. These acceptance limits have been selected from the data of the International Interlaboratory Comparison.
6. The test plates of the Phytotoxkit assay will be provided with “marks” for equidistant placing of the seeds, at a specific distance from the middle ridge of the test plate.

INTRODUCTION

The Phytotoxkit microbiotest is an assay very simple and practical to perform, and definitely more rapid and easy than any of the “conventional” toxicity tests on higher plants, in pots.

Since its appearance on the market about 6 years ago, the Phytotoxkit microbiotest is now in use in many laboratories in more than 20 countries, and the interest for this microbiotest is growing further continuously.

It has recently been mentioned by several users that, in analogy to other Toxkit microbiotests, it would be good to include in the test protocol of the Phytotoxkit microbiotest “validity criteria” for this assay. Furthermore, the addition to the test protocol of a reference test on a chemical compound would also be welcomed.

Extensive research on the former aspects has therefore recently been undertaken by the company MicroBioTests Inc., which is at the origin of the development of the Phytotoxkit microbiotest.

The results of this research have led to the selection of two validity criteria for this assay: the first one is related to the percentage germination of the seeds in the control test plates; the second one is dealing with the “mean length” which the roots of the 3 test plants must have in the control test plates, at the end of the 3 days test.

The experimentation on a “reference test” has been performed with boric acid, which is one of the chemicals which is advised for performance of reference tests on higher plants.

Eventually a test protocol for a reference test has been worked out to be applied “on one concentration” of boric acid; the percentage of growth inhibition of the roots of the plants in that particular concentration of the reference chemical, is then determined in comparison to the growth of the roots in the control test plates.

In order to evaluate the applicability of the selected validity criteria on one hand, and the precision of the reference test with boric acid on the other, an initiative has been taken to perform an international interlaboratory comparison on the Phytotoxkit microbiotest.

In case the outcome of the Phytotoxkit interlaboratory comparison is considered satisfactory, it is the intention to propose this microbiotest with higher plants to the ISO, as a new standard.

ORGANISATION OF AN INTERNATIONAL INTERLABORATORY COMPARISON ON THE PHYTOTOXKIT

This International Interlaboratory Comparison has been organised under the coordination and supervision of the “Consiglio Nazionale delle Ricerche (CNR) – Istituto per lo Studio degli Ecosistemi (I.S.E.)” in Pallanza (Italy).

In accordance to the ISO rules on the determination of accuracy of measurement methods and results (ISO 5725-2: 2002) between 8 and 15 laboratories should be participating in the exercise, each of which should already have experience with the test under analysis.

A list was therefore compiled of the laboratories, organisations, institutes and companies in different countries known to regularly apply the Phytotoxkit, and an invitation was sent out in March 2011 by I.S.E. for their possible participation in the International Interlaboratory Comparison.

It was indicated in the invitation that no subscription charge would be asked to the participants and that they only had to perform one test on one concentration of the selected reference chemical. All the materials needed for performing the assay would be included in a "testkit" which would be sent free of charge to the participants.

An "Operational Procedure" has been worked out by the organisers which describes in detail the experimental procedure of the International Interlaboratory Comparison.

The conditions for participation in this Interlaboratory Comparison are that the participants should strictly follow the "Operational Procedure" (which is included in the testkit), and that the detailed results of their toxicity test should be sent to the organiser I.S.E. "within the stipulated deadline" (i.e. within two months after reception of the testkit).

A request was made by I.S.E. to the company MicroBioTests in Belgium to work out a model for the testkit and its contents.

The company MicroBioTests was subsequently asked to prepare all the testkits and to send them to the participants at the timing indicated by I.S.E.

The following time schedule for the International Interlaboratory Comparison was eventually worked out:

- Shipment of the materials to the participants: First week of May 2011
- Time to perform the interlaboratory comparison: May – June 2011
- Sending of the results to the organiser: last week of June 2011

PARTICIPANTS IN THE INTERNATIONAL INTERLABORATORY COMPARISON ON THE PHYTOTOXKIT

About 40 laboratories, organisations, institutes and companies worldwide were contacted for participation in the International Interlaboratory Comparison on the Phytotoxkit.

Thirty laboratories, institutes, organisations and companies from 12 countries indicated their interest to participate in this International Interlaboratory Comparison and have sent their Participation Form to I.S.E., with their agreement to abide by the conditions for participation.

From the 30 potential participants, 28 have performed the assays and have sent their results to the organiser within the stipulated deadline.

The names of the participating laboratories, institutes, organisations and companies are given hereunder, per country, listed in alphabetical order.

BELGIUM

- Flemish Environment Agency VMM, Erembodegem
- MicroBioTests Inc., Mariakerke

- Scientific Institute of Public Service ISSeP, Liège

CANADA

- Environment Canada, Environmental Science and Technology Centre, Ottawa

ESTONIA

- National Institute of Chemical Physics and Biophysics, Tallinn

FRANCE

- Université Paul Verlaine, LIEBE (UMR7146), Metz

GREECE

- Technical University of Crete – Laboratory of Toxic and Hazardous Waste Management, Chania-Crete

GUATEMALA

- Servicios y Productos Ambientales SEPRA, Guatemala City

HUNGARY

- National Institute of Environmental Health, Budapest

ITALY

- Ambiente S.C., Carrara
- ARPA Sardegna, Cagliari
- ECOBIOQUAL, Torino
- R&C laboratory s.r.l., Altavilla Vicentina, Vicenza

POLAND

- Gdansk University of Technology, Chemical Faculty, Gdansk
- Institute of Soil Science and Plant Cultivation, State Research Institute, Department of Soil Science and Land Conservation, Pulawy
- Institute of Soil Science and Plant Cultivation, State Reseach Institute, Department of Herbology and Technology of Land Cultivation, Wroclaw
- Medical University of Gdansk, Department of Environmental Toxicology, Gdynia
- Medical University of Warsaw, Department of Environmental Health Sciences, Warsaw
- Military Institute of Hygiene and Epidemiology, Laboratory of Pharmacology and Toxicology, Warsaw
- Opole University, Department of Land Protection, Opole
- University of Agriculture in Krakow, Department of Agricultural and Environmental Chemistry, Krakow
- University of Life Sciences in Lublin, Institute of Soil Science and Environment Management, Lublin
- Wroclaw University of Environmental and Life Sciences, Department of Environmental Hygiene and Animal Welfare, Wroclaw
- Wroclaw University of Technology, Faculty of Environmental Engineering, Wroclaw

SOUTH AFRICA

- Golder Associates Research Laboratory Ltd, Florida
- Ambio Environmental Management (PTY) Ltd, Technology Transfer and Innovation, Vaal University of Technology, Vanderbijlpark

SOUTH KOREA

- University of Incheon, Green Pioneer R&D Center, Incheon

SWITZERLAND

- Ecole Polytechnique Fédérale de Lausanne, Ecotox Centre, Dubendorf-Zurich

REFERENCE CHEMICAL

Boric acid (H_3BO_3) is the reference chemical recommended by ASTM E 1963 (2002) and one of the three mentioned by ISO 22030 (2005) for plant toxicity tests. This compound was therefore selected as the reference chemical for the International Interlaboratory Comparison on the Phytotoxkit.

The reference chemical was included in the testkit with the instructions how to prepare the selected concentration for the assay, 250 mg boric acid per kg OECD artificial soil in the test plates.

TEST PROTOCOL OF THE PHYTOTOXKIT INTERNATIONAL INTERLABORATORY COMPARISON

The test protocol of the International Interlaboratory comparison is added in Annex 1 of this Report.

ENDPOINTS

For this International Interlaboratory Comparison the following endpoints have been considered for the three plant test species:

1. Seed germination: the number of seeds, in each of the three replicates for which a root was observed and measured at the end of the test
2. Mean root length: the mean length of all roots, in mm, for each of the three replicates, at the end of the test
3. Longest root length: the length of the longest root, in mm, in each of the three replicates at the end of the test.

MEASUREMENTS

As indicated in the test protocol, the participants were requested to measure the length of the roots of each germinated seed in each test plate (with the aid of the Image Tools programme[1]) and to type in their results on a specific Excel Result Sheet, which they were requested to send by e-mail to the organiser.

A copy of the Excel Result Sheets is given in Annex 3.

DATA TREATMENT

The Excel programme automatically calculates the number of germinated seeds, the mean length of the roots, the mean of the longest roots and the inhibition percentages of the latter 2 means, with respect to the corresponding values for the negative controls, with the following formulas:

- Mean number of germinated seeds in the 3 replicates: $\overline{N} = \frac{(N_1 + N_2 + N_3)}{3}$
- Mean length of the *n* measured roots in replicate *i*: $\overline{L_i} = \frac{\sum_{k=1}^{n} L_k}{n}$
- Mean of the 3 mean root length $\overline{L_i}$: $\overline{\overline{L}} = \frac{(\overline{L_1} + \overline{L_2} + \overline{L_3})}{3}$
- Mean of the 3 longest root length LR_i of each replicate *i*: $\overline{LR} = \frac{(LR_1 + LR_2 + LR_3)}{3}$

[1] UTHSCSA ImageTool (IT) is a free image processing and analysis program for Microsoft Windows 9x, Windows ME or Windows NT. It can be downloaded from http://ddsdx.uthscsa.edu/dig/itdesc.html

As recommended by ASTM E 1963 (2002), the percent effect %E for each of the 3 plant species exposed to boric acid (relative to the mean of the controls) has been calculated using the formula:

$$\%\ E = \frac{(control\ endpoint\ mean - treatment\ endpoint\ value)100}{control\ endpoint\ mean}$$

The mean and the standard deviation of the effect percentage is then computed for each seed and endpoint.

Finally, taking profit of the availability of the extensive set of germination and root elongation data, a "% Germination Index" has also been calculated following the procedure outlined in the Italian Official Method UNICHIM 1651 (2003) for the plant bioassay in Petri dishes.

The % Germination Index indicates the extent of the success of the whole process (seed germination *plus* root elongation) for the seeds exposed to the toxicant (or sample), in comparison to the success of the whole process in the negative control, that is in optimal conditions. Since the Index is a percentage, it allows to compare results obtained in different tests (hence, with different negative controls): the eventual differences in the negative controls are eliminated, imposing that the % Germination Index of these controls are equal to 100 %. The "figure" (low or high percentage) actually indicates "the opposite" of the % inhibition, i.e. the higher the % Germination Index, the less toxic effects have been found.

The formulas to calculate the % Germination Index are given and explained in Annex 2.

Statistical analysis

It was stipulated by the organiser that the statistical analysis of all the data would be made by I.S.E.

This statistical analysis produced the repeatability and reproducibility of the interlaboratory comparison, according to the ISO 5725-2 (2002) procedure. The full data treatment is reported *in extenso* in Annex 4, with figures and tables.

Remark: for the sake of uniformity, the term "mean" will be used throughout the report for the indication of the "overall mean" of all the mean results of the participating laboratories, and the term "average" for the means of the individual results produced by each laboratory.

Data analysis of the "negative controls"

The data on seed germination and root lengths have been summarized in the following figures, as histograms, which are ordered from the lowest to the highest average of each laboratory.

A horizontal line (in red) shows the overall mean for all 28 laboratories, and two horizontal broken line (in black) indicates the 95 % confidence limits calculated on the basis of the data set without h and k outliers (see Annex 4 for details).

The data on the repeatability and the reproducibility which are given in each section below originate from the corresponding tables in Annex 4.

Number of germinated seeds

A seed is considered germinated when an emergence protrudes from the seed at the end of the exposure period (this point is further explored in the General Discussion).

The statistics are based on the average and variability of the number of germinated seeds in the 3 replicates.

The starting number of seeds is 10 for each test; therefore, the 95 % upper confidence limit (which statistically can exceed 10) has been omitted in the pertinent figure.

Lepidium sativum (LES)

Figure 1 clearly shows the very good germination of the *Lepidium sativum* seeds; all the laboratories except one indeed obtained a mean germination higher than 80%.

The analysis of the individual data indicated that two results had to be discarded (lab 1 as h outlier, lab 14 as k outlier: see Annex 4 for details). However, the statistical treatment does not change the mean value, remaining at 9 seeds (= 90% germination, even discarding stragglers). Discarding the two outliers, there is a small improvement in both the intra- and interlaboratory variability.

The accepted results, based on 26 laboratories (out of the original 28 participating labs), have a repeatability (intralaboratory variability) of 8.5 % and a reproducibility (interlaboratory variability) of 8.9 %.

On the basis of the corrected data set (without h and k outliers), one laboratory produced a seed germination average which is lower than the 95% lower confidence limit.

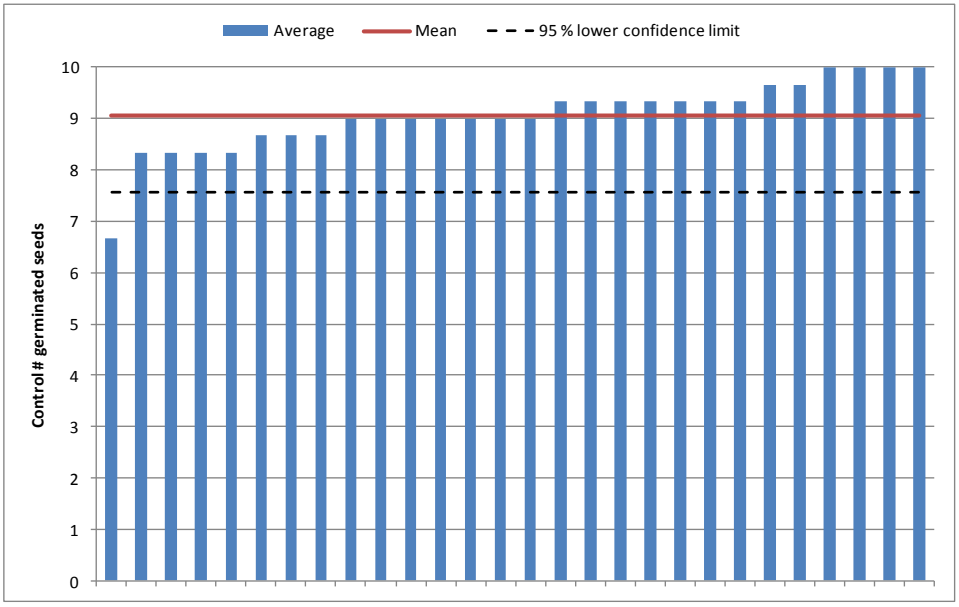

Figure 1 - Average number of germinated seeds of *Lepidium sativum* in the negative controls for each laboratory, with two horizontal lines indicating the “overall mean” and the 95% lower confidence limit.

Sinapis alba (SIA)

Similarly to *Lepidium sativum*, all the laboratories also obtained very good germination with the second dicotyl test species (> 80% in all but one laboratory). Two results have been discarded (lab 21 as h outlier, lab 11 as k outlier). Again, however, the statistical treatment does not change the mean value, remaining at 9 seeds (= 90% germination, even discarding stragglers).

The accepted results, based on 26 laboratories (out of the original 28 participating labs), have a repeatability of 7.9 % and a reproducibility of 8.5 %.

On the basis of the corrected data set (without h and k outliers), one laboratory produced a seed germination average lower than the 95% lower confidence limit.

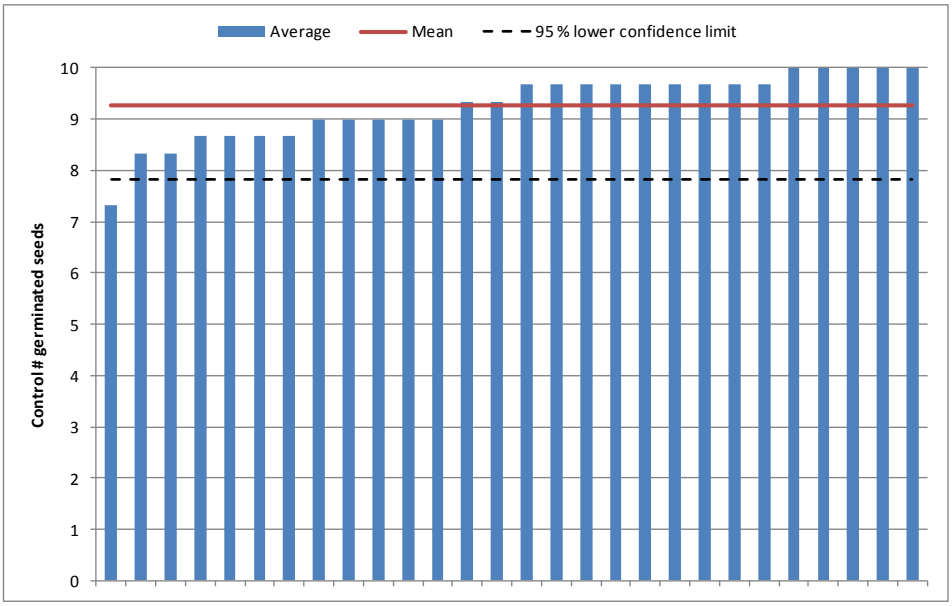

Figure 2 - Average number of germinated seeds of *Sinapis alba* in the negative controls for each laboratory, with two horizontal lines indicating the "overall mean" and the 95% lower confidence limit.

Sorghum saccharatum (SOS)

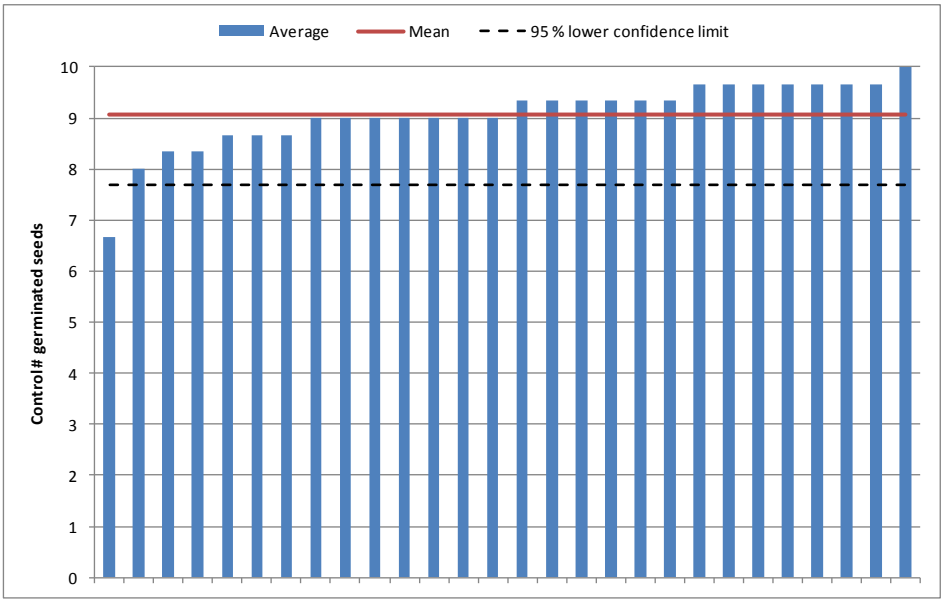

Figure 3 - Average number of germinated seeds of *Sorghum saccharatum* in the negative controls for each laboratory, with two horizontal lines indicating the "overall mean" and the 95% lower confidence limit.

The germination success of the monocotyl *Sorghum saccharatum* was also very good. All the laboratories except one have indeed reported germination figures at or higher than 80%.

Three results have been discarded (lab 21 as h and k outlier, and labs 14 and 17 as k outliers). The overall mean is 9 seeds (= 90% germination, even discarding stragglers). The accepted results, based on 25

laboratories (out of the original 28 participating labs), have a repeatability of 7.6 % and a reproducibility of 8.3 %.

On the basis of the corrected data set (without h and k outliers), one laboratory produced a seed germination average lower than the 95% lower confidence limit.

Discussion

The International Interlaboratory Comparison on the Phytotoxkit provided consistent results for the germination of all 3 seeds in the negative controls, with a high percentage of germination and a low intra- and interlaboratory variability.

Taking into account the 95 % lower confidence limit (overall average $\pm$ 1.96 s_R), it can be safely concluded that the Phytotoxkit can adopt a common validity criterion of 70 % germination in the negative control for all three seeds, with a reproducibility (interlaboratory variability) of 9.0 %.

NB : the validity criterion of 70 % germination in the negative control is the same as that indicated in OECD Guideline 208 (2006) for the Seedling Emergence and Seedling Growth Test.

Root length

The root lengths have been measured on the pictures taken at the end of the exposure period, using the Image Tools software. The statistics are based on the average and variability of the measured length in the 3 replicates.

Lepidium sativum (LES)

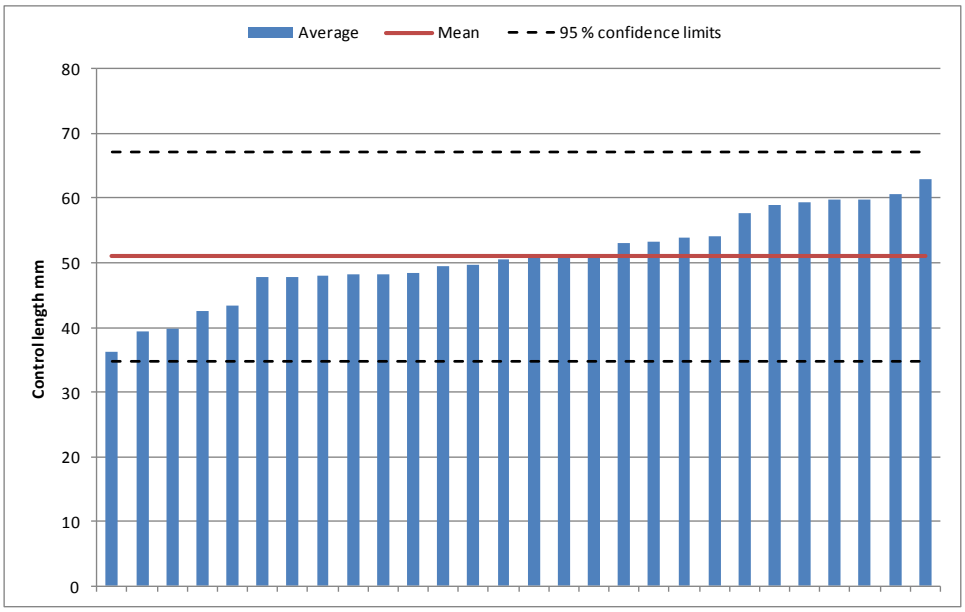

Figure 4 - Average length of the roots of *Lepidium sativum* in the negative controls for each laboratory, with three horizontal lines indicating the “overall mean” and the 95% confidence limits.

Figure 4 shows that the average root lengths reported by the participants ranged from 36 mm to over 60 mm. There are no k outliers. The accepted results, based on 28 laboratories, have an overall mean of 51 mm, a repeatability of 11.3 % and a reproducibility of 16.3 %.

On the basis of the corrected data set (without h and k outliers), all the averages are within the 95% confidence limits.

Sinapis alba (SIA)

Similarly to the average root lengths of *Lepidium sativum*, those of *Sinapis alba* are spread over a range of 34 mm to more than 60 mm.

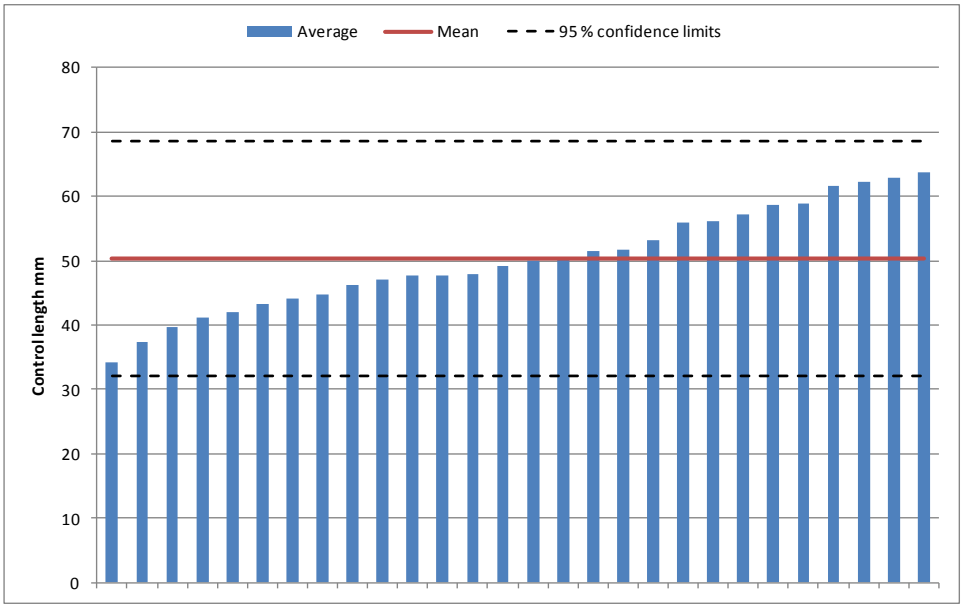

Figure 5 - Average length of the roots of *Sinapis alba* in the negative controls for each laboratory, with three horizontal lines indicating the "overall mean" and the 95% confidence limits.

Two results have been discarded (labs 25 and 27 as k outliers). The accepted results, based on 26 laboratories (out of the original 28 participating labs), have a mean of 50 mm, a repeatability of 10.1 % and a reproducibility of 18.4 %.

On the basis of the corrected data set (without h and k outliers), all the averages are within the 95% confidence limits.

Sorghum saccharatum (SOS)

Figure 6 clearly shows that the spread of the average root lengths measured by the participants is substantially larger than that of the two dicotyl test species. At the exception of one laboratory, which obtained an average root length of only 15 mm, all the other labs have reported root lengths in a range of about 30 mm up to 70 mm.

No outliers have been detected. The accepted results, based on all 28 laboratories, have a mean of 48 mm, a repeatability of 15.2 % and a reproducibility of 30.7 %.

On the basis of the corrected data set (without h and k outliers), one laboratory produced an average root length lower than the 95% lower confidence limit.

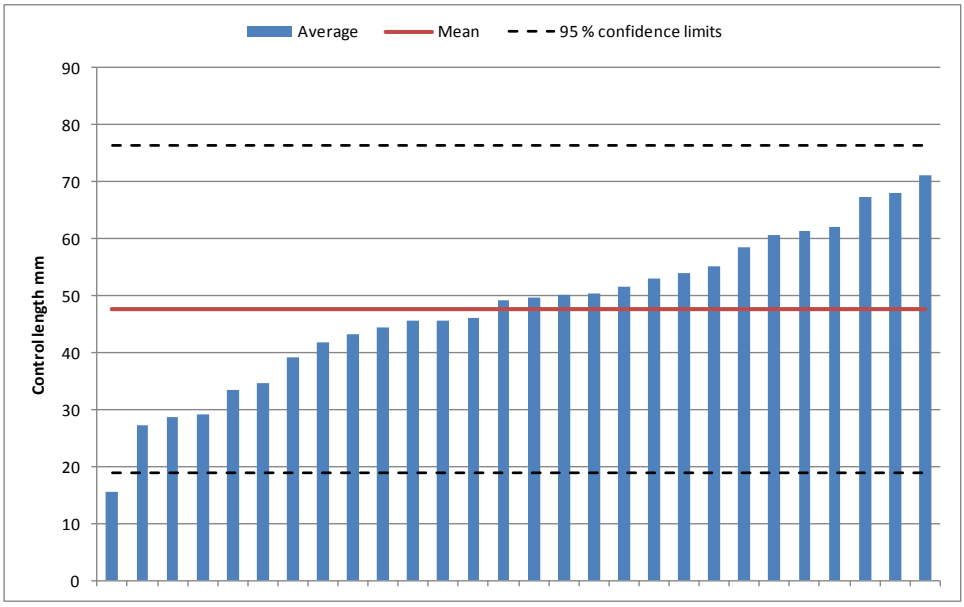

Figure 6 - Average length of the roots of *Sorghum saccharatum* in the negative controls for each laboratory, with three horizontal lines indicating the "overall mean" and the 95% confidence limits.

Discussion

The data given above for the mean root lengths, the range of the average root lengths, the 95% lower confidence limit, and the repeatability and reproducibility, are summarized for the 3 test species in an overview table.

Table 1 interestingly shows that despite the substantial difference in the range of the average root lengths between the two dicotyl and the monocotyl test species, the mean root length is virtually the same for the 3 test species. The 95% lower confidence limits are, however, different for the 3 plant species. For the two dicotyls the 95% lower confidence limit is about 35 mm and 31 mm respectively, whereas for the monocotyl it is as low as 19 mm.

Table 1 - Overview table of overall mean root length, the range of the average root lengths, the 95% lower confidence limits and the % repeatability and % reproducibility of the assays performed by the 28 participating laboratories

	Mean root length (mm)	Range of average root lengths (mm)	95% lower confidence limit (mm)	% Repeatability	% Reproducibility
Lepidium sativum	51	36 – 63	34.7	11.3	16.3
Sinapis alba	50	34 – 64	32.2	10.1	18.4
Sorghum saccharatum	48	15 – 71	19.0	15.2	30.7

Looking at the bars in Figure 6 it, however, appears that out of 28 laboratories, only 4 labs did not obtain an average root length of 30 mm for *Sorghum saccharatum* (which is most probably due to some experimental problems) and that 75% of the laboratories even obtained an average root length above 40 mm.

Since for the two dicotyl species the 95% lower confidence limit is above 30 mm, and taking into account that for the monocotyl the majority of the average root lengths are also above 30 mm, it will be proposed in the

Section General Discussion of this Report to take “the same” validity criterion for the mean root length in the controls for the 3 test species, namely 30 mm.

Longest root length

The longest root lengths in each test plate have also been calculated automatically by the Excel data treatment programme. The statistics is based on the average and variability of the measured length of the longest root in the 3 replicates.

Lepidium sativum (LES)

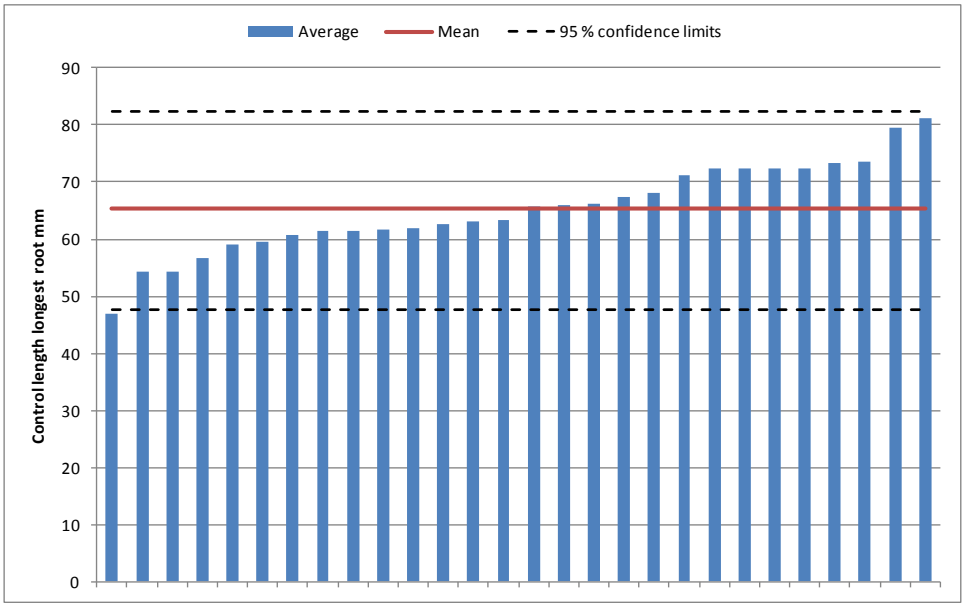

Figure 7 - Average length of the longest roots of *Lepidium sativum* in the negative controls for each laboratory, with three horizontal lines indicating the “overall mean” and the 95% confidence limits.

Figure 7 shows that the average lengths for the longest roots range from 47 mm to 81 mm.

One result has been discarded (Lab 9 as k outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a mean length of the longest root of 65 mm, a repeatability of 7.6 % and a reproducibility of 13.6 %.

On the basis of the corrected data set (without h and k outliers), one laboratory produced a longest root length lower than the 95% lower confidence limit.

Sinapis alba (SIA)

Figure 8 reveals that the spread between the data obtained by the participating laboratories for the average of the longest root is from 54 mm to about 80 mm.

One outlier has been detected (Lab 10 as k outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a mean length of the longest root of 68 mm, a repeatability of 8.0 % and a reproducibility of 13.4 %.

On the basis of the corrected data set (without h and k outliers), all the averages are within the 95% confidence limits.

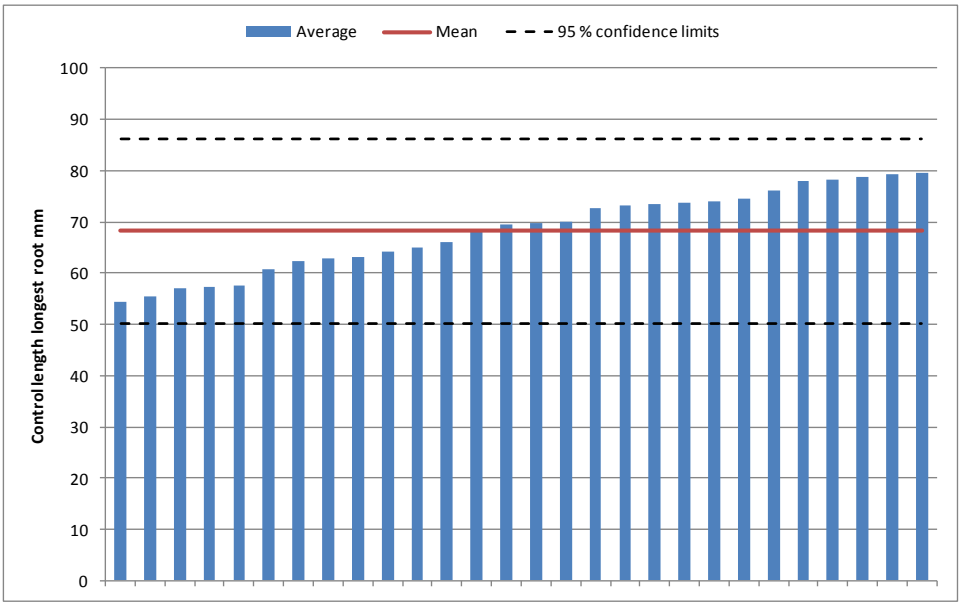

Figure 8 - Average length of the longest roots of *Sinapis alba* in the negative controls for each laboratory, with three horizontal lines indicating the "overall mean" and the 95% confidence limits.

Sorghum saccharatum (SOS)

Figure 9 shows that with one exception, all the laboratories have obtained an average length for the longest root in a range of 48 mm to 105 mm.

One result has been discarded (lab 23 as k outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a mean length of the longest root of 78 mm, a repeatability of 11.9 % and a reproducibility of 25.0 %.

On the basis of the corrected data set (without h and k outliers), one laboratory produced a longest root length lower than the 95% lower confidence limit.

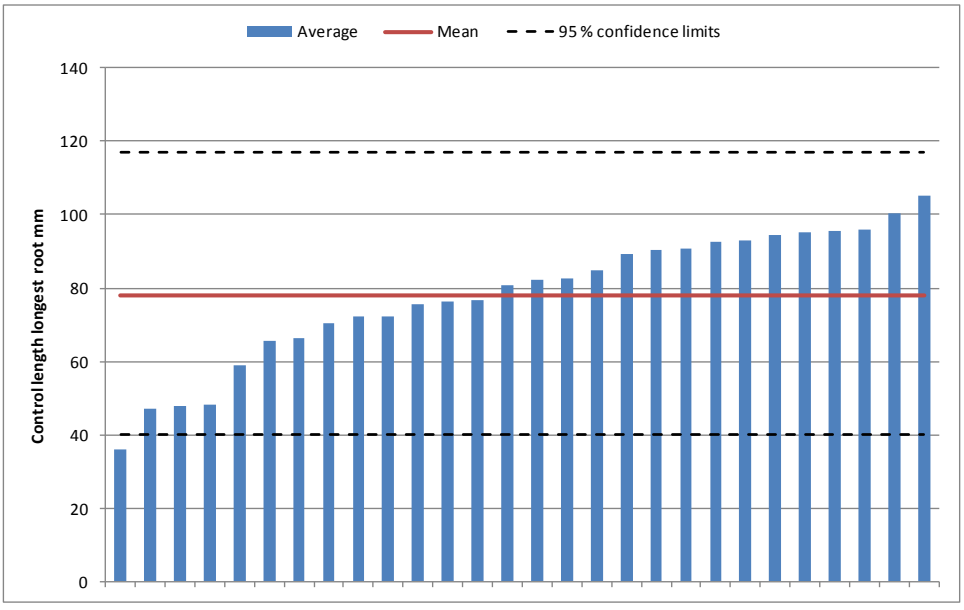

Figure 9 - Average length of the longest roots of *Sorghum saccharatum* in the negative controls for each laboratory, with two horizontal lines indicating the "overall mean" and the 95% lower confidence limit.

Discussion

The concept of “measurement of the longest root” has deliberately been included in the International Interlaboratory comparison, as a possible alternative to the time-consuming measurement of the root length of each germinated seed in each test plate.

The longest root is indeed easily visible in each test plate, and measuring “only” the longest root takes but a fraction of the time needed to measure the length of “all” the roots in each test plate.

This alternative will be addressed further in detail in the General Discussion section of this report.

Similarly to the Discussion Section on the mean root length, an overview table has been made for the data on the overall mean of the longest root lengths, the range of the average longest root lengths, the 95% lower confidence limits, and the % repeatability and reproducibility are summarized below for the 3 test species in an overview table.

Table 2 - Overview table of the overall mean of the longest root length, the range of the averages of the longest root lengths, the 95 % lower confidence limits and the % repeatability and % reproducibility of the assays performed by the 28 participating laboratories

	Mean longest root length (mm)	Range of average longest root lengths (mm)	95% lower confidence limit (mm)	% Repeatability	% Reproducibility
Lepidium sativum	65	47 - 81	47.7	7.6	13.6
Sinapis alba	68	54 – 80	50.2	8.0	13.4
Sorghum saccharatum	78	36 - 105	40.0	11.9	25.0

Table 2 shows that whereas the mean values for the longest root lengths are quite similar for the two dicotyl species (65-68 mm), the mean for the longest root length is substantially higher for the monocotyl test species (78 mm), but in turn the spread between the lowest and the highest values is also much higher than for *Lepidium sativum* and *Sinapis alba*.

The % repeatability and reproducibility are substantially better for the two dicotyls than for the monocotyl, which eventually resulted in a 95% lower confidence limit of 48 – 50 mm for these two test species, *versus* about 40 mm for *Sorghum saccharatum*.

As will be addressed further in the section General Discussion of this Report, a “validity criterion” can, however, easily be extrapolated from the data on the average longest root length shown in Figures 7, 8 and 9.

These figures indeed show that the lower 95% confidence limit is above 40 mm for the two dicotyls and quite near 40 mm for the monocotyl species; 40 mm will hence be proposed as the “validity criterion” for the average length of the longest root in the controls.

Data analysis of the results of the test plates spiked with boric acid

Number of germinated seeds

Lepidium sativum (LES)

One result has been discarded (Lab 25 as k outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a mean of 9 germinated seeds, a repeatability of 6.8 % and a reproducibility of 7.8 %.

On the basis of the corrected data set (without h and k outliers), all the averages are above the 95% lower confidence limit.

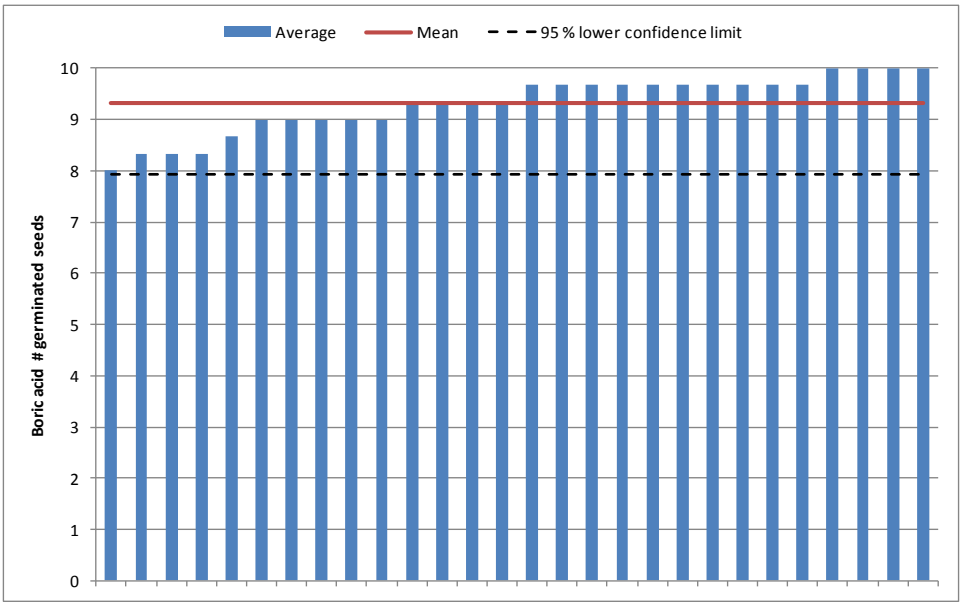

Figure 10 - Average number of germinated seeds of *Lepidium sativum* in the test plates spiked with boric acid, for each laboratory, with two horizontal lines indicating the "overall mean" and the 95% lower confidence limit.

Sinapis alba (SIA)

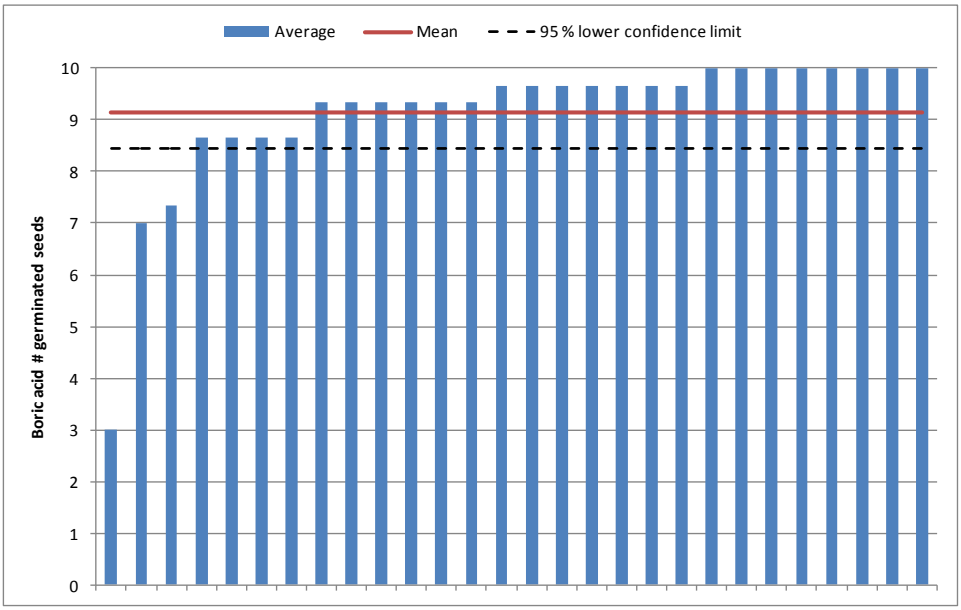

Figure 11 - Average number of germinated seeds of *Sinapis alba* in the test plates spiked with boric acid, for each laboratory, with two horizontal lines indicating the "overall mean" and the 95% lower confidence limit.

Five results have been discarded (labs 21, 4, and 5 as h outliers; labs 1 and 19 as k outliers). The accepted results, based on 23 laboratories (out of the original 28 participating labs), have a mean of 9 germinated seeds, a repeatability of 4.9 % and a reproducibility of 6.1 %.

On the basis of the corrected data set (without h and k outliers), Figure 11 shows that three labs reported a seed germination lower than the 95% lower confidence limit.

Sorghum saccharatum (SOS)

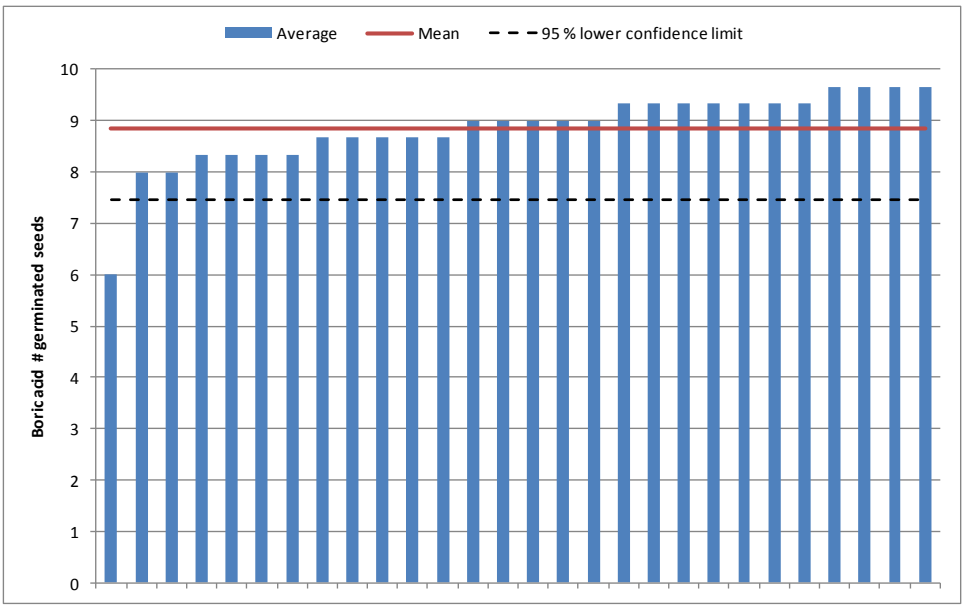

Figure 12 - Average number of germinated seeds of *Sorghum saccharatum* in the test plates spiked with boric acid, for each laboratory, with two horizontal lines indicating the "overall mean" and the 95% lower confidence limit.

Three results have been discarded (lab 21 as h outlier, and labs 4 and 11 as k outliers). The accepted results, based on 25 laboratories (out of the original 28 participating labs), have a mean of 9 germinated seeds, a repeatability of 8.7 % and a reproducibility of 8.8 %.

On the basis of the corrected data set (without h and k outliers), Figure 12 shows that one laboratory had obtained a seed germination lower than the 95% lower confidence limit.

Discussion

Figures 10, 11 and 12 clearly show that the spiking of the soil in the test plates with 250 mg boric acid per kg OECD soil did not substantially decrease the germination of the 3 types of seeds in comparison to the controls. Furthermore, the repeatability and reproducibility for the seed germination in the test plates spiked with boric acid appear to be quite similar to those reported above for the controls, all the figures being below 10%. The statistical aspects with regard to the percentage inhibition of seed germination will be addressed further on in detail in the section "Calculation of the inhibition percentages".

Root length

Lepidium sativum (LES)

As shown by Figure 13, the average root lengths in the test plates spiked with boric acid range from 19 mm to 34 mm.

One result has been discarded (lab 11 as k outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a mean root length of 27 mm, a repeatability of 11.9 % and a reproducibility of 17.1 %.

On the basis of the corrected data set (without h and k outliers), all the averages are within the 95% confidence limits.

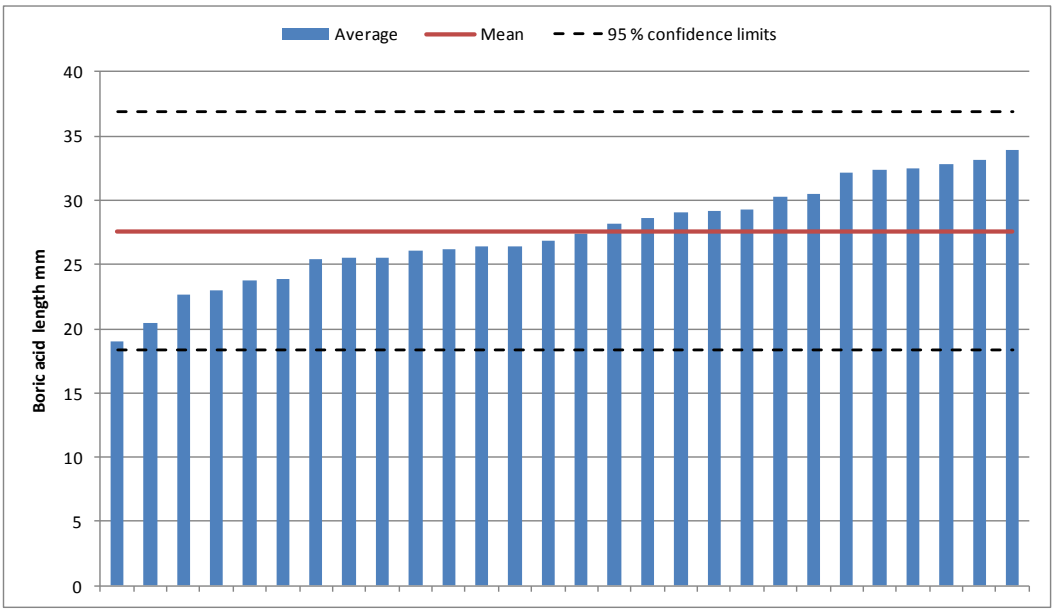

Figure 13 - Average length of the roots of *Lepidium sativum* in the test plates spiked with boric acid, for each laboratory, with three horizontal lines indicating the "overall mean" and the 95% confidence limits.

Sinapis alba (SIA)

Figure 14 shows that, with one exception, the spread between the lowest and the highest average root lengths is 18 mm – 37 mm.

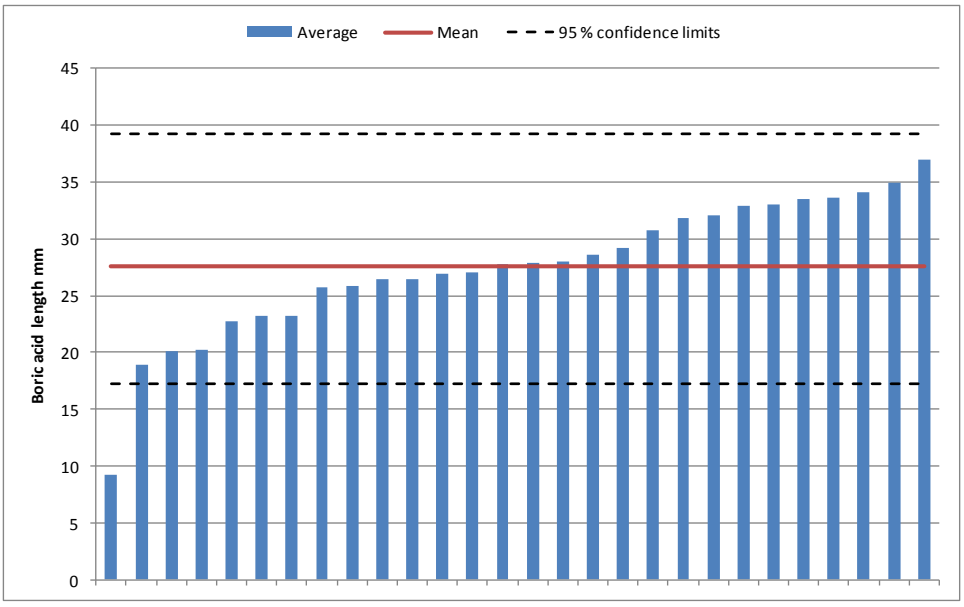

Figure 14 - Average length of the roots of *Sinapis alba* in the test plates spiked with boric acid, for each laboratory, with three horizontal lines indicating the "overall mean" and the 95% confidence limits.

One result has been discarded (lab 11 as h outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a mean root length of 28 mm, a repeatability of 12.1 % and a reproducibility of 19.8 %.

On the basis of the corrected data set (without h and k outliers), one laboratory produced an average root length lower than the 95% lower confidence limit.

Sorghum saccharatum (SOS)

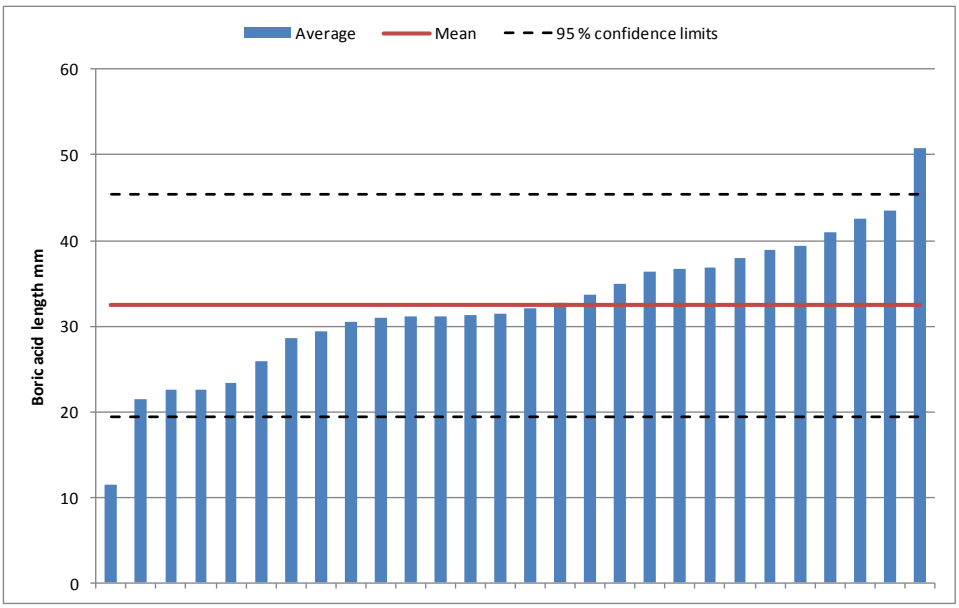

Figure 15 - Average length of the roots of *Sorghum saccharatum* in the test plates spiked with boric acid, for each laboratory, with three horizontal lines indicating the "overall mean" and the 95% confidence limits.

From Figure 15, it appears that at one exception, the average root lengths of *Sorghum saccharatum* in the test plates spiked with boric acid range from 22 mm to 51mm. Three results have been discarded (labs 5 and 11 as h outliers, lab 14 as k outlier). The accepted results, based on 25 laboratories (out of the original 28 participating labs), have a mean root length of 32 mm, a repeatability of 8.8 % and a reproducibility of 20.6 %.

On the basis of the corrected data set (without h and k outliers), one laboratory produced an average root length lower than the 95% lower confidence limit, and another an average root length higher than the 95% upper confidence limit.

Discussion

The overview table hereunder summarizes the data for the overall mean root lengths, the range of the average root lengths, the 95% lower confidence limits and the % repeatability and reproducibility for the test plates spiked with boric acid.

Figures 13, 14 and 15 and the data in the overview table clearly show the impact of the 250 mg boric acid per kg OECD artificial soil on the length of the roots of the 3 test species. The overall mean as well as the average root lengths are indeed substantially lower than those found in the controls.

The repeatability and the reproducibility of the results provided by the participants for the average length of the roots in the test plates spiked with boric acid are quite similar for the two dicotyl test species to those

indicated in Table 1 for the controls. For *Sorghum saccharatum* in turn a substantially better repeatability and reproducibility was found in the test plates spiked with boric acid.

Table 3 - Overview table of overall mean root length, the range of the average root lengths, the 95% lower confidence limits and the % repeatability and % reproducibility in the test plates spiked with boric acid

	Mean root length (mm)	Range of average root lengths (mm)	95% lower confidence limit (mm)	% Repeatability	% Reproducibility
Lepidium sativum	28	19 - 34	18.4	11.9	17.1
Sinapis alba	28	9 - 37	17.3	12.1	19.8
Sorghum saccharatum	32	12 - 51	19.3	8.8	20.6

The statistical aspects with regard to the percentage inhibition of the root length will be addressed further on in detail in the section “Calculation of the inhibition percentages”.

Longest root length

Lepidium sativum (LES)

With one exception, all the averages for the longest root in the test plates spiked with boric acid are in the range 30 mm to 45 mm.

One result has been discarded (lab 5 as h outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a mean length of the longest root of 38 mm, a repeatability of 10.6 % and a reproducibility of 12.8 %.

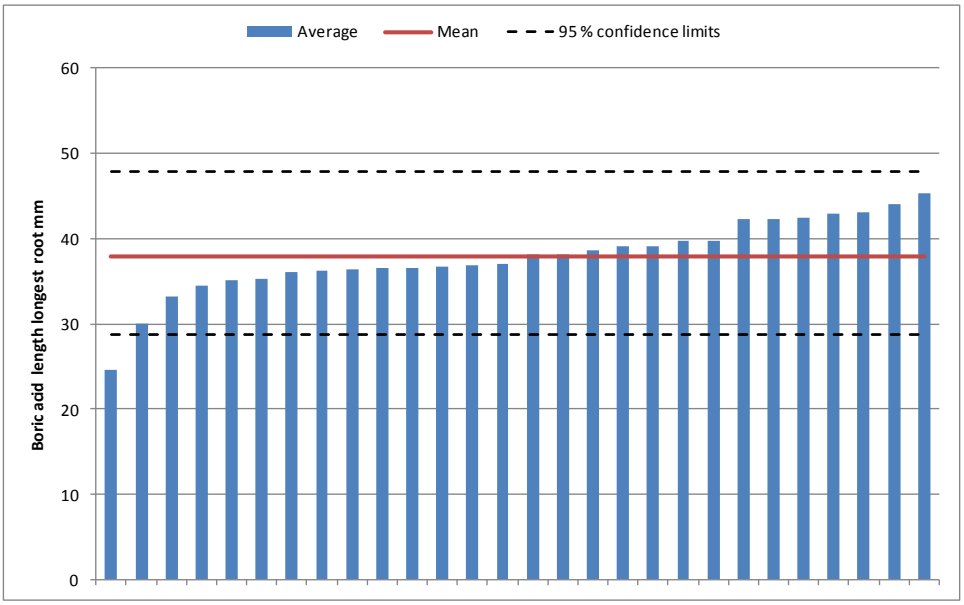

Figure 16 - Average length of the longest roots of *Lepidium sativum* in the test plates spiked with boric acid, for each laboratory, with three horizontal lines indicating the “overall mean” and the 95% confidence limits.

On the basis of the corrected data set (without h and k outliers), one laboratory produced a longest root length lower than the 95% lower confidence limit.

Sinapis alba (SIA)

The averages for the longest roots in the test plates spiked with boric acid range from 24 mm to 54 mm.

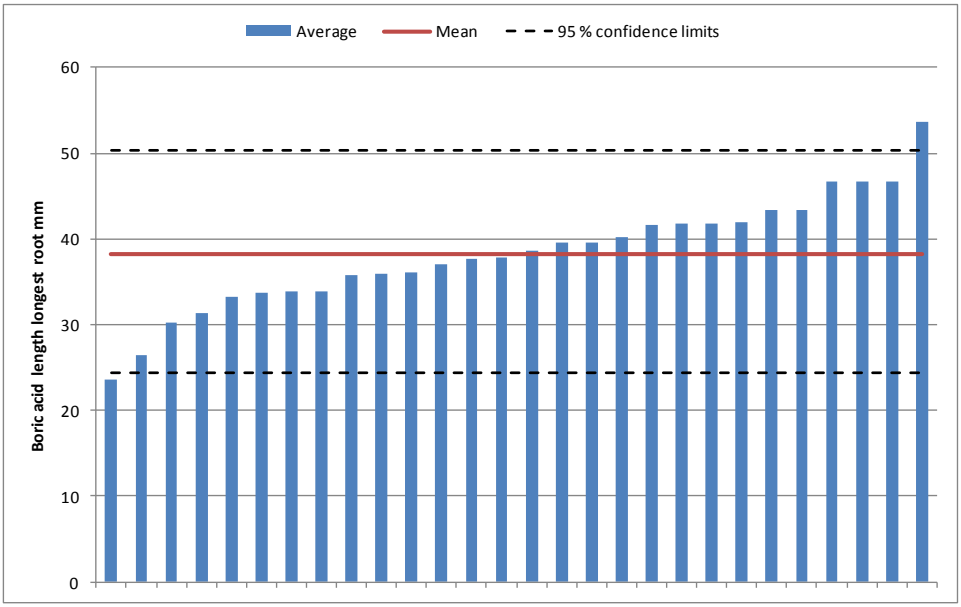

Figure 17 - Average length of the longest roots of *Sinapis alba* in the test plates spiked with boric acid, for each laboratory, with three horizontal lines indicating the "overall mean" and the 95% confidence limits.

Two results have been discarded (lab 22 as h outlier and lab 25 as k outlier). The accepted results, based on 26 laboratories (out of the original 28 participating labs), have a mean length of the longest root of 37 mm, a repeatability of 11.2 % and a reproducibility of 17.7 %.

On the basis of the corrected data set (without h and k outliers), one laboratory produced a longest root length lower than the 95% lower confidence limit, and another a longest root length higher than the 95% upper confidence limit.

Sorghum saccharatum (SOS)

Figure 18 shows that the average values for the longest roots in the test plates spiked with boric acid range from 22 mm to 62 mm. Two results have been discarded (lab 11 as h outlier, lab 9 as k outlier). The accepted results, based on 26 laboratories (out of the original 28 participating labs), have a mean length of the longest root of 46 mm, a repeatability of 8.8 % and a reproducibility of 17.9 %.

On the basis of the corrected data set (without h and k outliers), one laboratory produced a longest root length lower than the 95% lower confidence limit.

Discussion

Overview Table 4 shows the means for the longest root, the range of the average longest root, the 95% lower confidence limit and the % repeatability and reproducibility for the 3 test species in the test plates spiked with boric acid.

As can be seen from Figures 16, 17 and 18 and the overview table, similarly to the average root length, the spiking of the OECD artificial soil in the test plates with 250 mg boric acid had a substantial impact on the average length of the longest roots.

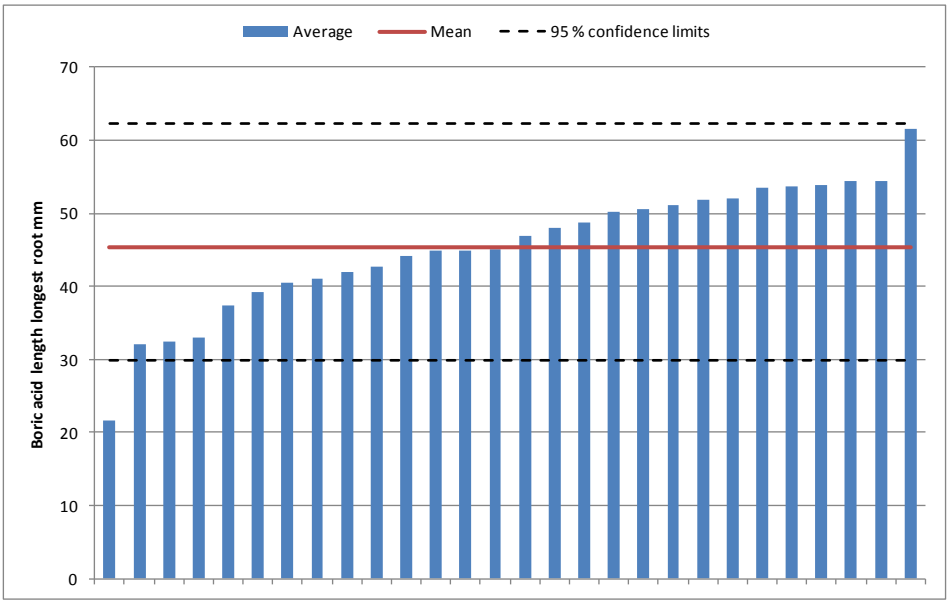

Figure 18 - Average length of the longest roots of *Sorghum saccharatum* in the test plates spiked with boric acid, for each laboratory, with three horizontal lines indicating the "overall mean" and the 95% confidence limits.

The statistical aspects with regard to the percentage inhibition of the length of the longest root will be addressed further on in detail in the section "Calculation of the inhibition percentages".

Table 4 - Overview table of the overall mean of the longest root length, the range of the averages of the longest root lengths, the 95 % lower confidence limits and the % repeatability and % reproducibility in the test plates spiked with boric acid

	Mean longest root length (mm)	Range of average longest root lengths (mm)	95% lower confidence limit (mm)	% Repeatability	% Reproducibility
Lepidium sativum	38	25 – 45	28.8	10.6	12.8
Sinapis alba	37	24 - 54	24.4	11.2	17.7
Sorghum saccharatum	46	22 - 62	29.9	8.8	17.9

Calculation of the inhibition percentages

As can clearly be seen in all the former figures and tables, the results of the germination success and those of the average root length and the average length of the longest root varied (in some cases even substantially) between the laboratories. The possible reason(s) for these differences will be addressed further on in the section General Discussion.

In view of these "lab to lab" differences, it is clear that the calculation of the inhibition percentages also had to be made on a "lab to lab" basis. These calculations were actually automatically made by the Excel programme of the Results Sheets, on the basis of "the data pairs" of each laboratory for the concerned endpoints.

From these individual laboratory inhibition percentages "a mean percentage" has subsequently been calculated for the 28 participating laboratories for each endpoint, with the 95% confidence limits.

% inhibition of the seed germination

As already mentioned in the section on the seed germination of the test plates spiked with boric acid, comparison of the figures on seed germination in the controls and in the test plates spiked with boric acid already visually showed that the germination success was not influenced to a substantial extent by the reference toxicant in the concentration used for the spiking. Furthermore, and as shown by the histograms of the three following figures, the percentage germination of the seeds in the test plates spiked with boric acid was for some laboratories even (slightly) higher than that in the controls (i.e. a "negative % inhibition).

Lepidium sativum (LES)

Two results have been discarded (lab 1 as h outlier, lab 25 as k outlier). The mean percentage inhibition of the seed germination in the test plates spiked with boric acid, based on 26 laboratories (out of the original 28 participating labs), was even (slightly) "below 0%" indicating that there was no inhibition, but even a slight biostimulation. The data in the corresponding table in Annex 4 shows a quite poor repeatability and reproducibility.

On the basis of the corrected data set (without h and k outliers), one laboratory produced a % Inhibition of the seed germination lower than the 95% lower confidence limit.

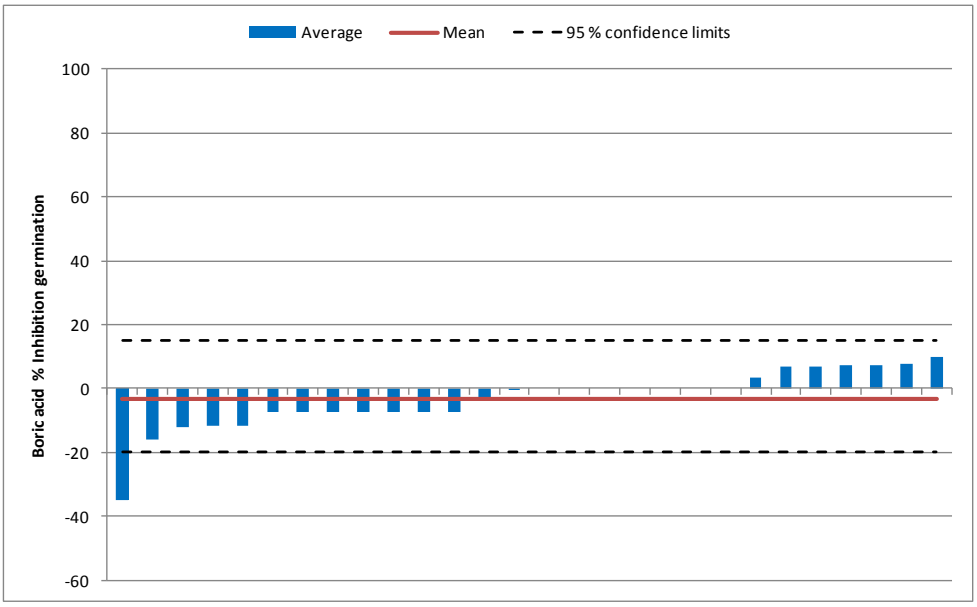

Figure 19 - % Inhibition of the seed germination of *Lepidium sativum* in test plates spiked with boric acid, for each laboratory, with three horizontal lines indicating the "overall mean" and the 95% lower and upper confidence limits.

Sinapis alba (SIA)

Two results have been discarded (lab 21 as h outlier, lab 1 as k outlier). Like for *Lepidium sativum*, the mean percentage inhibition of the accepted results, based on 26 laboratories (out of the original 28 participating labs), also shows that the germination of the seeds of *Sinapis alba* is not influenced by the reference toxicant in a concentration of 250 mg boric acid per kg OECD artificial soil. Similarly to the results of *Lepidium sativum*, the repeatability and reproducibility are quite low.

On the basis of the corrected data set (without h and k outliers), one laboratory produced a % Inhibition of the seed germination higher than the 95% upper confidence limit.

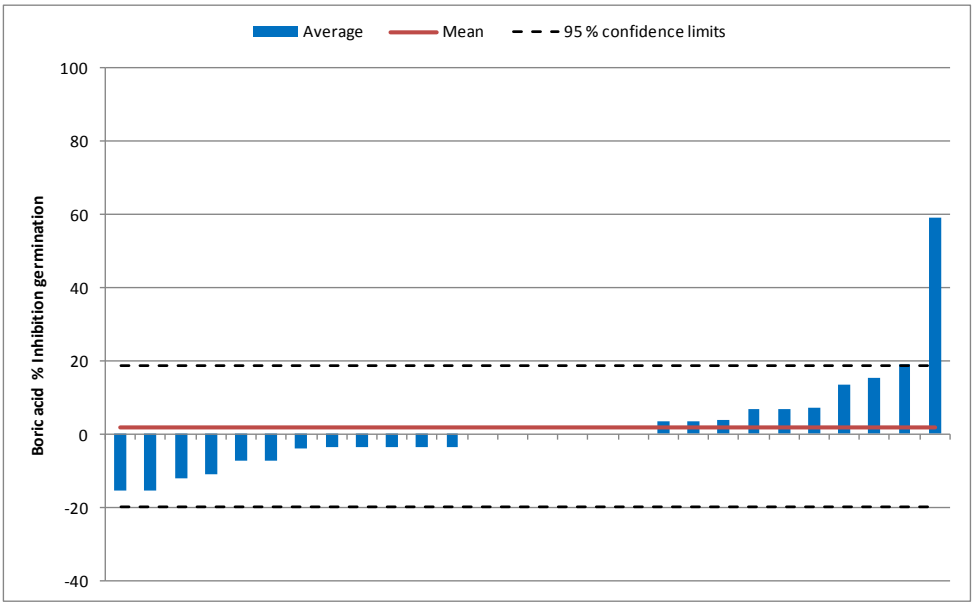

Figure 20 - % Inhibition of the seed germination of *Sinapis alba* in test plates spiked with boric acid, for each laboratory, with three horizontal lines indicating the "overall mean" and the 95% lower and upper confidence limits.

Sorghum saccharatum (SOS)

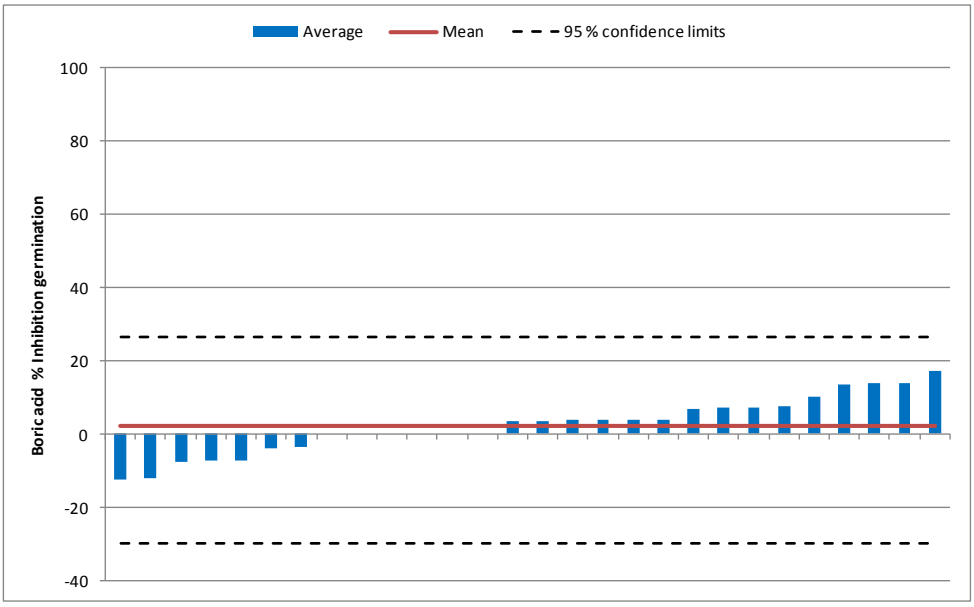

Figure 21 - % Inhibition of the seed germination of *Sorghum saccharatum* in test plates spiked with boric acid, for each laboratory, with three horizontal lines indicating the "overall mean" and the 95% lower and upper confidence limits.

Three results have been discarded (labs 4, 11, 21 as k outliers). Similarly to the results presented above for *Lepidium sativum* and *Sinapis alba*, the mean percentage inhibition for the germination of the seeds of *Sorghum saccharatum* in the test plates spiked with boric acid, calculated on the basis of the accepted results (25 laboratories out of the original 28 participating labs), is also virtually zero. The repeatability and the reproducibility are also quite poor for this test species.

On the basis of the corrected data set (without h and k outliers), all the averages are within the 95% lower and upper confidence limits.

Discussion

The percentage germination of seeds in polluted samples, in comparison to the controls, is clearly an interesting endpoint in plant tests. With the selected concentration of boric acid for the International Interlaboratory Comparison, the overall means for the germination inhibition, however, indicate that there is no impact of the reference toxicant on the germination of the seeds of the 3 plant species. This will be addressed further on in the section General Discussion of this report.

% inhibition average root length

Lepidium sativum (LES)

Figure 22 shows that the percentage inhibition for the average root length in the test plates spiked with boric acid ranges from 32 % to 60% between the laboratories.

One result has been discarded (lab 11 as k outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a mean % inhibition of the average root length of 45 %, a repeatability of 14.8 % and a reproducibility of 19.1 %.

On the basis of the corrected data set (without h and k outliers), all the averages are in the range of the 95% confidence limits.

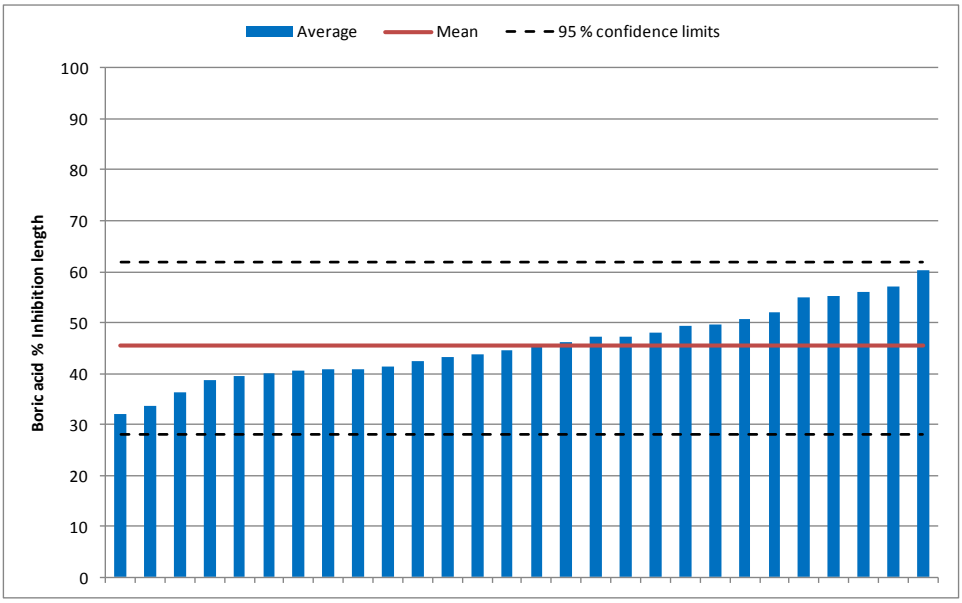

Figure 22 - % Inhibition of the average root length of *Lepidium sativum* in test plates spiked with boric acid for each laboratory, with three horizontal lines indicating the “overall mean” and the 95% confidence limits.

Sinapis alba (SIA)

From Figure 23 it can be seen that the percentage inhibition of the average root length in the test plates spiked with boric acid ranges from 22% to 75% between the laboratories

One result has been discarded (lab 11 as h outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a % inhibition of the mean root length of 44 %, a repeatability of 15.8 % and a reproducibility of 25.3 %.

On the basis of the corrected data set (without h and k outliers), one laboratory produced a % inhibition of the average root length lower than the 95% lower confidence limit, and another a % inhibition of the average root length higher than the 95% upper confidence limit.

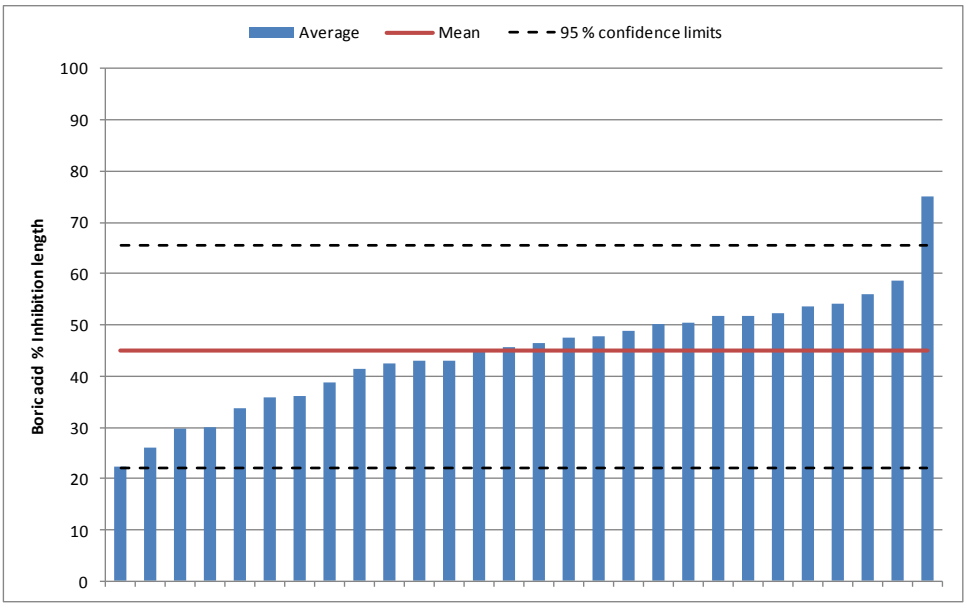

Figure 23 - % Inhibition of the average root length of *Sinapis alba* in the test plates spiked with boric acid for each laboratory, with horizontal lines indicating the "overall mean" and the 95% confidence limits.

Sorghum saccharatum (SOS)

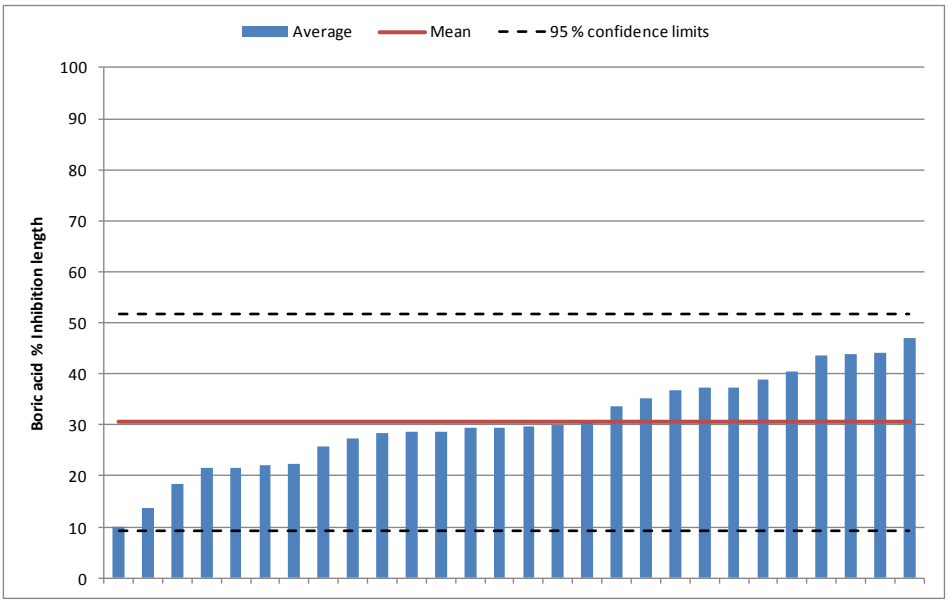

Figure 24 - % Inhibition of the average root length of *Sorghum saccharatum* in the test plates spiked with boric acid for each laboratory, with horizontal lines indicating the "overall mean" and the 95% confidence limits.

The % inhibition of the average root length in the test plates spiked with boric acid ranges from 10% to 47%.

Three results have been discarded (labs 11, 14, 21 as k outliers). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a % inhibition of the mean root length of 30%, a repeatability of 18.9 % and a reproducibility of 35.4 %.

On the basis of the corrected data set (without h and k outliers), all the averages are in the range of the 95% confidence limits.

Discussion

The data on the percentage inhibition of the average root length, the range of the % inhibition, the 95% confidence limits, and the repeatability and reproducibility percentages given above for the three test species have been put together in table 5.

Table 5 – Overview table of the mean % inhibition of the root length, the range of the % inhibition, the 95% confidence limits, and the repeatability and reproducibility percentages in the test plates spiked with boric acid.

	Mean % inhibition of mean root length	Range of % inhibition of mean root length	95% confidence limits	% Repeatability	% Reproducibility
Lepidium sativum	45	32 - 60	28.1 – 61.9	14.8	19.1
Sinapis alba	44	22 - 75	22.1 – 65.5	15.8	25.3
Sorghum saccharatum	30	10 - 47	9.4 – 51.7	18.9	35.4

From this table it appears that at a concentration of 250 mg boric acid per kg OECD artificial soil, the mean percentage inhibition of the average root length is virtually the same for *Lepidium sativum* and *Sinapis alba* (44-45%), but substantially lower for *Sorghum saccharatum* (30%).

The % repeatability is also quite similar for the dicotyls (15-16%) but somewhat higher for the monocotyl (19%). The % reproducibility is the best for *Lepidium sativum* (19%), but is lower for *Sinapis alba* and the lowest for *Sorghum saccharatum*.

Further discussion on the former data with regard to a reference test for the Phytotoxkit will be made in the section “General Discussion” of this report.

% inhibition longest root length

Lepidium sativum (LES)

As can be seen in Figure 25, the mean % inhibition for the length of the longest root in the test plates spiked with boric acid ranges from 30% to 51%.

No outlier has been detected. The accepted results, based on all 28 laboratories, have a % inhibition of the longest root length of 42 %, a repeatability of 14.4 % and a reproducibility of 17.6 %.

On the basis of the corrected data set (without h and k outliers), all the averages are in the range of the 95% confidence limits.

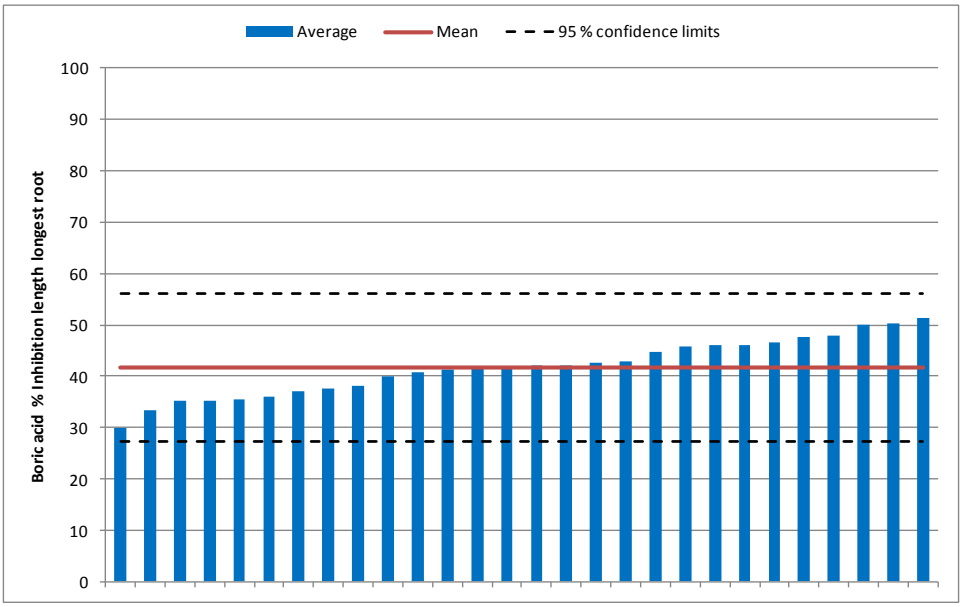

Figure 25 - % Inhibition of the longest root length of *Lepidium sativum* in the test plates spiked with boric acid for each laboratory, with horizontal lines indicating the "overall mean" and the 95% confidence limits.

Sinapis alba (SIA)

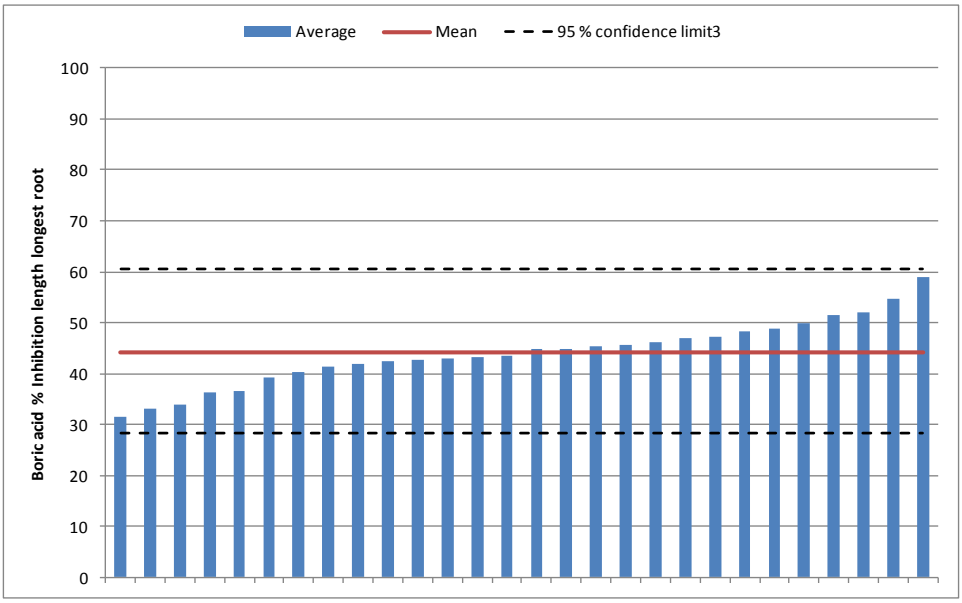

Figure 26 - % Inhibition of the longest root length of *Sinapis alba* in the test plates spiked with boric acid for each laboratory, with horizontal lines indicating the "overall mean" and the 95% confidence limits.

Figure 26 shows that the % inhibition for the length of the longest root in the test plates spiked with boric acid ranges from 31% to 59%.

One result has been discarded (lab 25 as k outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a % inhibition of the longest root length of 44 %, a repeatability of 14.5 % and a reproducibility of 18.6 %.

On the basis of the corrected data set (without h and k outliers), all the averages are in the range of the 95% confidence limits.

Sorghum saccharatum (SOS)

As can be seen in Figure 27, the data of the % inhibition for the length of the longest root in the test plates spiked with boric acid range from 31% to 53%.

One result has been discarded (lab 9 as k outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a % inhibition of the longest root length of 41 %, a repeatability of 12.9 % and a reproducibility of 18.1 %.

On the basis of the corrected data set (without h and k outliers), all the averages are in the range of the 95% confidence limits.

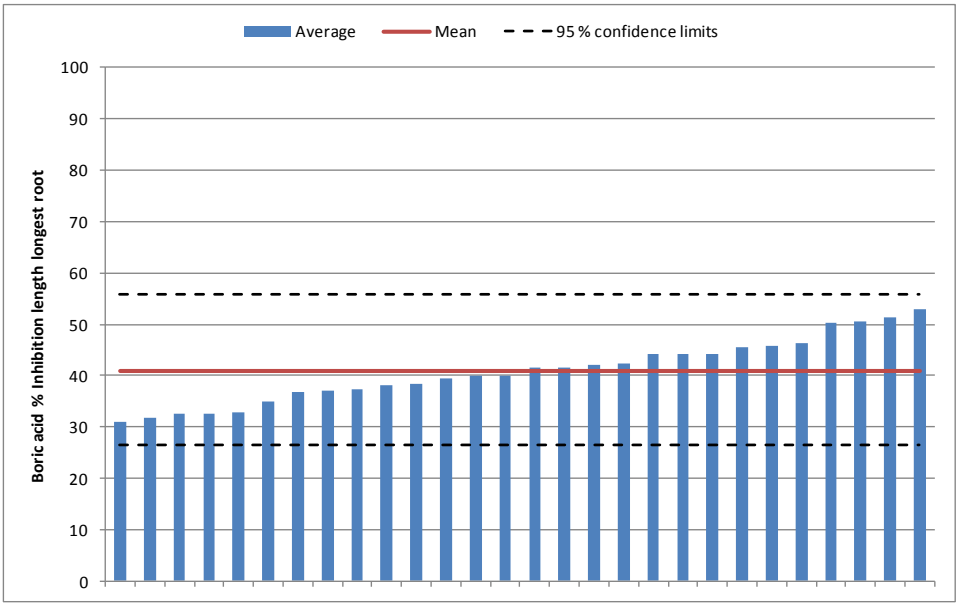

Figure 27 - % Inhibition of the longest root length of *Sorghum saccharatum* in the test plates spiked with boric acid for each laboratory, with horizontal lines indicating the "overall mean" and the 95% confidence limits.

Discussion

Table 6 shows that, contrary to Table 5 (which indicates the % inhibition for the average root length), the % inhibition for the length of the longest roots in the test plates spiked with boric acid is nearly identical for the 3 test species.

For the two dicotyl species, the % inhibition for the longest roots is also nearly the same as that for the mean root lengths.

Table 6 also shows that the range of the % inhibition for the length of the longest root is also substantially smaller than the range reported in Table 5 for the range of the % inhibition for the average root length. In addition it can also be seen from Tables 5 and 6 that the % repeatability and reproducibility are either similar or substantially better for the data based on the % inhibition for the length of the longest roots.

Finally these two tables also show that the 95% confidence limit values are in a more narrow range for the measurements based on the length of the longest root.

Table 6 – Overview table of the mean % inhibition of the length of the longest root, the range of the % inhibition, the 95% confidence limits, and the repeatability and reproducibility percentages in the test plates spiked with boric acid.

	Mean % inhibition of mean length of the longest root	Range of % inhibition of the mean length of the longest root	95% confidence limits	% Repeatability	% Reproducibility
Lepidium sativum	42	30 - 51	27.3 – 56.2	14.4	17.6
Sinapis alba	44	31 - 59	28.2 – 60.5	14.5	18.6
Sorghum saccharatum	41	31 - 53	26.6 – 55.9	12.9	18.1

% Germination Index

Lepidium sativum (LES)

As shown by Figure 28, the % Germination Index calculated for the individual laboratories ranges from 39% to 79%. One result has been discarded (lab 14 as h outlier).

The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a % Germination Index of 55 %, a repeatability of 13.0 % and a reproducibility of 18.2 %.

On the basis of the corrected data set (without h and k outliers), one laboratory produced a % Germination Index higher than the 95% upper confidence limit.

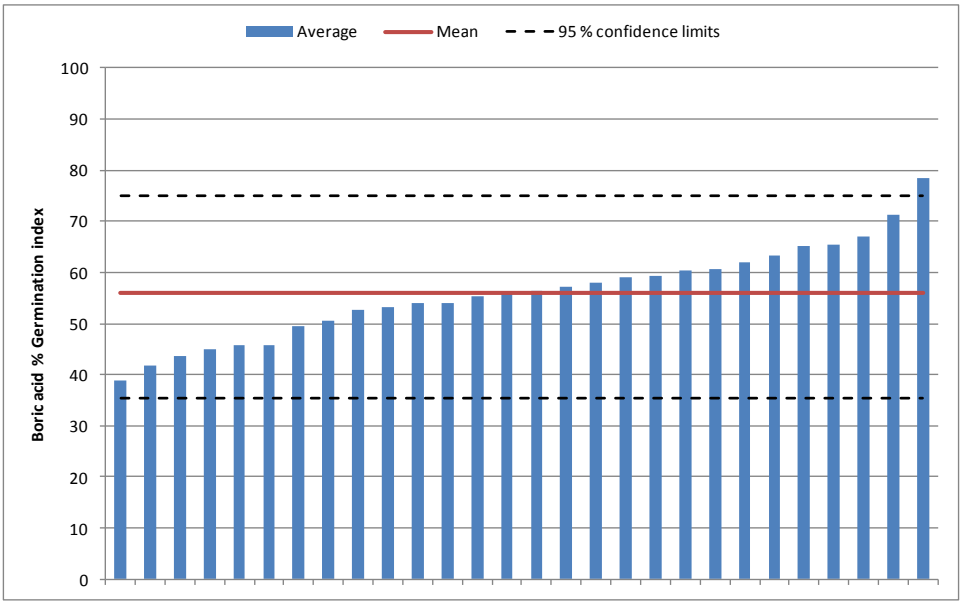

Figure 28 - % Germination Index of *Lepidium sativum* in the test plates spiked with boric acid for each laboratory, with horizontal lines indicating the "overall mean" and the 95% confidence limits.

Sinapis alba (SIA)

Figure 29 shows that the % Germination Index calculated for the individual laboratories ranges from 19% to 85%

One result has been discarded (lab 21 as k outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a % Germination Index of 56 %, a repeatability of 14.2 % and a reproducibility of 25.5 %.

On the basis of the corrected data set (without h and k outliers), one laboratory produced a % Germination Index lower than the 95% lower confidence limit, and another a % Germination Index higher than the 95% upper confidence limit.

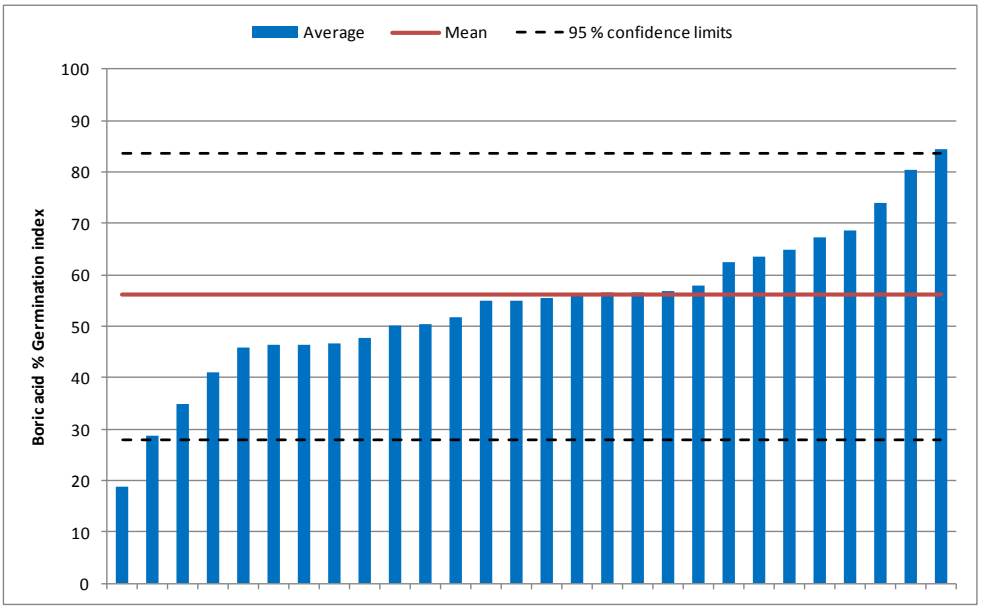

Figure 29 - % Germination Index of *Sinapis alba* in the test plates spiked with boric acid for each laboratory, with horizontal lines indicating the "overall mean" and the 95% confidence limits.

Sorghum saccharatum (SOS)

The % Germination Index calculated for the individual laboratories ranges from 46% to 91%. One result has been discarded (lab 21 as k outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a % Germination Index of 68 %, a repeatability of 14.0 % and a reproducibility of 20.3 %.

On the basis of the corrected data set (without h and k outliers), all the averages are within the 95% confidence limits.

Discussion

An overview table has been made to show the mean % Germination Index, the range of the % Germination Index for each laboratory, the 95% confidence limits and the % repeatability and reproducibility.

Table 7 shows that the mean % Germination Index is virtually the same for the two dicotyl species (55-56%), but substantially higher for the monocotyl (68%).

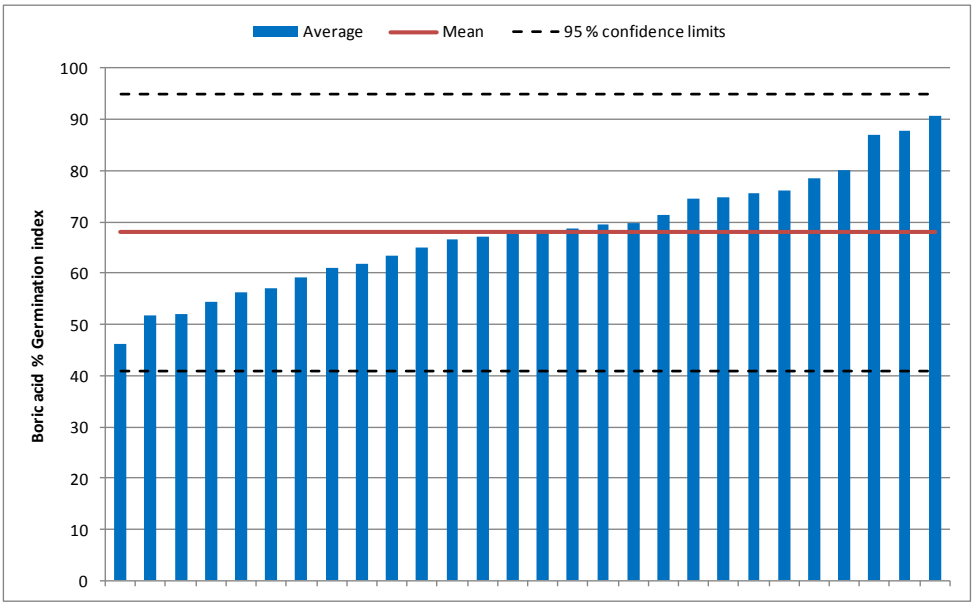

Figure 30 - % Germination Index of *Sorghum saccharatum* in the test plates spiked with boric acid for each laboratory, with horizontal lines indicating the "overall mean" and the 95% confidence limits.

Table 7 – Overview table of the mean % Germination Index, the range of the % Germination Index for each laboratory, the 95% confidence limits and the % repeatability and reproducibility.

	Mean % Germination Index	Range of % Germination Index	95% confidence limits	% Repeatability	% Reproducibility
Lepidium sativum	55	39 – 79	35.5 – 75.0	13.0	18.2
Sinapis alba	56	19 – 84	27.9 – 83.6	14.2	25.5
Sorghum saccharatum	68	46 - 91	40.9 – 95.0	14.0	20.3

Since as shown in Figures 19, 20 and 21 the germination of the seeds was not inhibited by the concentration of boric acid used (virtually 0% inhibition of seed germination) the values for the mean % Germination Index are actually for the 3 test species in basic agreement with those reported for the mean % inhibition of the mean root lengths.

For the present Interlaboratory Comparison, the % Germination Index therefore reflects "the opposite" of the mean % inhibition of the mean root lengths.

For *Lepidium sativum* the % Germination Index is 55%, whereas the % inhibition of the mean root lengths is 45%; for *Sinapis alba* these 2 figures are 56% and 44% and for *Sorghum saccharatum* 68% and 30% respectively.

The % Germination Index summarizes all the information on the impact of toxicants on seed germination and root length, and facilitates the comparison among tests on different treatments and/or samples.

Youden plots

To further explore the submitted data, and looking for possible systematic “errors”, the measurements of the mean root length and the longest root length have been plotted (for each seed in negative controls and treatments) in Youden plots.

In these plots, dots identify the data of the individual laboratories, the broken horizontal line indicates the overall mean for the longest root length, the broken vertical line the mean root length, and the broken box the 95 % confidence limits for the two length measurements.

Lepidium sativum (LES)

Control

This Figure shows that the data of all the laboratories are within the 95 % confidence limits box.

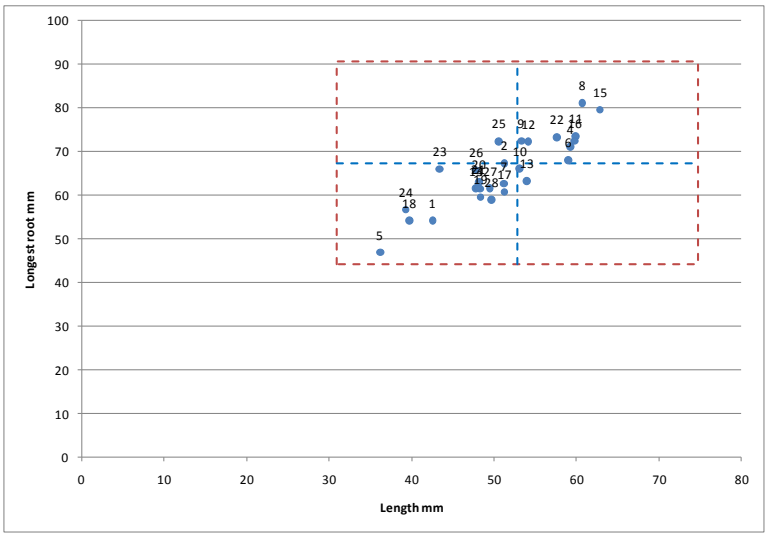

Figure 31 – Youden plot for the data on the longest root in the controls

Boric acid

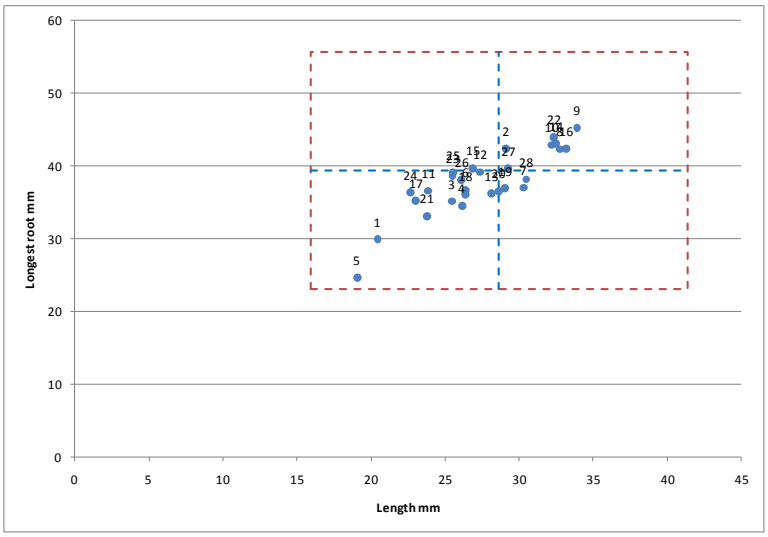

Figure 32 – Youden plot for the data on the longest root in the test plates spiked with boric acid

Figure 32 also shows that all the data of the individual laboratories are within the 95 % confidence limits box.

However, labs 5 and 1 in both cases are in the lower left quadrant, suggesting a possible systematic underestimation of lengths.

Sinapis alba (SIA)

Control

Figure 33 shows that the data of all the laboratories are within the 95 % confidence limits box.

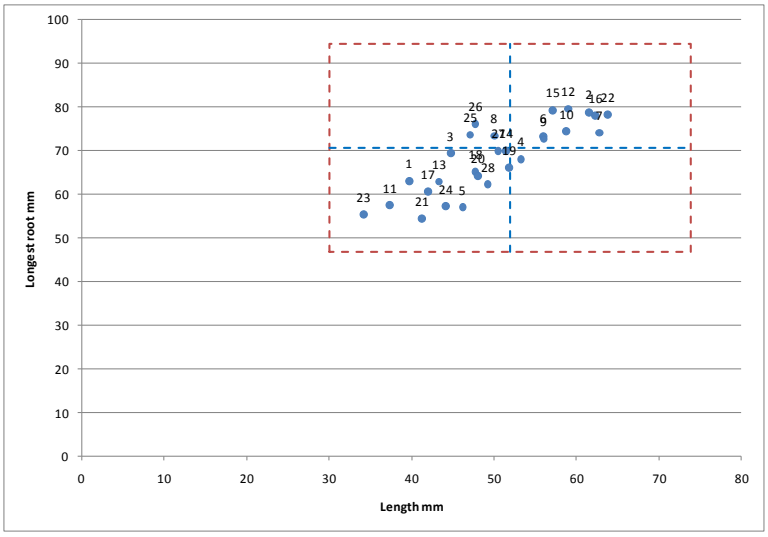

Figure 33 – Youden plot for the data on the longest root in the controls

Boric acid

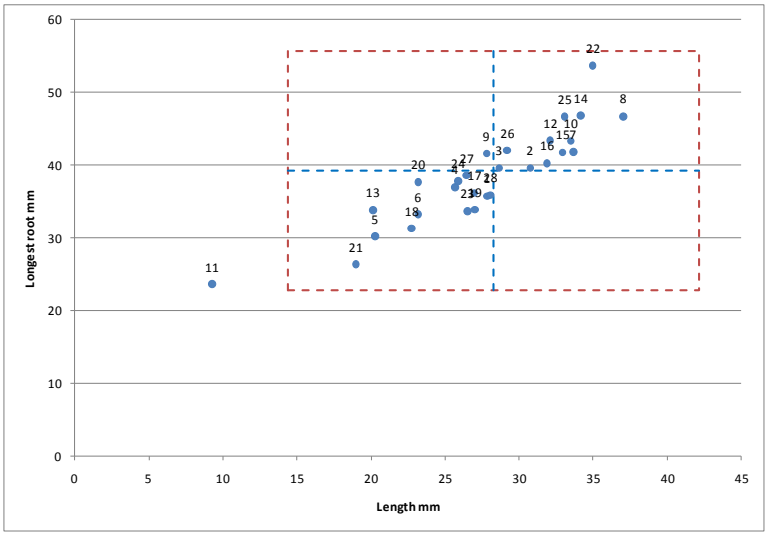

Figure 34 – Youden plot for the data on the longest root in the test plates spiked with boric acid

This figure shows that Lab 11 is outside the 95 % confidence limits, in the lower left quadrant. There is likely an underestimation of the lengths.

Sorghum saccharatum (SOS)

Control

Figure 35 reveals that Lab 11 is outside the 95 % confidence limits, in the lower left quadrant, probably because of an underestimation of the lengths.

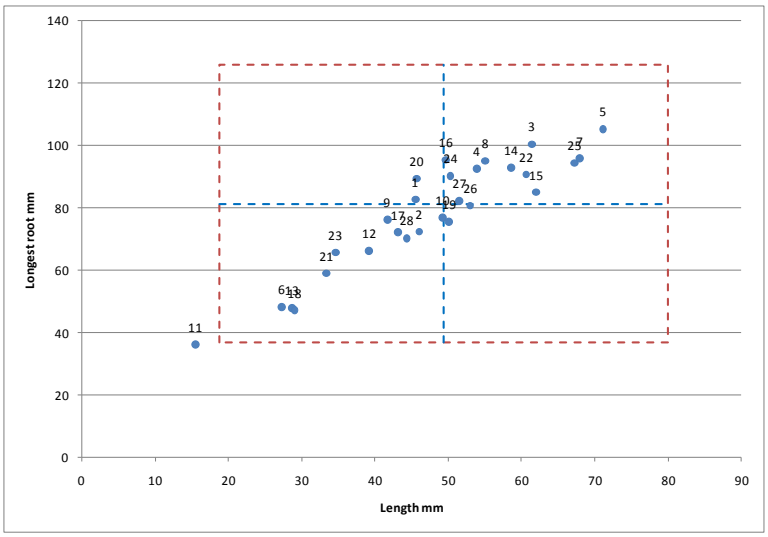

Figure 35 – Youden plot for the data on the longest root in the controls

Boric acid

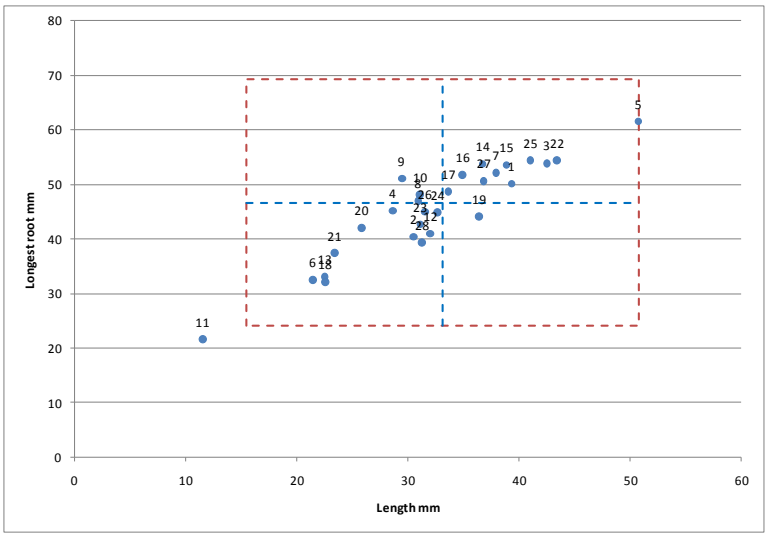

Figure 36 – Youden plot for the data on the longest root in the test plates spiked with boric acid

As can be seen from this figure, Lab 11 is outside the 95 % confidence limits, in the lower left quadrant, also probably because of an underestimation of the lengths.

Discussion

All the Youden plots show that there is a rather good spreading on the diagonal, meaning that the length measurements are fairly consistent for all the laboratories (with the possible exception of lab 11).

As a measure of this agreement, the determination coefficient has been calculated for all the data pairs.

Table 8 gives the linear regressions of the length of the longest root for the 3 test species in the control test plates and in the test plates spiked with boric acid.

Table 8 – Linear regressions of the length of the longest root for the 3 test species in the control test plates and in the test plates spiked with boric acid

	Test plates	R^2
Lepidium sativum	Control	0.7539
	Boric acid	0.7422
Sinapis alba	Control	0.6944
	Boric acid	0.8049
Sorghum saccharatum	Control	0.8571
	Boric acid	0.8519

As can be seen from this table, the relationships are all statistically highly significant, and explain at least 69 % of the variability (*Sinapis alba - control*), up to 85 % (*Sorghum saccharatum* - boric acid).

GENERAL DISCUSSION, CONCLUSIONS AND SUGGESTIONS FOR IMPROVEMENT OF THE PHYTOTOXKIT TEST PROCEDURE

Overall evaluation of the International Interlaboratory Comparison on the Phytotoxkit microbiotest

The substantial international participation in the Phytotoxkit Interlaboratory Comparison (28 laboratories, institutes, organisations and companies from 12 countries) is a clear sign for the interest in a simple and practical microbiotest for rapid assessment of the hazard of pollutants on higher plants.

Although the International Interlaboratory Comparison had deliberately been restricted to one assay with only one concentration of the reference toxicant, this nevertheless in practice meant that each participating laboratory had to prepare 18 test plates, take 18 “image recordings” of the test plates and subsequently perform about 180 individual germination and root length measurements after the incubation period.

The organisers therefore want to express herewith their sincere appreciation to all the participants for their time and efforts in performing this (still extensive) reference assay.

A first analysis of the Results Sheets turned in by the participating laboratory, already revealed that all of them had managed to perform the reference test properly and had provided satisfactory data.

The automatic processing of all the individual measurements by the Excel programme of the Results Sheets has undoubtedly contributed positively to the uniform presentation of the obtained data.

The outcome of the statistical processing of all the data (which involved the analysis of nearly 10.000 individual data on germination and root lengths) is given in the Figures and the Tables in Annex 4 of this Final Report, but in order to allow for a comprehensible evaluation, the averages for the seed germination, the average root length and the average length of the longest root have also been put in Figures in the body text of this Final Report. The averages are presented in histograms in increasing order (i.e. from the lowest to the highest value), with horizontal bars for the overall mean and the 95% confidence limits.

The visual analysis of these Figures shows that, although the participants had all been using the same materials included in the test kit, and had been asked to strictly followed the prescribed test method, there was (a more or less substantial) spread between the lowest and the highest results of the average root length and the average length of the longest roots.

Yet this was not the case for the seed germination data for which a high percentage of germination has been obtained by all the participants for the 3 test species, both in the control test plates and in those spiked with boric acid.

The differences in the averages for the root length are without any doubt due to experimental (small) “lab to lab” differences in the preparation and the set up of the test plates, and the scoring of the results.

The statistical analysis of the data reported by each laboratory revealed that there is no mathematical relationship between “the level” of the % inhibition and the mean root lengths in the controls and the test plates spiked with boric acid. In other words, the laboratories which had obtained “smaller” root lengths than other labs (in both the controls and the spiked test plates) are not those which had the lowest % inhibition values. This statement applies for both “the average root lengths” and “the average lengths of the longest roots”.

Furthermore, and as already reported in the discussion section on the seed germination, all the laboratories found (and hence confirmed the findings made during the preparation of the Interlaboratory Comparison) that at a concentration of 250 mg boric acid per kg OECD artificial soil there is no effect of this reference chemical on the germination success of the seeds of the 3 plant species.

The global evaluation of the results of the International Interlaboratory Comparison summarized in Tables 5 and 6 shows that the average % inhibitions for both root length and longest root length of the 3 plant species, are all in the range 40 to 45%, except for *Sorghum saccharatum* for which the % inhibition of the longest root is somewhat lower (30%). As already mentioned in the corresponding places in the different sections, virtually all the averages are within the 95% confidence limits of these mean values.

The figures calculated for the % Germination Index, which reflect the impact of the toxicant on both seed germination and root growth, confirmed the findings on the “no inhibition of seed germination” and the inhibition percentages as calculated from the mean root lengths.

It is quite interesting to note in this regard that on the basis of the mean percentage length inhibition of the roots, “the sensitivity” of the 3 plant species for the selected concentration of boric acid is “quite similar”, especially for the calculations based on the “longest root”.

All the figures and tables of this Report, based on about 10.000 data, thus confirm that the selection of 250 mg boric acid per kg OECD artificial soil was a good choice for a reference test, since it has lead to “meaningful” % inhibition of the root length for the 3 plant species, as reported by the 28 participating laboratories.

In comparison to other Interlaboratory Comparisons, it may be underlined that the intralaboratory variability for the mean % inhibition of both the average root length and the mean length of the longest root are below 20% and hence quite satisfactory.

The interlaboratory variability figures are somewhat higher (20% to 35%) for the mean % inhibition of the root length, but less than 20% for the mean % inhibition of the longest root, and hence also satisfactory.

On the basis of all the former statements, it can be concluded that the International Interlaboratory Comparison of the Phytotoxkit microbiotest has given very reliable results.

Validity criteria for the Phytotoxkit microbiotest

Similarly to other toxicity tests on higher plants prescribed by national or international organisations, a figure has to be selected for the percentage germination of the test seeds which must be obtained in the negative controls.

As shown by the figures on the germination of the seeds of the 3 plant species used in the Phytotoxkit assay, all the participants obtained more than 80% germination with the two dicotyls and the monocotyl.

Since the lower 95% confidence limit for the germination success is above 70% for the 3 types of seeds, it was already suggested in the section Discussion on the germination of the seeds in the controls to take 70% as the validity criterion for seed germination, i.e. the same percentage as indicated in OECD Guideline 208 (2006) for the Seedling Emergence and Seedling Growth Test.

Remark: With regard to seed germination, it must, however, be mentioned that in the present International Interlaboratory Comparison, the participants were not requested to evaluate themselves "visually" which and how many seeds had germinated. The seed germination was indeed calculated automatically by the Excel programme of the Results Sheets, and is based on the number of seeds for which "root length measurements" had been performed.

From the answers to the questionnaire sent by the organisers to the participants on completion of their assay, and which deals with a number of technical aspects of the test (see Section "Questionnaire" hereafter), it appears that some participants have measured root lengths as of 1 mm, whereas others only took into consideration root lengths of several mm for their own measurements. This clearly also had a repercussion on the germination success figures in the Results Sheets.

It is proposed herewith that for the validity criterion on the seed germination in the Phytotoxkit microbiotest, the same value as that used in the Italian Official Method UNICHIM 1651 (2003) for the plant bioassay in Petri dishes to indicate whether or not a seed has germinated, namely "1 mm root length".

Actually the 1 mm root length criterion has also to be applied for the analysis of the seed germination in the spiked test plates, for subsequent calculation of the % inhibition of the seed germination.

Similarly to other standard methods for plant tests, it is furthermore suggested to take a second validity criterion in consideration for the Phytotoxkit microbiotest, namely the "minimum mean length of the roots" of the germinated seeds in the negative controls.

As indicated in Table 1 of this Report, the mean root length of the 3 test species in the controls is around 50 mm and the 95% lower confidence limit for the two dicotyls is above 30 mm.

For *Sorghum saccharatum* in turn, the 95% lower confidence limit was around 20 mm. Figure 6, however, shows that from the 28 participants, 24 had obtained an average root length in the controls exceeding 30 mm and it is hence suggested, for reasons of uniformity and practicality, to take "30 mm mean root length" in the controls as the second validity criterion for each of the three plant species of the Phytotoxkit microbiotest.

Measurement of the average root length versus measurement of the average length of the longest root

Extensive research on the Phytotoxkit microbiotest prior to the launching of the International Interlaboratory Comparison had already indicated that there seemed to be a correspondence between the inhibition percentages of the average root length and those based on the measurement of "only" the longest root in each test plate.

The present Interlaboratory Comparison has confirmed this, and as shown by Figures 5 and 6 of this Report, both mean inhibition percentages are quite close to each other for the two dicotyl species (i.e. around 40%).

Whereas for *Sorghum saccharatum*, a lower mean inhibition percentage was found for the average root length (30%), in turn the measurement of only the longest root gave virtually the same percentage inhibition as for the two dicotyl test species. Tables 5 and 6 furthermore also show that the % reproducibility of the measurements of only the longest root is also substantially better (18%) than that of the measurements of the mean root lengths (35%).

As already indicated in the corresponding Discussion Section of this report, finding "the longest root" in each test plate is quite easy and the length measurement of "only" the longest root in each test plate (instead of the length measurements of the roots of all the germinated seeds) is clearly a substantial time saving.

As mentioned in the same Discussion Section, it is actually also possible to select a minimum mean length of the longest root in the controls, as the second validity criterion for the Phytotoxkit assay.

The 95% confidence limits for the mean length of the longest roots in the controls are indeed above 40 mm for the two dicotyl species, and virtually 40 mm for the monocotyl.

It is therefore suggested to take 40 mm as the second validity criterion for the assay if only the longest roots are measured in each test plate.

Questionnaire, problems and suggestions for improvement of the Phytotoxkit test procedure

After reception of all the Results Sheets of the individual participant, a questionnaire has been sent by the organiser of the International Interlaboratory Comparison to all the participants.

This questionnaire dealt with a number of technical aspects (use of the materials, experimental conditions, etc) as well as the request for information on possible problems during the test performance, and suggestions for improvement of the test procedure.

Twenty one participants sent in their answers and a synthesis of the replies to each question is given hereunder.

1. Did you make and use the "rectangular plastic strip" and the "flat bottom pestle" which was included in the kit, for flattening the dry soil, after it was poured in the test plates from the bags ?

All the replies indicated that the participants had made use of these additional materials.

2. Do you consider this new procedure practical and time saving in comparison to the conventional procedure of flattening the soil with a spatula ?

Eighteen participants replied with a "yes" and three with a "no". The reasons for the "no" are addressed hereunder.

3. Did you experience problems in the application of the test procedure of the International Interlaboratory Comparison ?

From the 21 answers, 18 indicated that they had not had any problem.

Two participants replied that the size of the rectangular plastic strip included in the testkit was slightly too large, and hence did not fit correctly in the test plate, with subsequent problems for the flattening of the dry soil. This problem has been addressed in the meantime and a simple technique has been worked out for improvement.

One participant had problems with the flattening of the dry soil and preferred the "original" method, i.e. flattening of the hydrated soil with a spatula.

Two participants mentioned that it was not easy to place the 10 seeds at a uniform distance from each other at the start of the test. This remark has been addressed in the meantime and the solution suggested by one participant will be implemented, namely by making 10 “marks” at equal distance of each other on the middle ridge of the test plates, for the exact positioning of the seeds. In addition, a lateral mark will be made on both sides of the test plates to indicate “the distance” at which the seeds shall be placed from the middle ridge of the plate. Both improvements are shown in the Figure below.

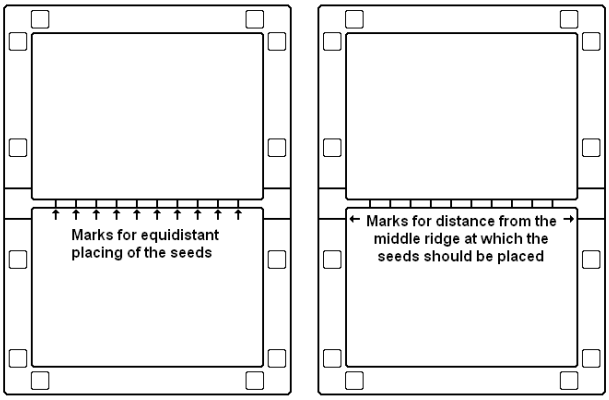

Figure 37 – Phytotoxkit test plate showing the marks for the positioning of the seeds

Another remark is related to the difficulty in finding exactly “the place of the root” from where to start the length measurement. This is especially the case if the coat of the seed “has moved” from its original position to a different place in the test plate (which may happen with the seed coats of the two dicotyl test species).

A simple tip for the two dicotyl species is to find the “boundary” between the root and the shoot. This boundary is situated exactly at the point where tiny “secondary roots” start to develop laterally on the (primary) root. In addition it is also relatively easy to see the boundary from the somewhat larger “thickness” of the shoot versus the root.

The photo hereunder clearly shows the location of the root/shoot boundaries of germinated seeds of *Sinapis alba*.

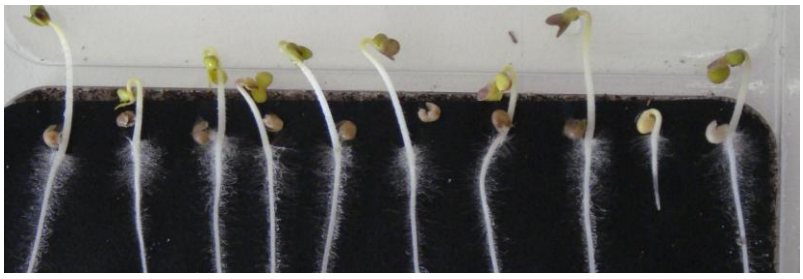

Figure 38 – Photo of part of a test plate with germinated seeds of *Sinapis alba*, showing the lateral secondary roots on the (primary) roots

A final remark addresses the fact that with some germinated seeds of *Sorghum saccharatum*, the root originates “on the back side” of the seed, making the root length measurement less precise. Although this is correct, it appears that in practice the measurement error is in most cases negligible.

4. Number of hours at which the pictures of the test plates have been taken after the prescribed (72h) incubation period.

Virtually all the replies indicated that the pictures of the test plates had been taken within one hour (maximum 3 hours) after the 72h incubation period.

5. Did you experience a problem in the length measurement of the roots of the germinated seeds ?

From the 21 answers, 4 pointed to some problems with the length measurements of the roots.

One of the reported problems is that the roots of adjacent seeds sometimes "join each other" at a certain place, making it difficult to measure exactly their respective lengths.

Furthermore, some roots, and especially many roots of *Sorghum saccharatum*, don't grow "straight", which also makes the length measurements more difficult.

Some participants have been addressing these problems themselves, by (after removal of the cover of the test plate) "straightening" the roots with tweezers, prior to take a picture of the test plate.

6. Temperature inside the incubator during the incubation period of the the test plates.

All the answers except two indicated that the temperature inside the incubator was 25°C.

Two laboratories replied that the incubation temperature was 24°C.

Assuming that the incubation temperatures are probably also the same as for the laboratories which did not reply to the questionnaire, it seems that they were all within $\pm$ 2°C of the prescribed incubation temperature. Former research on the Phytotoxkit microbiotest has shown these small temperature differences do not influence the outcome of the test (i.e. the inhibition percentages).

7. How much did the temperature fluctuate during the incubation period.

Eighteen participants indicated that the temperature inside their incubator only fluctuated 1°C during the incubation period and three participants reported a temperature fluctuation of 2°C.

Similarly to the former consideration, it was also found in former research that temperature fluctuations which do not exceed 2°C during the incubation period do not affect the test results significantly.

8. What was the minimum length of the root of a germinated seed which has been taken into consideration for length measurement (= the data of which have been included in the Results Sheet).

Unfortunately no specific instructions had been given in this regard in the Procedure for the performance of the reference test in the International Interlaboratory Comparison.

As a result, it appeared from the answers to the questionnaire that not all the participants have used the same "minimum root lengths" as the starting point for their measurements.

Nine participants e.g. replied that they had measured the roots as of 1 mm length and three others started the measurements as of 2 mm root length. The other nine laboratories, had taken into consideration root lengths of 3 mm or more, even up to 8 mm in one laboratory.

As indicated above in the "Remark" in Section 2 "Validity criteria of the Phytotoxkit microbiotest" these differences in "the starting point" for root length measurements undoubtedly had an influence on the "Germination figures" which were calculated automatically by the Excel programme of the Results Sheets. Fortunately, as shown by the data on the germination success of the seeds, there was no inhibition of the seed germination at the concentration of boric acid used in this Interlaboratory Comparison.

The differences in the starting point for length measurements may, however, possibly also have had a repercussion on the inhibition percentages reported by the individual laboratories.

Yet, the analysis of the individual percentage inhibition of the average root lengths and the "minimum root length" used by same laboratory showed that (for the data of the present Interlaboratory Comparison) there was no correspondence between the % inhibition results from the laboratories which had started the measurements as of 1 mm root length, and those who had only taken into consideration "longer" root lengths.

Anyhow, in order to avoid the former problems it will be indicated in the amended version of the Phytotoxkit microbiotest, that the same "starting point" (= starting length) has to be used for root length measurements.

In section 2 of the General Discussion dealing with the validity criteria for the Phytotoxkit microbiotest, it is indicated that roots should be measured as of 1 mm length for the determination of the Germination success. Since this value (1 mm root length) has been used by many laboratories in the present Interlaboratory Comparison for their own measurements of root lengths, it is therefore proposed to use this value (1 mm root length) as the starting point for the measurement of the root lengths.

9. Are the data in your Results Sheets "root lengths" (only) or are they "root + shoot lengths".

Although the test protocol of the International Interlaboratory Comparison clearly specified that the measurements should only involve "root lengths", it appeared from the data that two laboratories had submitted data for "root + shoot lengths". Since the pictures of the test plates were still available, measurements of "root lengths only" were subsequently made by these two participants, and their data could be taken into consideration for the statistical analysis.

10. Suggestions for improvement of the test procedure for a reference test for the Phytotoxkit microbiotest and suggestions for improvement of the Phytotoxkit test procedure

Several questions/remarks on the test protocol of either the reference test or the basic test procedure of the Phytotoxkit assay have been forwarded by some participants. These specific remarks have already been addressed in the former sections of the General Discussion, along with the suggestions for adaptation and improvement.

The International Interlaboratory Comparison of the Phytotoxkit assay has undoubtedly contributed very positively to the improvement and the refining of the test procedure and the amendments will be incorporated in the updated version of the Standard Operational Procedure of the Phytotoxkit which will be prepared shortly, and which will also include the adapted experimental protocol for a reference test.

5. Reference test for the Phytotoxkit microbiotest

The choice of boric acid as the compound for a reference test was based on the fact that this chemical is also recommended as reference chemical in several national and international standards on toxicity tests with higher plants.

As indicated in the first section of this General Discussion, a concentration of 250 mg boric acid to spike the OECD reference soil in the test plates also appeared to have been a good choice, since it has given very reliable results and average inhibition percentages which are relatively near the overall mean value.

With regard to the "acceptability" of the results of a Phytotoxkit reference test performed with 250 mg boric acid per kg OECD artificial soil, clearly the 2 validity criteria of the basic test procedure also apply, namely a mean germination percentage of 70% for the 3 test seeds and a minimum length of 30 mm of the roots in the controls.

In addition, and in analogy to reference tests prescribed in other toxicity tests, it seems appropriate to also include for the reference test of the Phytotoxkit assay, “a range” within which limits the % inhibition of root length obtained with the prescribed concentration of boric acid must be situated.

Similarly to the “acceptance limits” for reference tests, which have been derived from recent Interlaboratory Comparisons on the “*Thamnocephalus platyurus*” microbiotest and the “*Heterocypris incongruens*” microbiotest, it is hereby proposed to use the same mathematical approach for the calculation of the acceptance range for the Phytotoxkit reference test, i.e. the overall mean value $\pm$ 2 S_R (reproducibility).

These limits have been calculated from the data in the Tables in Annex 4 dealing with the % inhibition of the mean root length and the % inhibition of the mean length of the longest root for the 3 plant species, and the ranges are presented in Tables 8 and 9 hereunder.

Table 8 – “Acceptance limits” for the percentage inhibition of the mean root length in a reference test with 250 mg boric acid in 1 kg OECD artificial soil

	Acceptance limits (%)
Lepidium sativum	28 - 62
Sinapis alba	22 - 65
Sorghum saccharatum	9 - 52

Table 9 – “Acceptance limits” for the percentage inhibition of the mean length of the longest root in a reference test with 250 mg boric acid in 1 kg OECD soil

	Acceptance limits (%)
Lepidium sativum	27 - 58
Sinapis alba	28 - 60
Sorghum saccharatum	27 - 56

ADAPTATION OF THE PHYTOTOXKIT STANDARD OPERATIONAL PROCEDURE

From the facts and figures of the International Interlaboratory Comparison, a substantial number of considerations, suggestions and choices have been addressed in detail in the Discussion Sections of this Final Report, and will be taken into consideration for the improvement of the Phytotoxkit Standard Operational Procedure.

A synthesis is given hereunder of the major points which will be reflected in the updated version of the Operational Procedure of the Phytotoxkit microbiotest.

1. Seeds are considered as germinated when an emergence of at least 1 mm protrudes from the seed at the end of the exposure period.
2. The first validity criterion for the Phytotoxkit assay is that 70% of the seeds of the three test species must have germinated in the negative controls at the end of the exposure period.

3. The second validity criterion for the Phytotoxkit test is that the mean root length in the negative controls must be at least 30 mm for the three test species.
4. Calculation of the % inhibition of the growth of the roots can be made on either the measurement of the length of “all the roots” of the germinated seeds in the test soil and in the negative controls, or alternatively on the measurement of the length of “only the longest root” in each test plate.
5. Length measurements of the roots must be made on all the roots of the germinated seeds, as of a length of 1 mm emergence of the root from the seed.
6. In case “the longest root” concept is used for calculation of the % inhibition of root growth, the second validity criterion for the assay is that the mean length of the longest roots in the negative controls must be at least 40 mm for the three plant species.
7. Calculation of the % inhibition can also be based on the % Germination Index which integrates the data on seed germination and root length. The procedure for calculation of the % Germination Index will be given in the updated Phytotoxkit Standard Operational Procedure.
8. A detailed description of a reference test with boric acid will be included in the updated Standard Operational Procedure of the Phytotoxkit microbiotest, based on spiking of the test plates with 250 mg boric acid per kg OECD artificial soil.
9. Besides the two validity criteria for all Phytotoxkit assays, a third validity criterion has to be applied for the reference tests with boric acid. This criterion, which is based on the extensive data set of the International Interlaboratory Comparison, specifies the “acceptance limits” of the reference test, the values of which are given in Tables 8 and 9 of the Final Report.
10. A technical improvement will be made on the Phytotoxkit test plates by the addition of “marks” on the middle ridge, for an “equidistant” placing of the 10 seeds, and of a mark on the lateral sides of the test plate (near the middle ridge) to indicate the distance from the middle ridge at which the seeds should be placed.
11. A technical improvement will also be made on the “rectangular plastic strips” which can be used for easy flattening of the dry soil in the test plates, so that these rectangular strips fit perfectly in the bottom part of the test plate.

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INTERNATIONAL INTERLABORATORY COMPARISON OF THE PHYTOTOXKIT

Annex 1

PROCEDURE FOR THE PERFORMANCE OF A REFERENCE TEST WITH 250 MG BORIC ACID/KG OECD ARTICIFICIAL SOIL

CONTENTS OF THE KIT FOR PERFORMING THE REFERENCE TEST

- 18 Phytotoxkit test plates
- 3 carboard boxes
- 18 bags with OECD artificial soil
- 18 black filter papers
- 18 labels
- 3 tubes with seeds of *Lepidium sativum*, *Sinapis alba* and *Sorghum saccharatum*
- 1 vial with stock solution of boric acid
- 10 plastic strips
- 1 small flat-bottom pestle
- 1 pair of plastic tweezers
- 1 syringe (50 ml volume)
- 1 brochure with the Standard Operational Procedure of the Phytotoxkit microbiotest
- 1 CD Rom with the test protocol, the Image Tools programme and the Excel programme for the data treatment

Preparation of a test solution of 714 mg/l boric acid

1. Put about half a liter of deionized water in a 1 liter volumetric flask.
2. Pour the total content of the vial with the stock solution of boric acid into the 1 liter volumetric flask.
3. Fill the (empty) vial of stock solution of boric acid “two times” with deionized water and pour the contents into the 1 liter volumetric flask.

NB: This “rinsing” operation ensures the total transfer of boric acid from the vial with stock solution into the volumetric flask.

4. Add deionised water to the volumetric flask to the 1 liter mark and shake to homogenise the contents.

Labeling of the Phytotoxkit test plates

1. Take “the bottom part” of the 18 test plates and put a label on the side border of each of them with the following specific inscriptions for the assays with *Lepidium sativum* (LES), *Sinapis alba* (SIA) and *Sorghum saccharatum* (SOS):

- 3 test plates: LES control 1; LES control 2; LES control 3
- 3 test plates: LES 250 mg – Replic 1; LES 250 mg – Replic 2; LES 250 mg – Replic 3

2. Label the 6 test plates for SIA and the 6 test plates for SOS the same way as for LES.

Making of rectangular plastic strips for the Phytotoxkit test plates

1. Take one of the plastic strips and remove the orange protective cover from the (small) taped part at one end of the strip.
2. Lift up the other end of the strip and place (fix) this end "exactly" on the total surface of the tape at the other end of the plastic strip, in order to "close" the plastic strip into a circle.

NB: the rectangular plastic strips must all be of exactly the same length to fit exactly to the 4 walls of the bottom compartment of the test plates. It is therefore of crucial importance when performing step 2 that the "entire tape" is covered by the (free end) of the plastic strip, but not more nor not less !

3. Fold the circular plastic strip to about 90 degrees at each of the 4 scratched lines on the strip, in order "to transform" the circular strip into a rectangular strip of (exactly)12.5 x 8.5 cm.
4. Make rectangular strips with the other 9 plastic strips in the same way.

Addition of the OECD artificial soil to the test plates

1. Take one of the test plates and place a rectangular plastic strip in the bottom compartment.
2. Take a bag with OECD artificial soil and empty the contents in the bottom compartment of the test plate which is provided with a rectangular strip.
3. Place the test plate on a flat surface and shake it gently to distribute the dry soil evenly over the whole surface area of the bottom compartment of the test plate.
4. Flatten the soil and compact it slightly with the small flat-bottom pestle.
5. Remove the rectangular plastic strip by lifting it up carefully in order not to disturb the surface of the flattened soil.

   NB: the surface can be flattened again with the small flat-bottom pestle.

6. Proceed exactly in the same way to fill the other 17 test plates with OECD artificial soil.

Hydration of the control test plates

1. Pour about half a liter of deionized water in a beaker.
2. Take the 50 ml syringe and suck up 35 ml deionized water.
3. Take one of the test plates labelled "control" and spread the 35 ml deionized water evenly over the whole surface of the soil.
4. Proceed the same way for the 8 other control test plates.

Hydration of the test plates with toxicant

1. Pour about half a liter of the boric acid toxicant solution in a beaker.
2. Take the 50 ml syringe and suck up 35 ml toxicant solution.
3. Take one of the test plates labelled "250 mg" and spread the 35 ml toxicant solution evenly over the whole surface of the soil.
4. Proceed the same way for the 8 other test plates which carry a label "250 mg".

   NB: the addition (spiking) of 35 ml toxicant solution to a test plate containing 90 ml OECD soil results in a (nominal) toxicant concentration of 250 mg boric acid per kg (dry) OECD soil (see *explanation below, in the section "Calculation of the concentration of boric acid for spiking of the OECD artificial soil").*

Placing of the black filter paper

1. Take a black filter paper and put it on top of one of the hydrated test plates.
2. Wait until the filter is completely wet and flatten the surface with a spatula.
3. Proceed the same way for all the other hydrated test plates.

Placing of the seeds on the black filter paper

1. Take the 6 test plates labelled LES.
2. With the aid of the blue plastic tweezers, put *10 seeds* of this plant in each test plate.
3. The seeds shall be placed on top of the black filter paper in one row and at equal distance of each other.
4. Proceed the same way for the 6 test plates labelled SIA and for the 6 test plates labelled SOS.

Closing of the test plates

1. Cover the test plates with the flat transparent cover and click the protruding parts of the cover into the corresponding holes of the bottom part.

   NB: this "closing" operation has to be started at the middle of the test plates in order to avoid that the position of the seeds changes during the closing operation.

Pre-hydration of the seeds in the test plates

1. Put all the test plates on a table, with the side of the black filter and the seeds facing the table.
2. Leave the test plates in this position *for about one hour*, for a first pre-hydration of the seeds.

Incubation of the test plates

1. Place the 6 test plates labelled LES in one of the cardboard boxes, and those with the label SIA and SOS in the second and third cardboard box respectively.
2. Put the cardboard boxes with the test plates "vertically" in the incubator.
3. Incubate the test plates for 72 hours at 25°C in darkness.

Image recording at the end of the exposure period

1. Follow the instructions given in the Standard Operational Procedure (SOP) of the Phytotoxkit (page 19) and take a photo of each test plate with a digital camera.

   NB: As indicated in the SOP of the Phytotoxkit on page 20, the transfer of the test plates from the incubator to the ambient environment very often leads to the development of condensation on the inside of the lid of the test plates which interferes with the visibility of the roots.

   It is therefore advised to lift up (very carefully) and remove the lid of the test plate prior to shooting the picture.

2. Transfer the pictures of the digital camera to a computer and store them as JPEG files in the selected Directory.

Measurement of the lengths of the roots in the test plates

1. Load the Image Tool programme and the Excel programme from the CD Rom on your computer.
2. Follow the instructions given in the Standard Operational Procedure of the Phytotoxkit (pages 20-22) for the measurement of the length of the roots with the aid of the Image Tools programme.

IMPORTANT REMARK

The Excel programme which processes the results of the length measurements will only work if the length data of the roots (as measured with the Image Tools programme) are transferred to this Excel programme with “a decimal point” (= a dot) and not as a “comma”. Depending of the computer, the configuration for the decimal has been set either with a dot or with a comma ! In case the decimal setting in your computer is “with a comma”, you have first to change the comma setting “to a dot setting”, via the control panel of your computer.

N.B : You may possibly have to set your computer to the “English version” to be able to do the following steps !

- Click on “Start” and go to “Control panel”
- Click on “Country settings”
- Click on “Adapt”
- Click on “Number”
- Change the “comma” sign for the decimal by the “dot” sign
- Click on “Execute”
- Click on “OK”

1. Measure the length of all the roots in the first control plate of *Lepidium sativum* (LES).
2. Close the frame with the picture of the test plate to see the sheet with the individual result numbers.
3. Click on “Edit” and on “Copy Results”.
4. Open the Excel programme “Results of the International Phytotoxkit Intercalibration Exercise”.

   NB: The Excel programme will show the sheet “Control plates” with 9 coloured boxes (3 boxes for the 3 control plates of each seed, each in a different colour).

5. Fill out your personal data on top of the sheet : Name – Laboratory – Institute – Company – Date of test performance.
6. Go to the box “Control LES 1” and click on “Paste here”.
7. Click on the icon “Paste” of the computer, which will then show all the length results in the box Control LES 1.
8. Proceed the same way for the second and third control test plate of *Lepidium sativum* and for the 3 control test plates of *Sinapis alba* (SIA) and *Sorghum saccharatum* (SOS).
9. Proceed the same way for the 3 test plates with boric acid for each of the 3 plants.
10. Transfer the data for each test plate and plant in the corresponding coloured boxes of the second sheet of the Excel programme = “Test plates boric acid”.

Visualisation of the treated data

Once all the measurements and transfers have been made, open the third sheet of the Excel programme = “Results”.

In this sheet one will see the treated data for each test plate:

1. the percentage seeds that have germinated
2. the mean length of the roots in each test plate, with the standard deviation and the variation coefficient (CV)
3. the length of the longest root in each test plate
4. in the darkened box of the 3 test plates with boric acid:

   a) the % inhibition calculated on the basis of the mean root length for the 3 replicates of each plant

   b) the % inhibition calculated on the basis of the mean length of the longest root, for the 3 replicates of each plant.

Save the Results Sheets in a file (under another name to keep a copy of the original blank Excel programme !).

Calculation of the concentration of boric acid for spiking of the OECD soil, to obtain a 250 mg boric acid concentration per kg (dry) OECD artificial soil in the Phytotoxkit test plates

One bag of reference soil contains 90 ml dry OECD artificial soil, which (with an error of 1-2 %) corresponds to 100 g dry soil.

250 mg boric acid /kg OECD artificial soil = 25 mg boric acid/100 g OECD soil

For a full hydration of the OECD artificial soil transferred from one bag (= 100 g soil) into the Phytotoxkit test plate one needs to add 35 ml solution to the soil.

The 35 ml solution to spike the (100 g) OECD artificial soil in the test plate must therefore contain 25 mg boric acid.

Converted to a 1 litre toxicant solution this means: $\frac{25 \times 1000}{25}$ = 714 mg boric acid.

The concentration of 714 mg/l boric acid is obtained by dilution of the (total) volume of (concentrated) stock solution of boric acid to 1 litre (into a 1 litre volumetric flask.

A concentration of 250 mg boric acid per kg OECD artificial soil is then obtained by spiking the test plates with 35 ml of this solution.

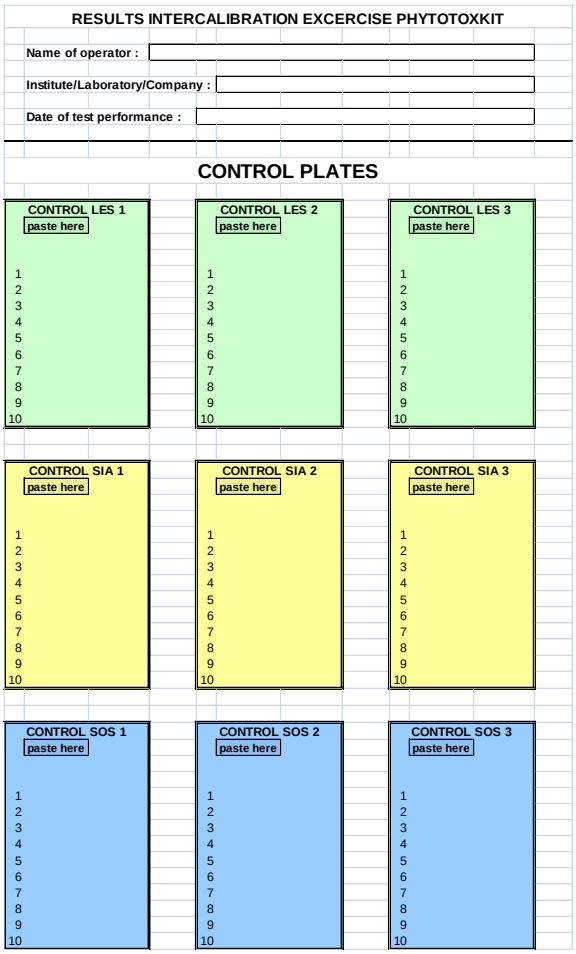
INTERNATIONAL INTERLABORATORY COMPARISON OF THE PHYTOTOXKIT

Annex 2

RESULT SHEETS

Measurement of the roots is performed with the Image Tool programme according to the instructions given in the corresponding section of Annex 1 – Procedure for the performance of a reference test with 250 mg boric acid per kg OECD artificial soil.

As per the instructions given in the same section, the data are subsequently transferred to the sheets “Control plates” and “Test plates with boric acid”, of the Results Sheets, copy of which is given below.



RESULTS INTERCALIBRATION EXCERCISE PHYTOTOXKIT

Name of operator :

Institute/Laboratory/Company :

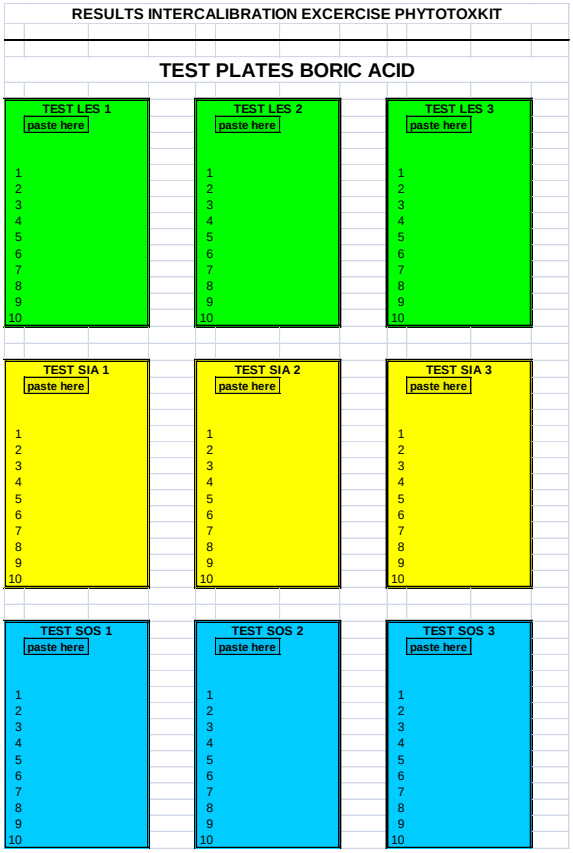
Date of test performance :

CONTROL PLATES

CONTROL LES 1	CONTROL LES 2	CONTROL LES 3
paste here	paste here	paste here
1	1	1
2	2	2
3	3	3
4	4	4
5	5	5
6	6	6
7	7	7
8	8	8
9	9	9
10	10	10

CONTROL SIA 1	CONTROL SIA 2	CONTROL SIA 3
paste here	paste here	paste here
1	1	1
2	2	2
3	3	3
4	4	4
5	5	5
6	6	6
7	7	7
8	8	8
9	9	9
10	10	10

CONTROL SOS 1	CONTROL SOS 2	CONTROL SOS 3
paste here	paste here	paste here
1	1	1
2	2	2
3	3	3
4	4	4
5	5	5
6	6	6
7	7	7
8	8	8
9	9	9
10	10	10



RESULTS INTERCALIBRATION EXCERCISE PHYTOTOXKIT

TEST PLATES BORIC ACID

TEST LES 1	TEST LES 2	TEST LES 3
paste here	paste here	paste here
1	1	1
2	2	2
3	3	3
4	4	4
5	5	5
6	6	6
7	7	7
8	8	8
9	9	9
10	10	10

TEST SIA 1	TEST SIA 2	TEST SIA 3
paste here	paste here	paste here
1	1	1
2	2	2
3	3	3
4	4	4
5	5	5
6	6	6
7	7	7
8	8	8
9	9	9
10	10	10

TEST SOS 1	TEST SOS 2	TEST SOS 3
paste here	paste here	paste here
1	1	1
2	2	2
3	3	3
4	4	4
5	5	5
6	6	6
7	7	7
8	8	8
9	9	9
10	10	10

The length data are then visualised on these sheets, and the Excel programme will automatically process the figures and calculate the % germination of the seeds, and the % inhibition of the mean root length and the % inhibition of the mean length of the longest roots for the 3 plant test species.

The third sheet of the Excel programme, copy of which is also given below, will show the original data and the calculated % inhibitions.

RESULTS INTERCALIBRATION EXCERCISE PHYTOTOXKIT

Name of operator :

Institute / Laboratory / Company :

Date of test performance :

LES - CONTROL				
	LES control 1	LES control 2	LES control 3	
	Length (mm)	Length (mm)	Length (mm)	
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				MEAN
# germinated	0	0	0	0
Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Std. Dev.	#DIV/0!	#DIV/0!	#DIV/0!	
VC%	#DIV/0!	#DIV/0!	#DIV/0!	
longest root	0.00	0.00	0.00	0.00

LES - 250 mg boric acid/kg OECD					
	LES boric acid 1	LES boric acid 2	LES boric acid 2		
	Length (mm)	Length (mm)	Length (mm)		
1					
2					
3					
4					
5					
6					
7					
8					
9					
10				MEAN	**% inhibition**
# germinated	0	0	0	0	
Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	**#DIV/0!**
Std. Dev.	#DIV/0!	#DIV/0!	#DIV/0!		
VC%	#DIV/0!	#DIV/0!	#DIV/0!		
longest root	0.00	0.00	0.00	0.00	**#DIV/0!**

SIA - CONTROL				
	SIA control 1	SIA control 2	SIA control 3	
	Length (mm)	Length (mm)	Length (mm)	
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				MEAN
# germinated	0	0	0	0
Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Std. Dev.	#DIV/0!	#DIV/0!	#DIV/0!	
VC%	#DIV/0!	#DIV/0!	#DIV/0!	
longest root	0.00	0.00	0.00	0.00

SIA - 250 mg boric acid/kg OECD					
	SIA boric acid 1	SIA boric acid 2	SIA boric acid 3		
	Length (mm)	Length (mm)	Length (mm)		
1					
2					
3					
4					
5					
6					
7					
8					
9					
10				MEAN	**% inhibition**
# germinated	0	0	0	0	
Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	**#DIV/0!**
Std. Dev.	#DIV/0!	#DIV/0!	#DIV/0!		
VC%	#DIV/0!	#DIV/0!	#DIV/0!		
longest root	0.00	0.00	0.00	0.00	**#DIV/0!**

SOS - CONTROL				
	SOS control 1	SOS control 2	SOS control 3	
	Length (mm)	Length (mm)	Length (mm)	
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				MEAN
# germinated	0	0	0	0
Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Std. Dev.	#DIV/0!	#DIV/0!	#DIV/0!	
VC%	#DIV/0!	#DIV/0!	#DIV/0!	
longest root	0.00	0.00	0.00	0.00

SOS - 250 mg boric acid/kg OECD					
	SOS boric acid 1	SOS boric acid 2	SOS boric acid 3		
	Length (mm)	Length (mm)	Length (mm)		
1					
2					
3					
4					
5					
6					
7					
8					
9					
10				MEAN	**% inhibition**
# germinated	0	0	0	0	
Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	**#DIV/0!**
Std. Dev.	#DIV/0!	#DIV/0!	#DIV/0!		
VC%	#DIV/0!	#DIV/0!	#DIV/0!		
longest root	0.00	0.00	0.00	0.00	**#DIV/0!**

INTERNATIONAL INTERLABORATORY COMPARISON OF THE PHYTOTOXKIT

Annex 3

CALCULATION OF THE % GERMINATION INDEX

The % Germination Index" indicates the extent of the success of the whole process (seed germination *plus* root elongation) for the seeds exposed to the toxicant (or sample), in comparison to the success of the whole process in the negative control, that is in optimal conditions.

First, the Germination Index GI_i is calculated for the controls *i* (for each lab and seed):

$$GI_{ci} = N_{ci} \times \overline{L_{ci}}$$

where N_{ci} is the number of germinated seeds in replicate *i* of control *c*, and $\overline{L_{ci}}$ is the mean root length in replicate *i* of control *c*.

Then, the mean Germination Index for the controls $\overline{GI_c}$ is calculated as $\overline{GI_c} = \frac{(GI_{c1} + GI_{c2} + GI_{c3})}{3}$, the Percentage Germination Index $\%GI_{ci}$ of the Germination Indexes of replicates *i* as $\%GI_{ci} = \frac{GI_{ci}}{\overline{GI_c}} \times 100$, and the mean % Germination Index $\overline{\%GI_c}$ for the controls *i* as $\overline{\%GI_c} = \frac{(\%GI_{c1} + \%GI_{c2} + \%GI_{c3})}{3}$.

The same calculations are made for the treatments (boric acid) t_i:

$$GI_{ti} = N_{ti} \times \overline{L_{ti}}$$

where N_{ti} is the number of germinated seeds in replicate *i* of treatment *t*, and $\overline{L_{ti}}$ is the mean root length in replicate *i* of treatment *t*.

$$\overline{GI_t} = \frac{(GI_{t1} + GI_{t2} + GI_{t3})}{3}$$

$$\%GI_{ti} = \frac{GI_{ti}}{\overline{GI_c}} \times 100$$

$$\overline{\%GI_t} = \frac{(\%GI_{t1} + \%GI_{t2} + \%GI_{t3})}{3}$$

INTERNATIONAL INTERLABORATORY COMPARISON OF THE PHYTOTOXKIT

Annex 4

STATISTICAL ANALYSIS OF THE RESULTS OF THE INTERNATIONAL INTERLABORATORY COMPARISON ON THE PHYTOTOXKIT

It was agreed with the participants that their results would be treated confidentially without mentioning names of the participating laboratories, organisations, institutes and companies in the presentation and discussion of the results. Therefore, in the following each laboratory is identified only by a randomly given code (the same for all data set).

For each data set, a figure reports the mean, minimum and maximum values for each (coded) lab; the last (right) figures refer to the overall mean, along with the 95 % confidence limits.

The repeatability and reproducibility of the interlaboratory comparison have been calculated according to the ISO 5725-2 (2002) procedure, providing the following results:

s_L^2 the estimate of the between-laboratory variance;

s_W^2 the estimate of the within-laboratory variance;

s_r^2 the arithmetic mean of the within-laboratory variances (after outliers have been excluded);

s_R^2 the estimate of the reproducibility variance: $s_R^2 = s_L^2 + s_r^2$.

To check the consistency of the data, the Mandel's h and k statistics have been used: the first (h) provides the between-laboratory consistency statistic, and the second (k) the within-laboratory consistency statistic.

The Grubb's test have then been applied to identify stragglers (if the test statistic is greater than its 5 % critical value and less than or equal to its 1 % critical value, the item tested is called a straggler and is indicated by a single asterisk), and outliers (if the test statistic is greater than its 1 % critical value, the item is called a statistical outlier and is indicated by a double asterisk).

If some straggler and/or outlier can be explained by a technical error, for example in transcribing a test result, after the proper correction the data is retained and the Grubb's test repeated. If it proves impossible to replace the suspect test result, then it should be discarded as a "genuine" outlier, while stragglers are retained as correct items. Therefore, the final statistics of this interlaboratory comparison, to be used in conclusions, for each endpoint and seed include stragglers, but not outliers.

Two figures show the results of the Grubb's test for h and k statistics, followed by the statistical analysis, for all data and the same without h or k outliers (data higher than overall mean + 3 times the interlaboratory standard deviation s_R, or lower than overall mean - 3 times the interlaboratory standard deviation s_R), and without h or k stragglers (data higher than overall mean + 2 times the interlaboratory standard deviation s_R, or lower than overall mean - 2 times the interlaboratory standard deviation s_R).

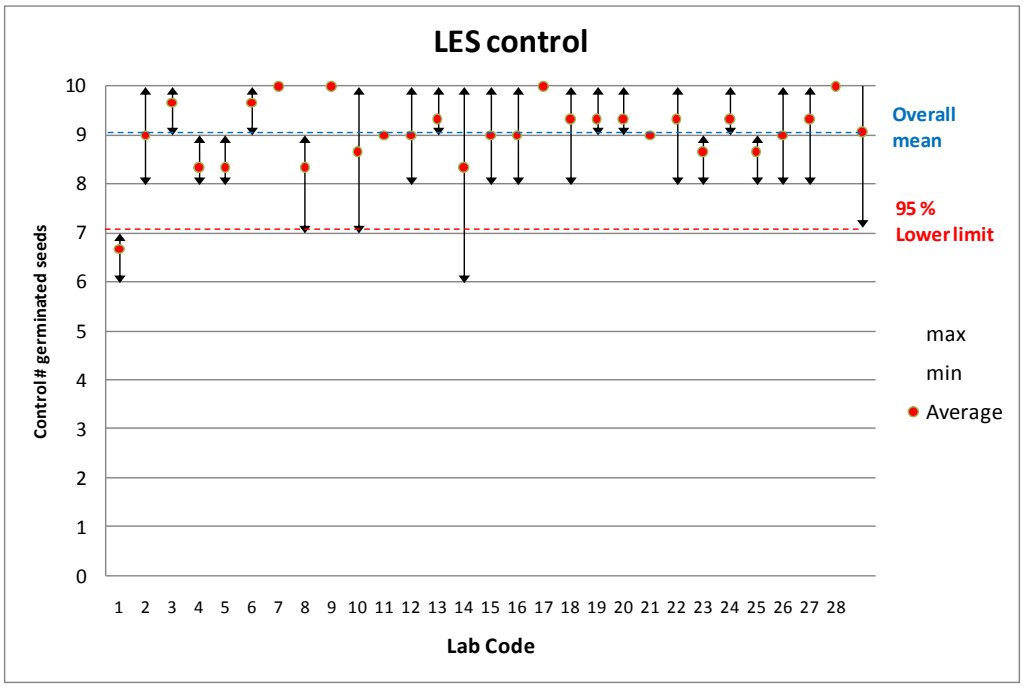
Negative control

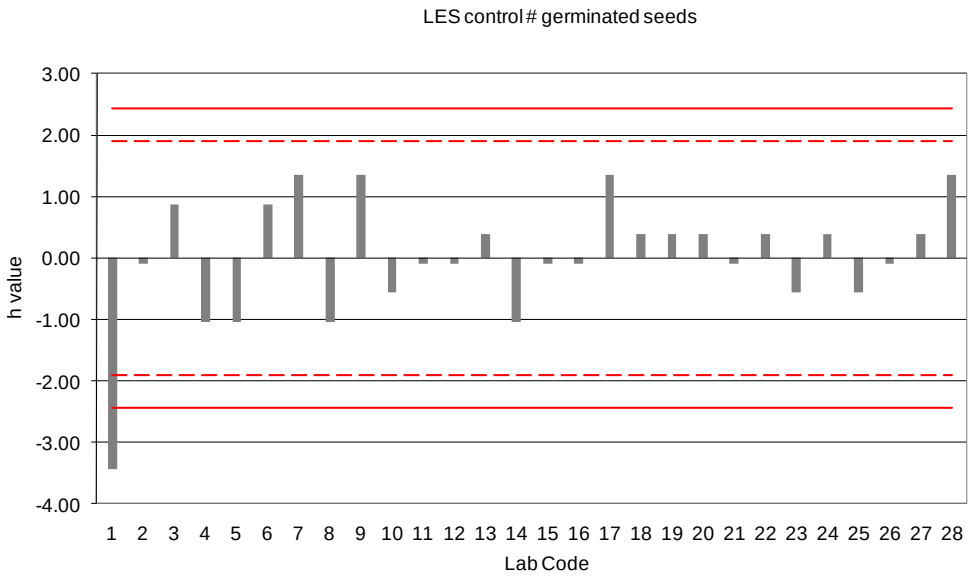
Number of germinated seeds

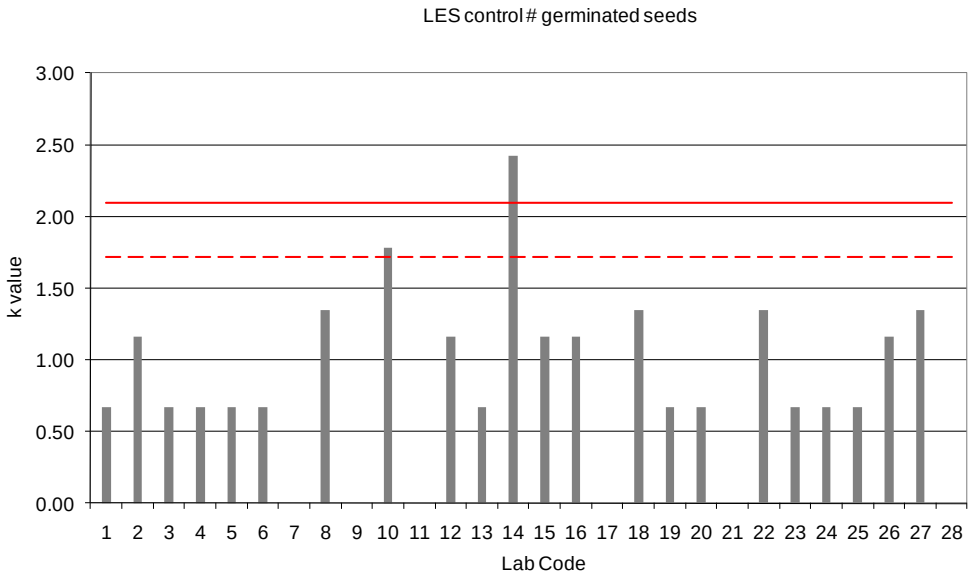
For each data set, a figure reports the mean, minimum and maximum values for each (coded) lab; the last (right) figures refer to the overall mean, along with the 95 % confidence limits.

The starting number of seeds is 10 for each test; therefore, the 95 % upper confidence limit (statistically exceeding 10) has been omitted in the pertinent figure.

Lepidium sativum (LES)

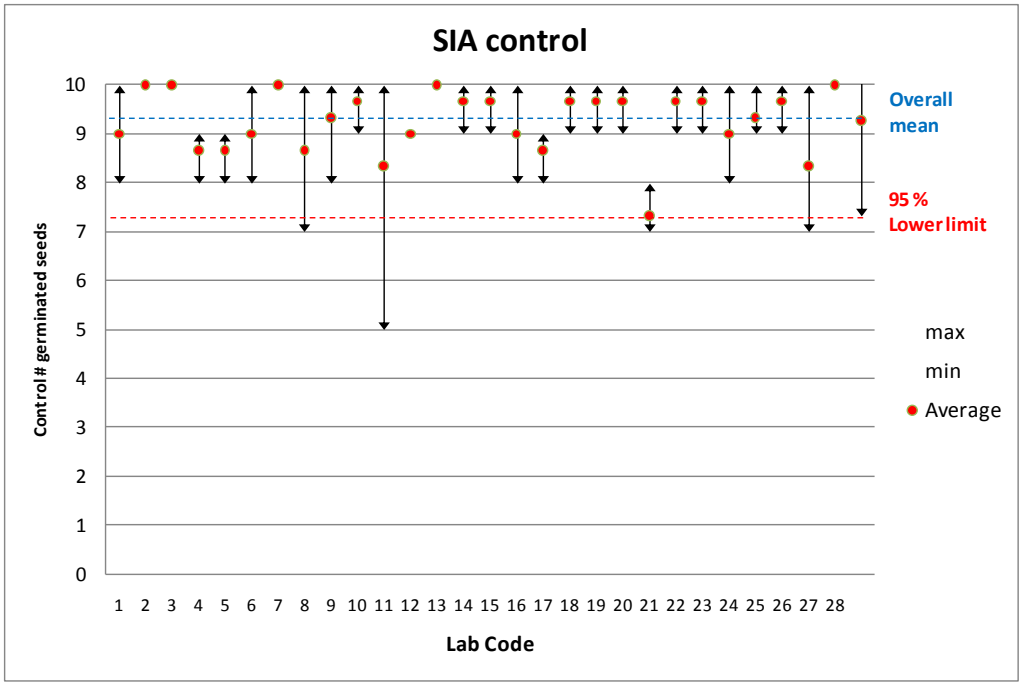


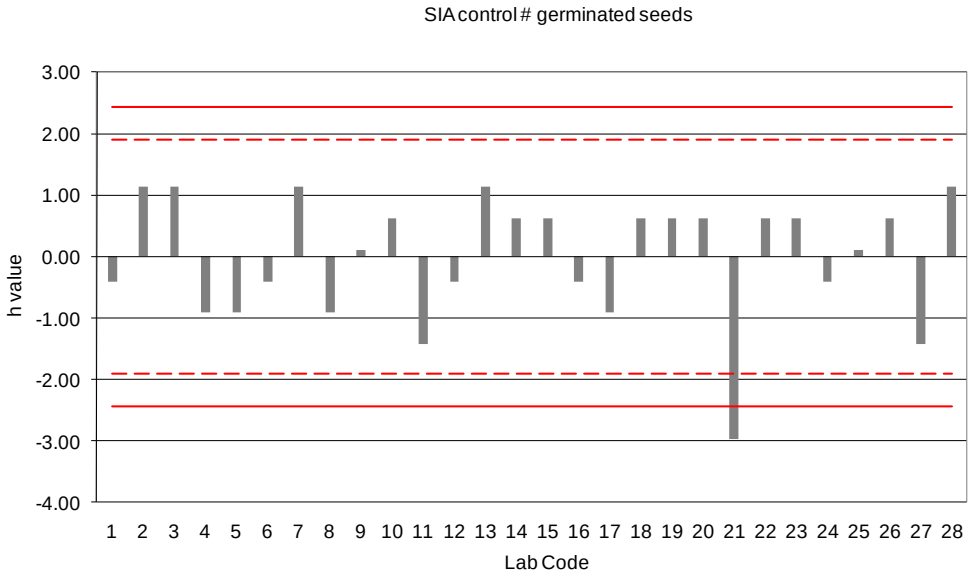


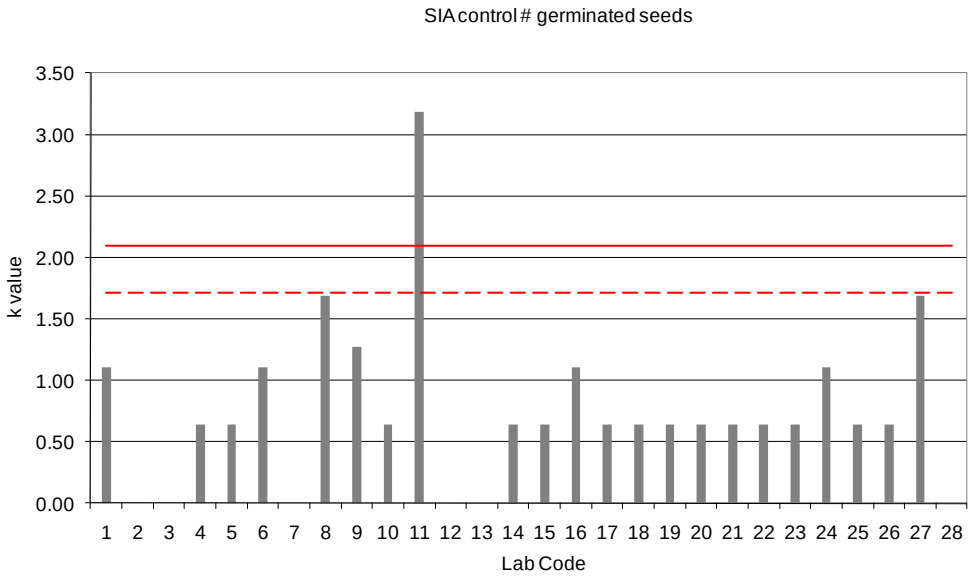


LES control # germinated seeds						
	All data	**Without k and h outliers**		**Without k and h stragglers**		
N labs	28	26		25		
N repeats	3	3		3		
Mean	9.1	9.2		9.2		
s_r	0.9	0.8		0.7		
CV%	9.5	8.5		8.0		
s_R	1.0	0.8		0.8		
CV%	10.9	8.9		8.6		
h straggler	1	0		0		
h outlier	1	0		0		
k straggler	2	1		0		
k outlier	1	0		0		
95 % Upper limit	11.0	10.8		10.7		
95 % Lower limit	7.1	7.6		7.7		

2 results have been discarded (Lab 1 as h outlier, lab 14 as k outlier). However, the statistical treatment does not change the mean value, remaining at 9 seeds (even discarding stragglers). Discarding the 2 outlier, there is a small improvement in both the intra- and interlaboratory variability. The accepted results, based on 26 laboratories (out of the original 28 participating labs), have a repeatability of 8.5 % and a reproducibility of 8.9 %.

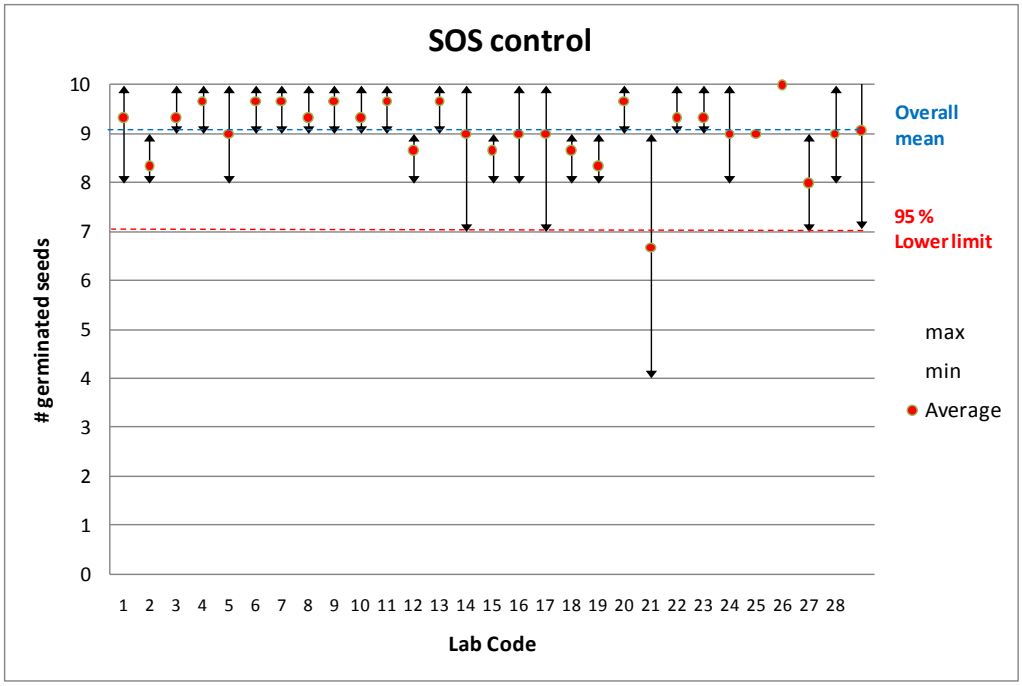

SIA control
Control # germinated seeds
Lab Code
Overall mean
95 % Lower limit
max
min
Average


SIA control # germinated seeds
h value
Lab Code

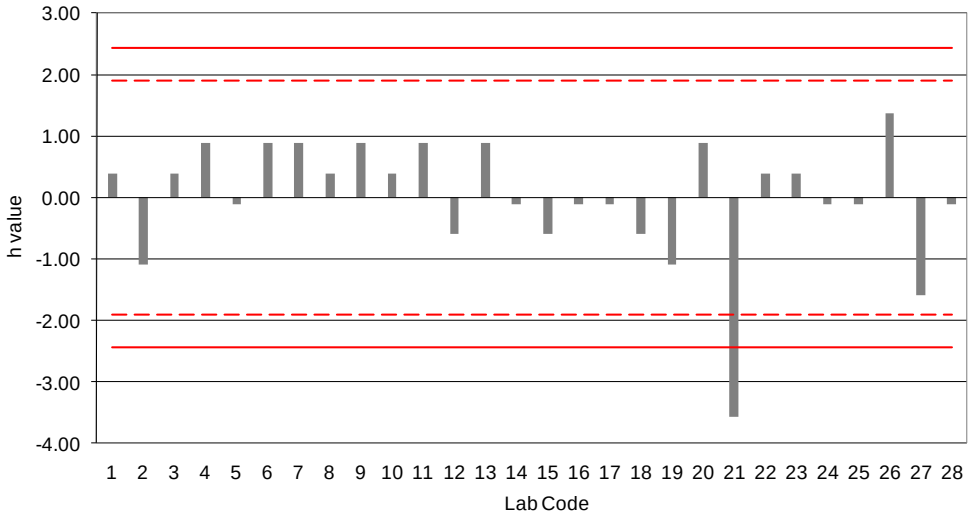


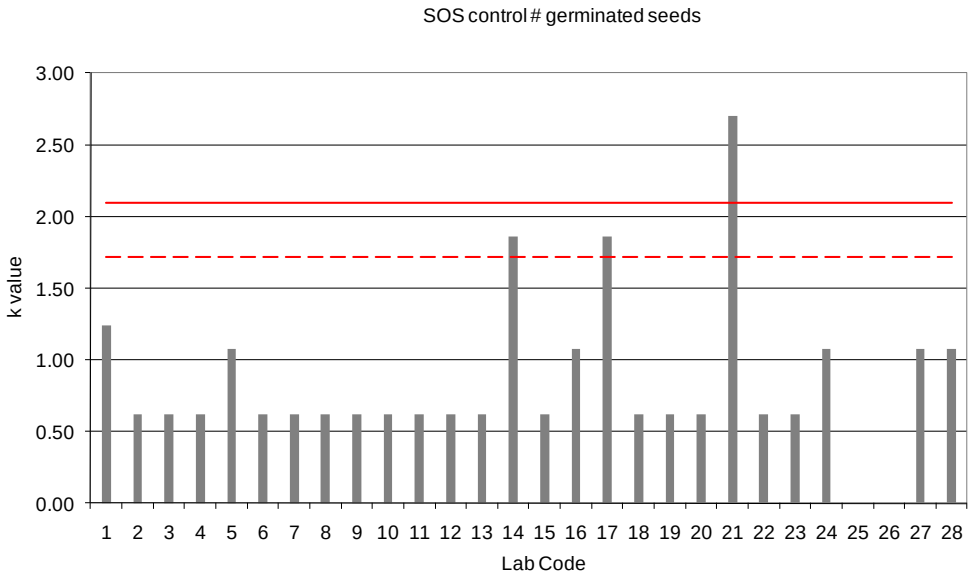
SIA control # germinated seeds						
	All data		**Without k and h outliers**		**Without k and h stragglers**	
N labs	28		26		23	
N repeats	3		3		3	
Mean	9.3		9.4		9.4	
s_r	0.9		0.7		0.6	
CV%	9.8		7.9		6.4	
s_R	1.0		0.8		0.7	
CV%	10.6		8.5		7.2	
h straggler	1		1		0	
h outlier	1		0		0	
k straggler	1		2		0	
k outlier	1		0		0	
95 % Upper limit	11.2		10.9		10.8	
95 % Lower limit	7.3		7.8		8.1	

2 results have been discarded (Lab 21 as h outlier, lab 11 as k outlier). Again, however, the statistical treatment does not change the mean value, remaining at 9 seeds (even discarding stragglers). The accepted results, based on 26 laboratories (out of the original 28 participating labs), have a repeatability of 7.9 % and a reproducibility of 8.5 %.



SOS control # germinated seeds





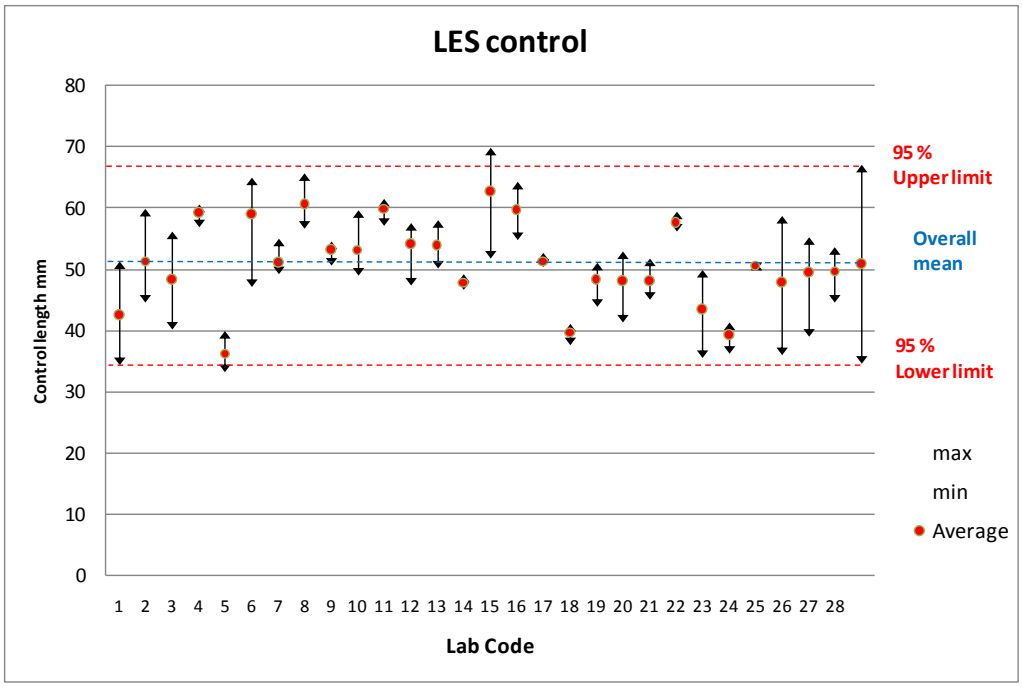
SOS control # germinated seeds						
	All data		**Without k and h outliers**		**Without k and h stragglers**	
N labs	28		25		22	
N repeats	3		3		3	
Mean	9.1		9.2		9.3	
s_r	0.9		0.7		0.7	
CV%	10.3		7.6		7.4	
s_R	1.0		0.8		0.7	
CV%	11.2		8.3		7.4	
h straggler	1		1		0	
h outlier	1		0		0	
k straggler	3		0		0	
k outlier	1		0		0	
95 % Upper limit	11.1		10.7		10.6	
95 % Lower limit	7.1		7.7		8.0	

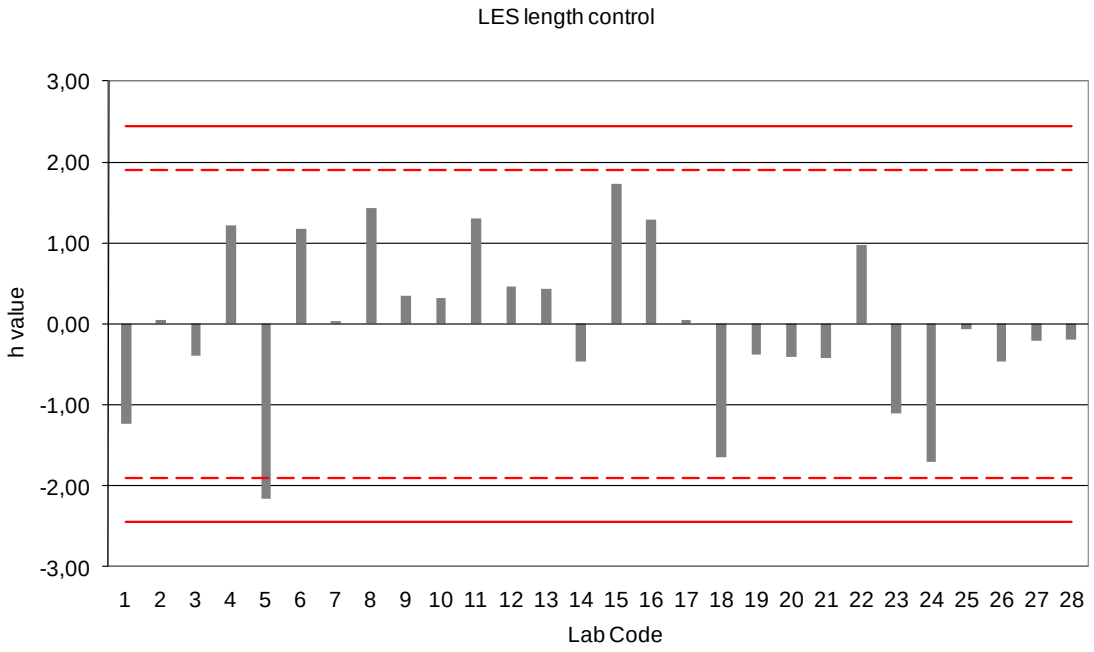
3 results have been discarded (Lab 21 as h and k outlier, and labs 14 and 17 as k outliers). The overall mean is 9 seeds (even discarding stragglers). The accepted results, based on 25 laboratories (out of the original 28 participating labs), have a repeatability of 7.6 % and a reproducibility of 8.3 %.

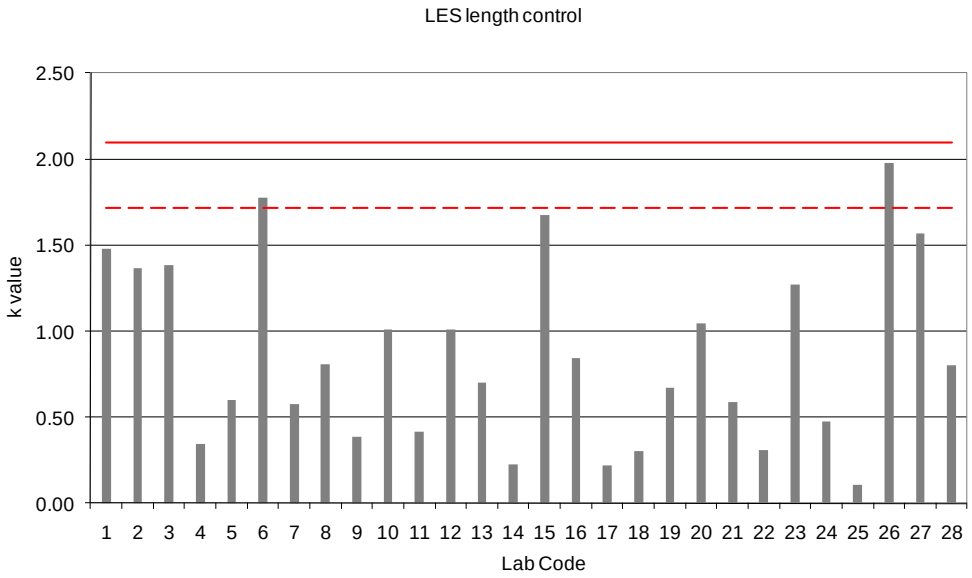
On the basis of these results, it can be safely concluded, taking into account the 95 % lower confidence limits, that the Phytotoxkit can adopt a validity criteria of 70 % germination in the negative control for all three seeds, with a reproducibility of 9.0 %.

Root length

Lepidium sativum (LES)

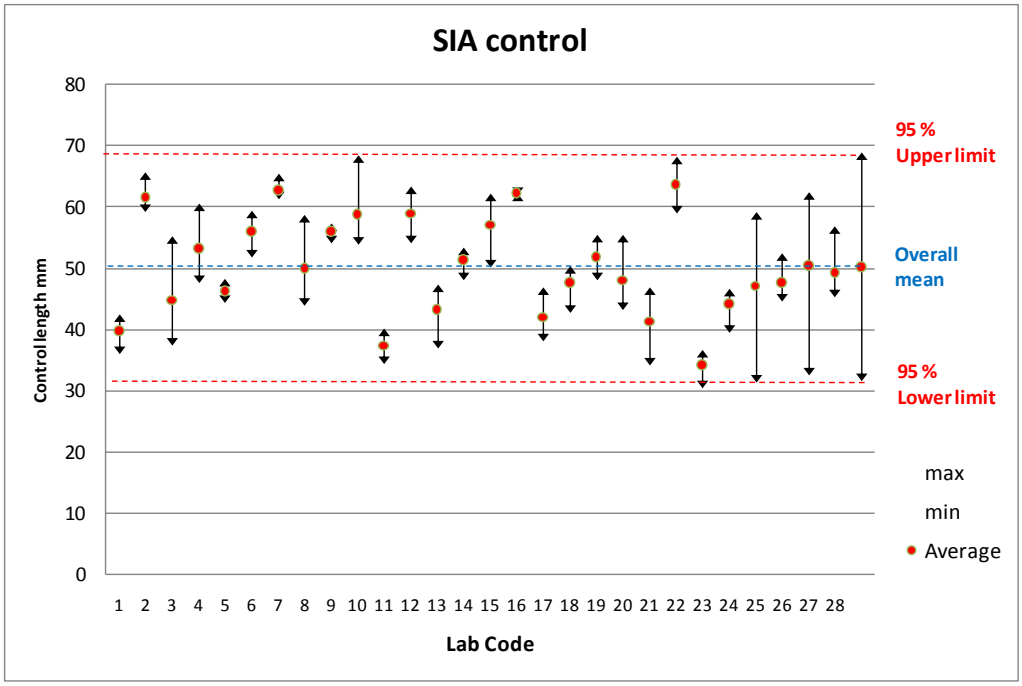


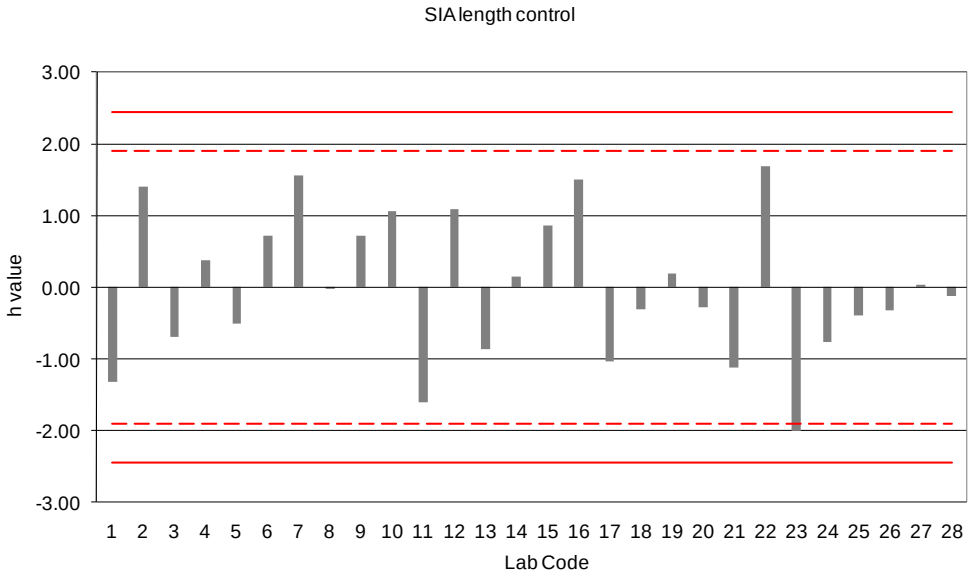


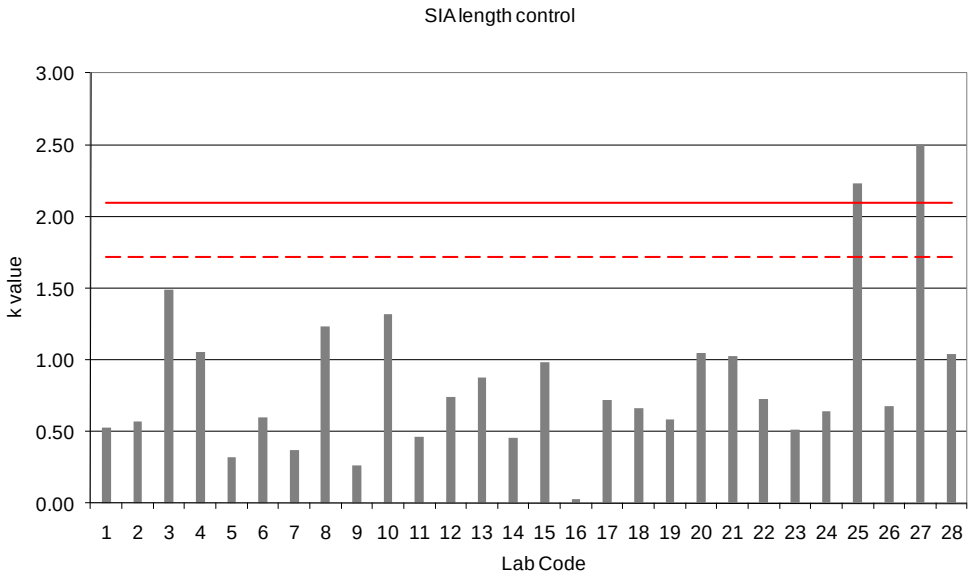


LES length control							
	All data		**Without k and h outliers**			**Without k and h stragglers**	
N labs	28		28			17	
N repeats	3		3			3	
Mean	51.0		51.0			53.4	
s_r	5.8		5.8			3.8	
CV%	11.3		11.3			7.1	
s_R	8.3		8.3			5.5	
CV%	16.3		16.3			10.3	
h straggler	1		1			0	
h outlier	0		0			0	
k straggler	2		2			0	
k outlier	0		0			0	
95 % Upper limit	67.2		67.2			64.2	
95 % Lower limit	34.7		34.7			42.5	

There are no k outliers. The accepted results, based on 28 laboratories, have an overall mean of 51 mm, a repeatability of 11.3 % and a reproducibility of 16.3 %.

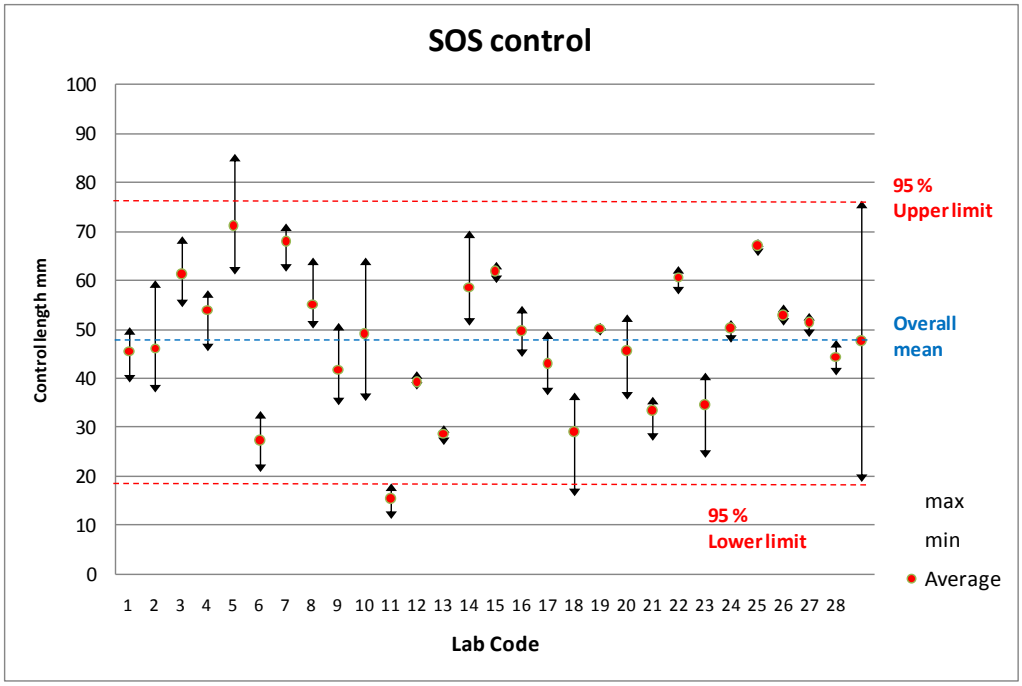

SIA control
Control length mm
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code
95 %
Upper limit
Overall
mean
95 %
Lower limit
max
min
Average

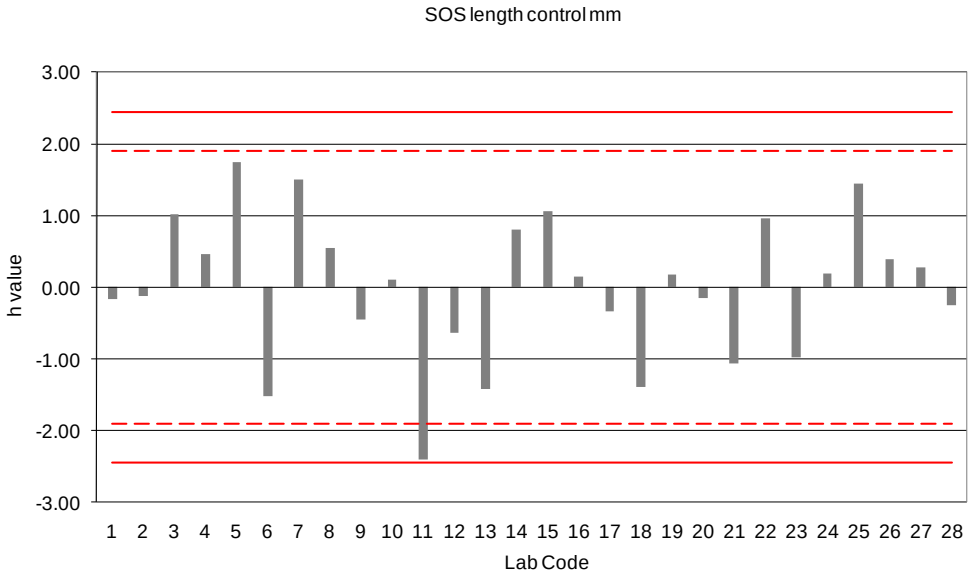

SIA length control
h value
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code

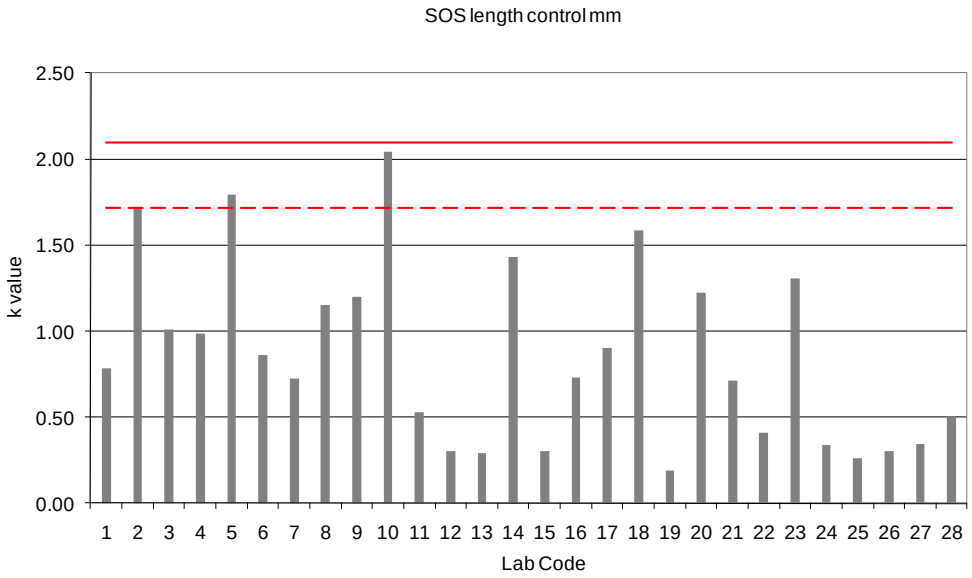


SIA length control								
	All data		**Without k and h outliers**			**Without k and h stragglers**		
N labs	28		26			23		
N repeats	3		3			3		
Mean	50.3		50.4			51.0		
s_r	6.4		5.1			4.7		
CV%	12.6		10.1			9.2		
s_R	9.5		9.3			8.7		
CV%	19.0		18.4			17.1		
h straggler	1		1			0		
h outlier	0		0			0		
k straggler	2		1			0		
k outlier	2		0			0		
95 % Upper limit	69.0		68.6			68.1		
95 % Lower limit	31.5		32.2			33.9		

2 results have been discarded (Labs 25 and 27 as k outliers). The accepted results, based on 26 laboratories (out of the original 28 participating labs), have a mean of 50 mm, a repeatability of 10.1 % and a reproducibility of 18.4 %.


SOS control
Control length mm
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code
95 %
Upper limit
Overall
mean
95 %
Lower limit
max
min
Average


SOS length control mm
h value
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code

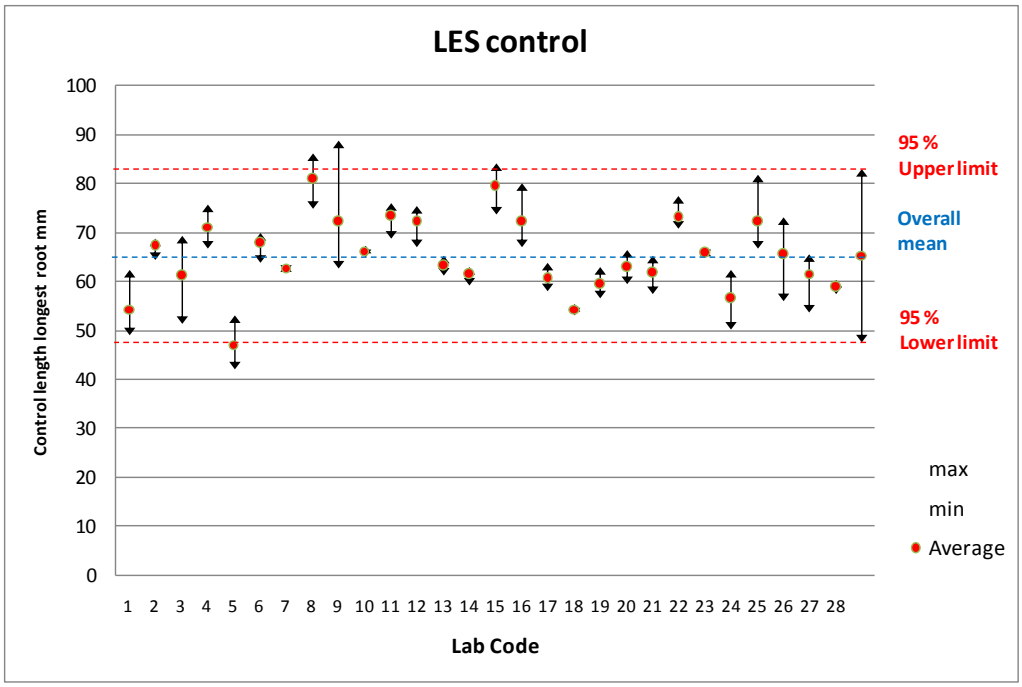


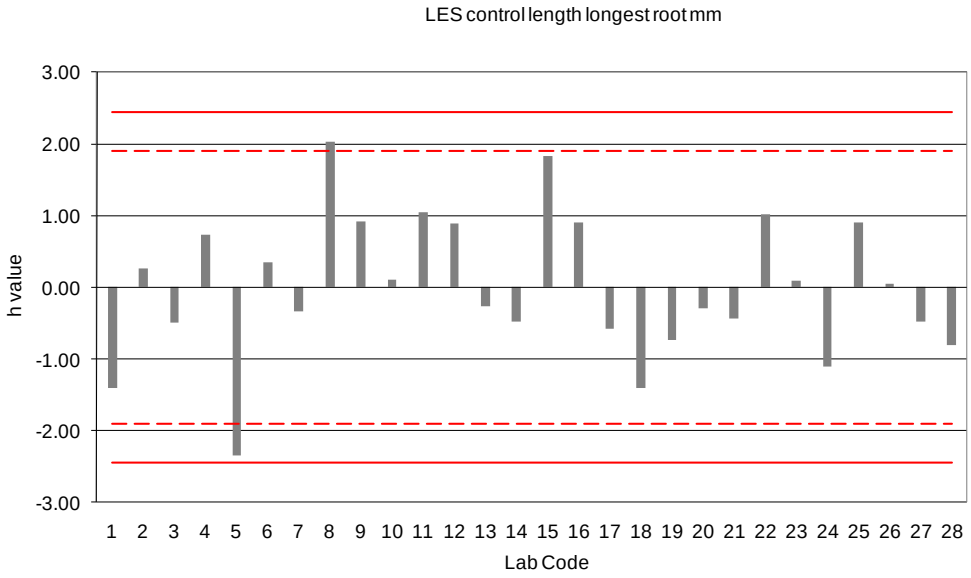
SOS length control mm								
	All data		**Without k and h outliers**			**Without k and h stragglers**		
N labs	28		28			18		
N repeats	3		3			3		
Mean	47.7		47.7			52.4		
s_r	7.2		7.2			5.3		
CV%	15.2		15.2			10.2		
s_R	14.7		14.7			9.7		
CV%	30.7		30.7			18.4		
h straggler	1		1			0		
h outlier	0		0			0		
k straggler	3		3			0		
k outlier	0		0			0		
95 % Upper limit	76.5		76.5			71.3		
95 % Lower limit	19.0		19.0			33.4		

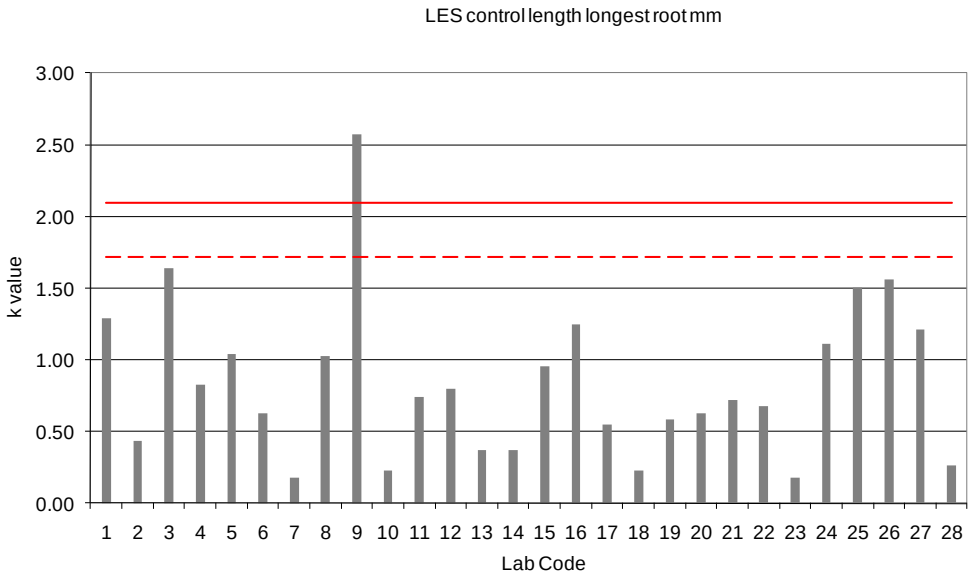
No outliers have been detected. The accepted results, based on all 28 laboratories, have a mean of 48 mm, a repeatability of 15.2 % and a reproducibility of 30.7 %.

Longest root length

Lepidium sativum (LES)

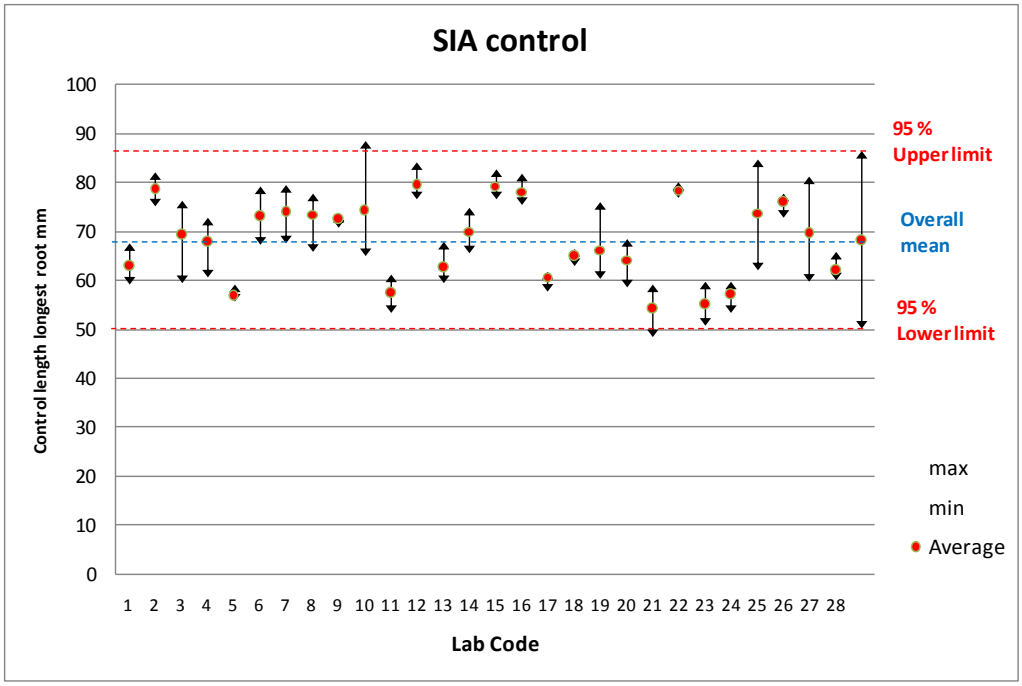


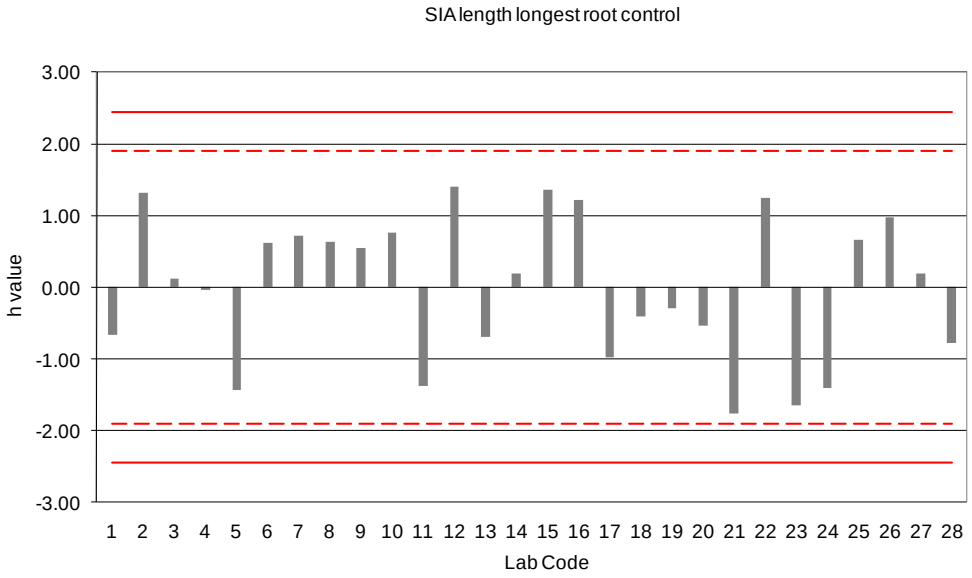


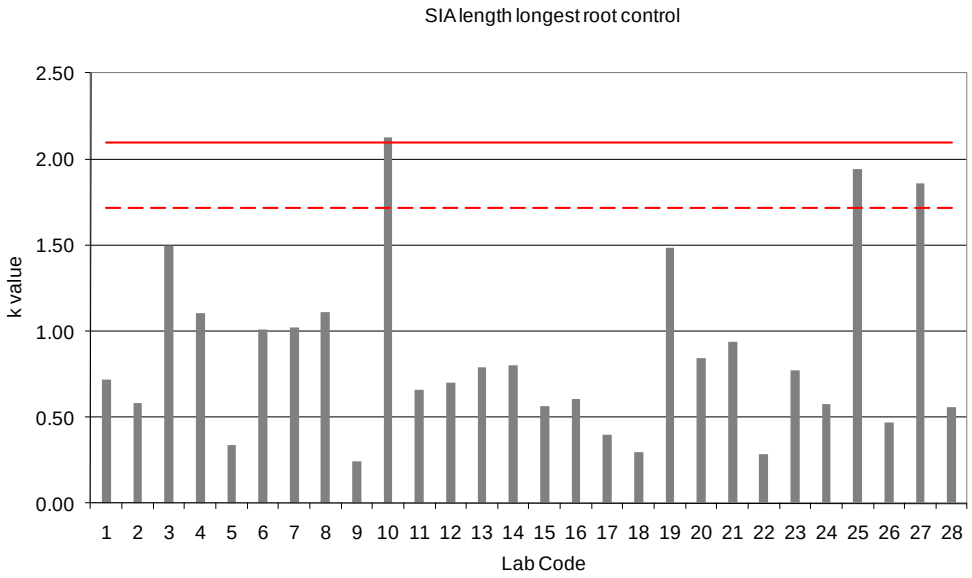


LES control length longest root mm						
	All data		**Without k and h outliers**		**Without k and h stragglers**	
N labs	28		27		16	
N repeats	3		3		3	
Mean	65.3		65.0		65.6	
s_r	5.5		4.9		3.1	
CV%	8.5		7.6		4.7	
s_R	9.0		8.8		5.5	
CV%	13.8		13.6		8.4	
h straggler	2		2		0	
h outlier	0		0		0	
k straggler	1		2		0	
k outlier	1		0		0	
95 % Upper limit	83.0		82.3		76.4	
95 % Lower limit	47.6		47.7		54.8	

1 result has been discarded (Lab 9 as k outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a mean length of the longest root of 65 mm, a repeatability of 7.6 % and a reproducibility of 13.6 %.

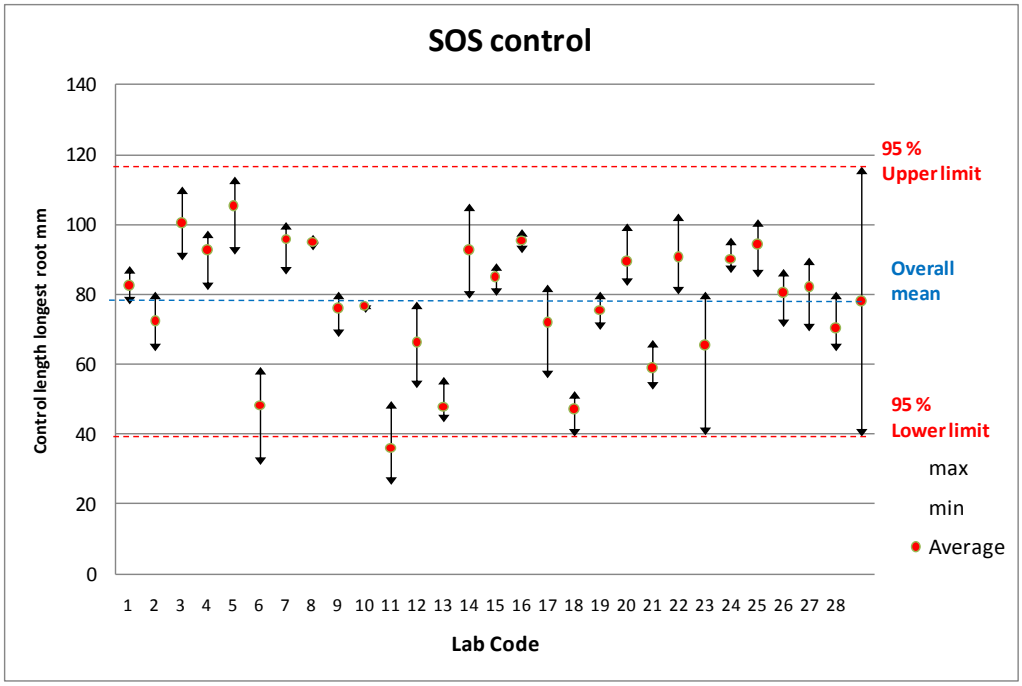

SIA control
Control length longest root mm
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code
95 %
Upper limit
Overall
mean
95 %
Lower limit
max
min
Average

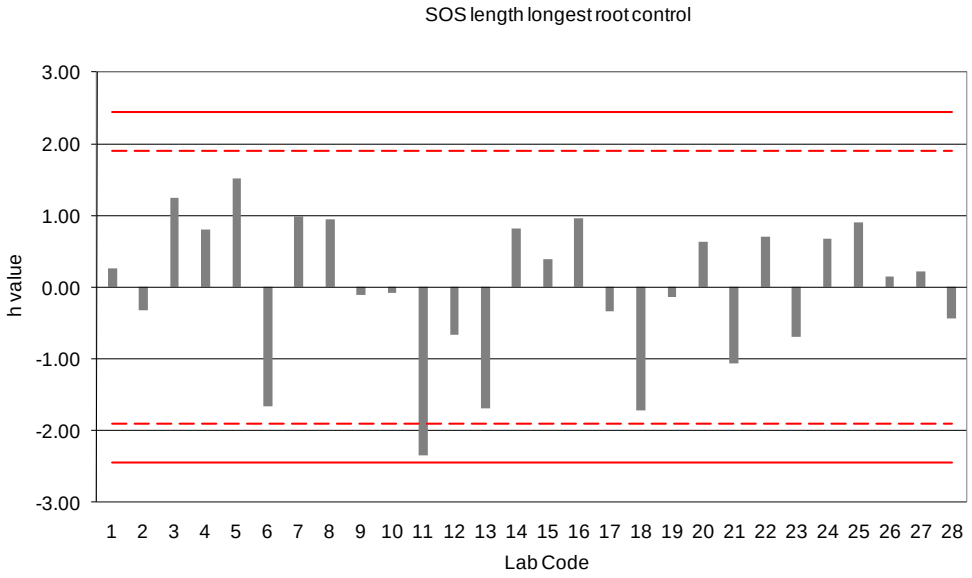

SIA length longest root control
h value
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code

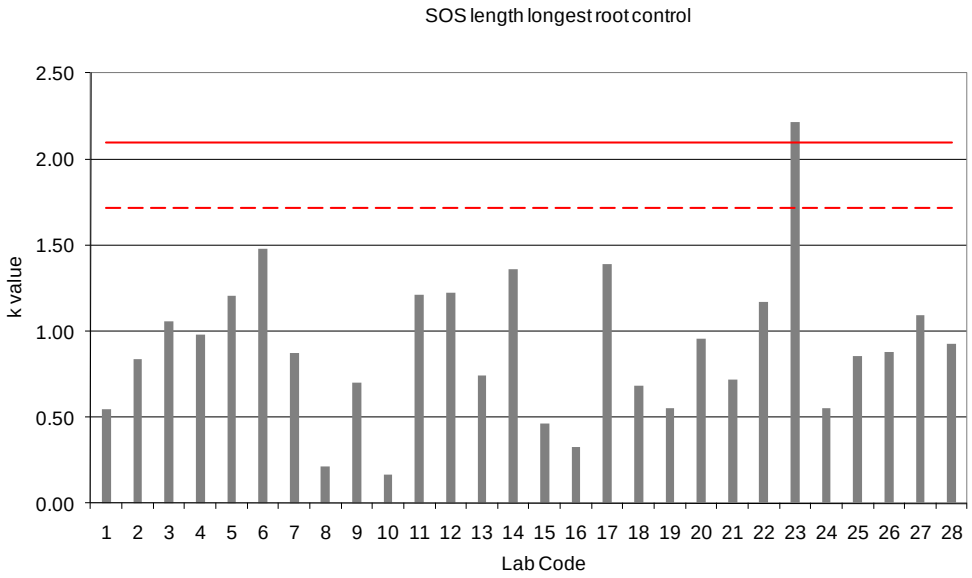


SIA length longest root control						
	All data		**Without k and h outliers**		**Without k and h stragglers**	
N labs	28		27		23	
N repeats	3		3		3	
Mean	68.4		68.1		67.9	
s_r	5.8		5.4		4.2	
CV%	8.5		8.0		6.2	
s_R	9.3		9.2		9.2	
CV%	13.5		13.4		13.6	
h straggler	0		0		0	
h outlier	0		0		0	
k straggler	3		2		0	
k outlier	1		0		0	
95 % Upper limit	86.5		86.1		86.0	
95 % Lower limit	50.2		50.2		49.8	

1 outlier has been detected (Lab 10 as k outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a mean length of the longest root of 68 mm, a repeatability of 8.0 % and a reproducibility of 13.4 %.


SOS control
Control length longest root mm
140
120
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code
95 %
Upper limit
Overall
mean
95 %
Lower limit
max
min
Average


SOS length longest root control
h value
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code



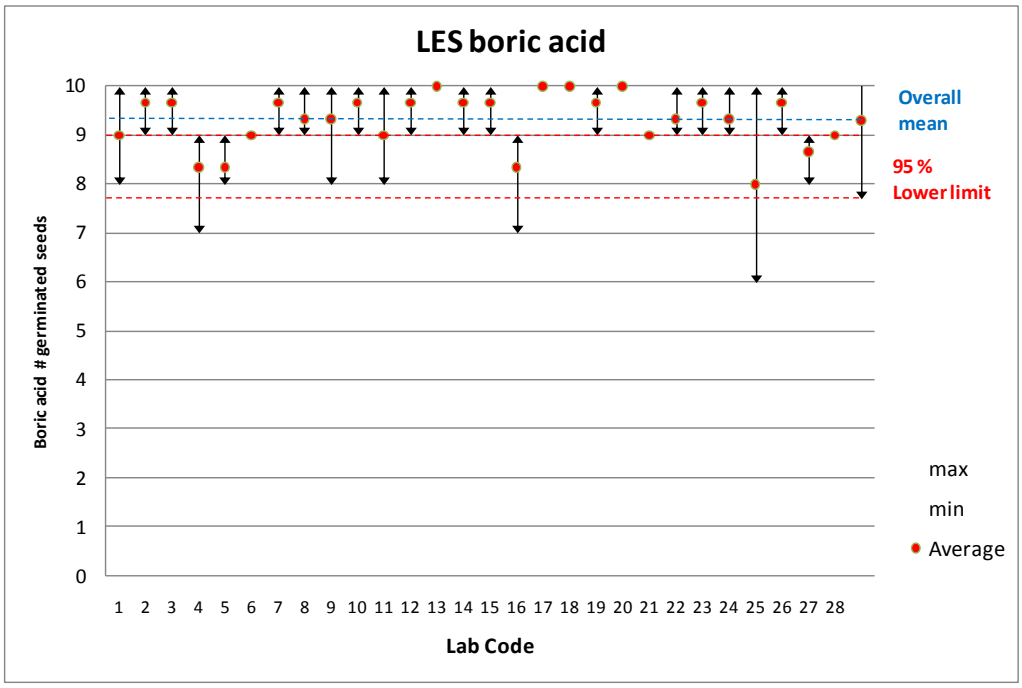
SOS length longest root control						
	All data		**Without k and h outliers**		**Without k and h stragglers**	
N labs	28		27		22	
N repeats	3		3		3	
Mean	78.1		78.6		85.6	
s_r	10.1		9.3		9.1	
CV%	12.9		11.9		10.6	
s_R	19.7		19.6		13.1	
CV%	25.3		25.0		15.3	
h straggler	1		1		0	
h outlier	0		0		0	
k straggler	1		0		0	
k outlier	1		0		0	
95 % Upper limit	116.8		117.1		111.2	
95 % Lower limit	39.4		40.0		60.0	

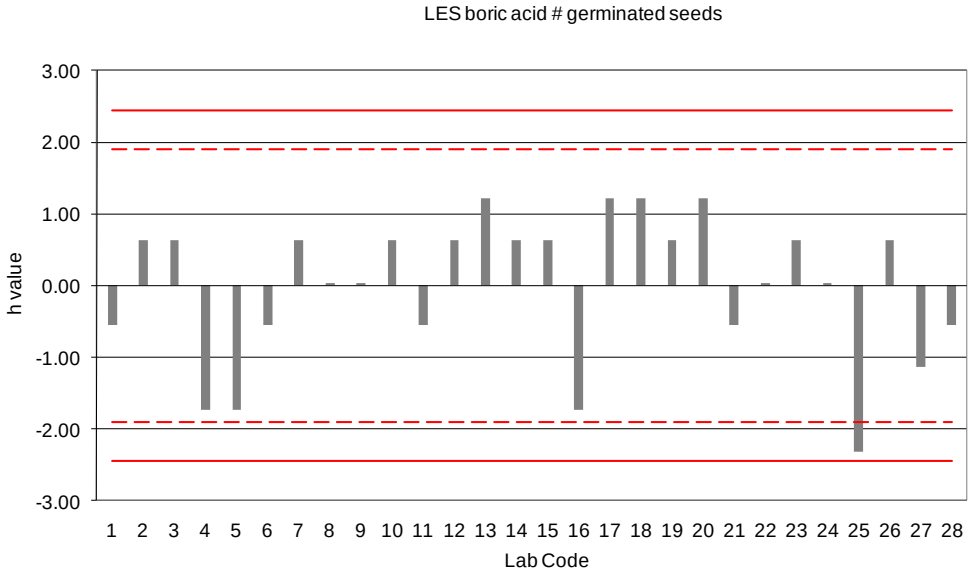
1 result has been discarded (Lab 23 as k outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a mean length of the longest root of 78 mm, a repeatability of 11.9 % and a reproducibility of 25.0 %.

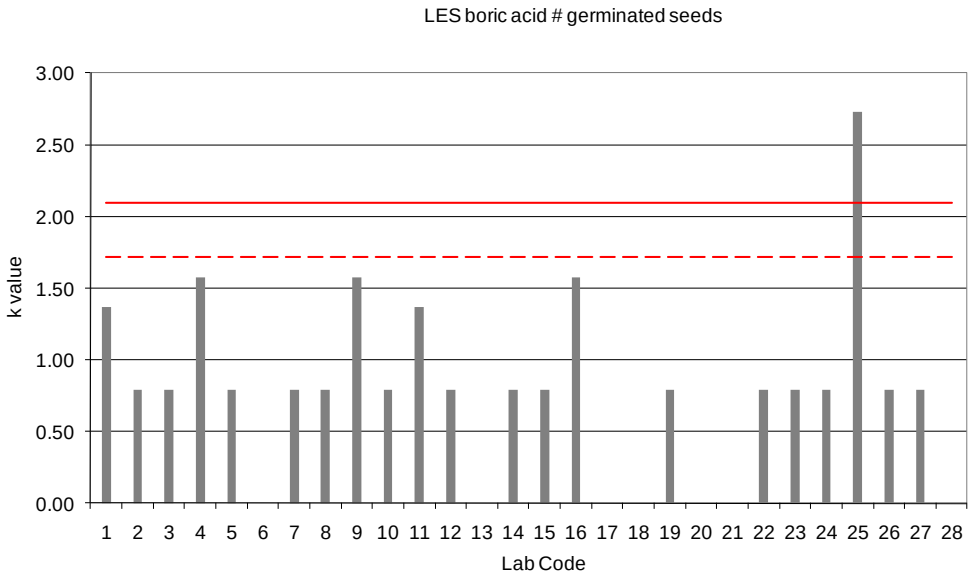
Boric acid

germinated seeds

Lepidium sativum (LES)

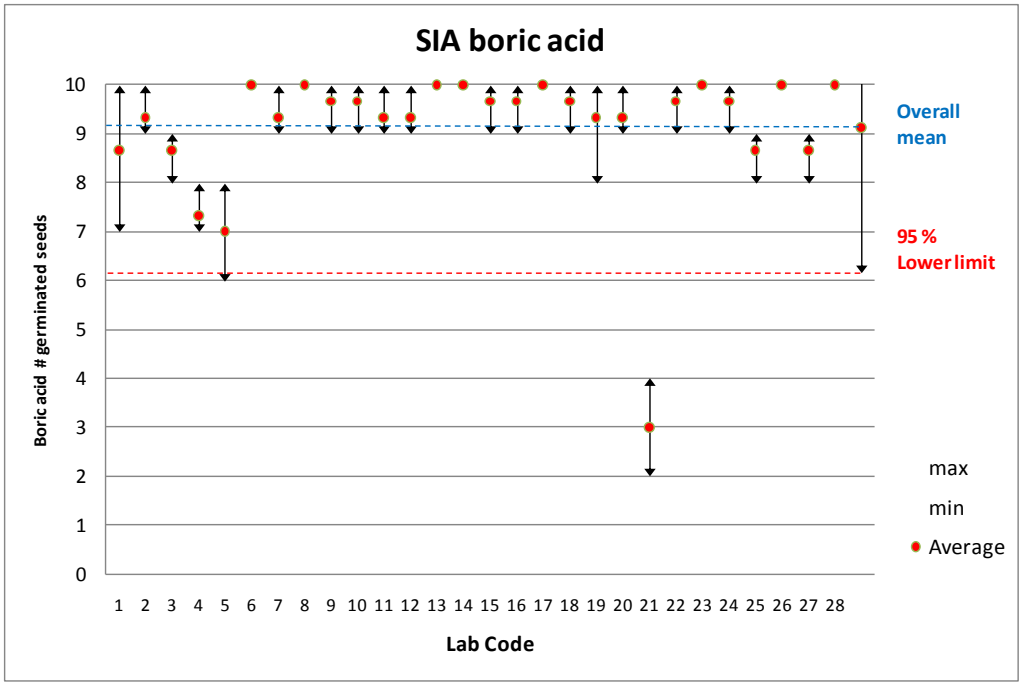




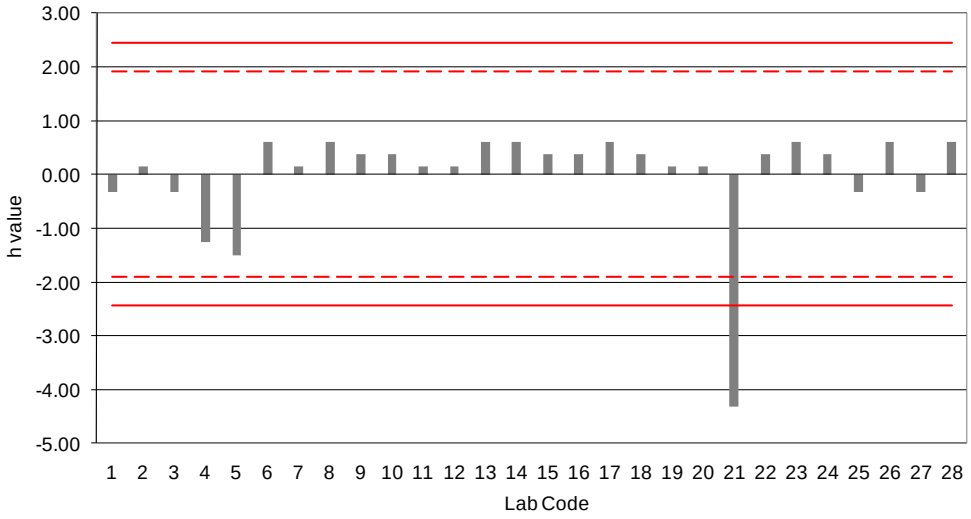


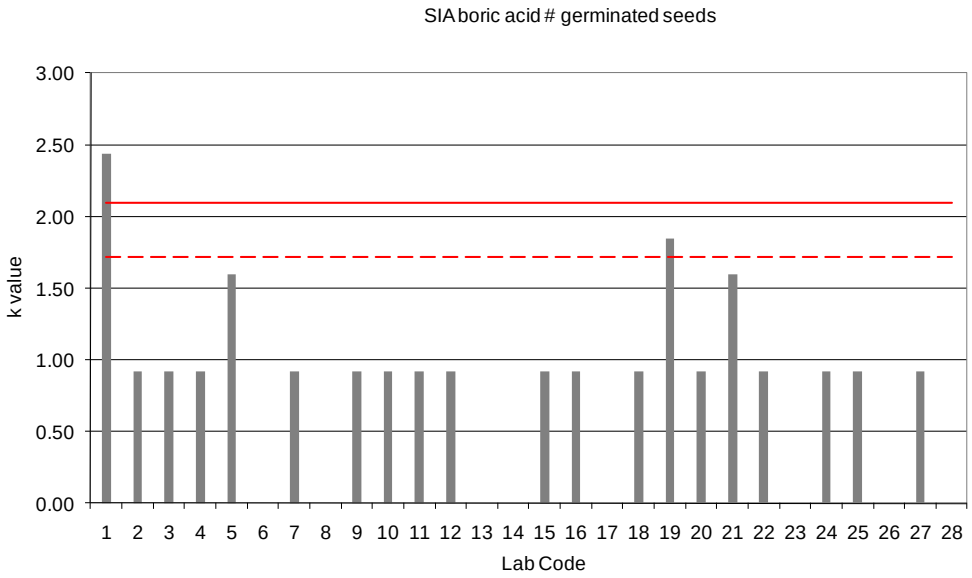
LES boric acid # germinated seeds						
	All data		**Without k and h outliers**		**Without k and h stragglers**	
N labs	28		27		20	
N repeats	3		3		3	
Mean	9.3		9.4		9.6	
s_r	0.7		0.6		0.5	
CV%	7.9		6.8		4.9	
s_R	0.8		0.7		0.5	
CV%	8.8		7.8		5.2	
h straggler	1		3		0	
h outlier	0		0		0	
k straggler	1		3		0	
k outlier	1		0		0	
95 % Upper limit	10.9		10.8		10.6	
95 % Lower limit	7.7		7.9		8.6	

1 result has been discarded (Lab 25 as k outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a mean of 9 germinated seeds, a repeatability of 6.8 % and a reproducibility of 7.8 %.



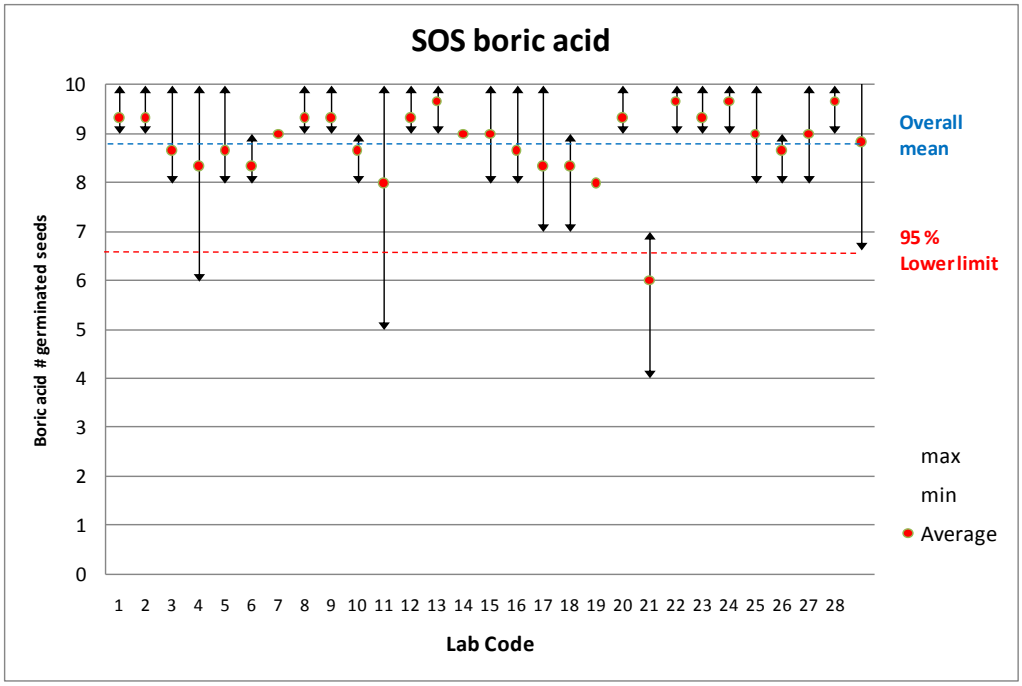
SIA boric acid # germinated seeds

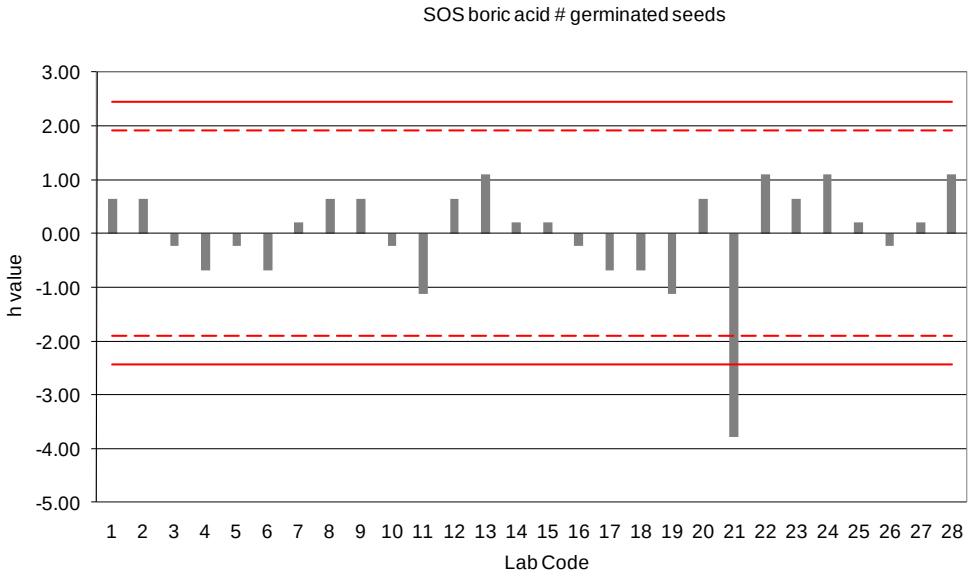


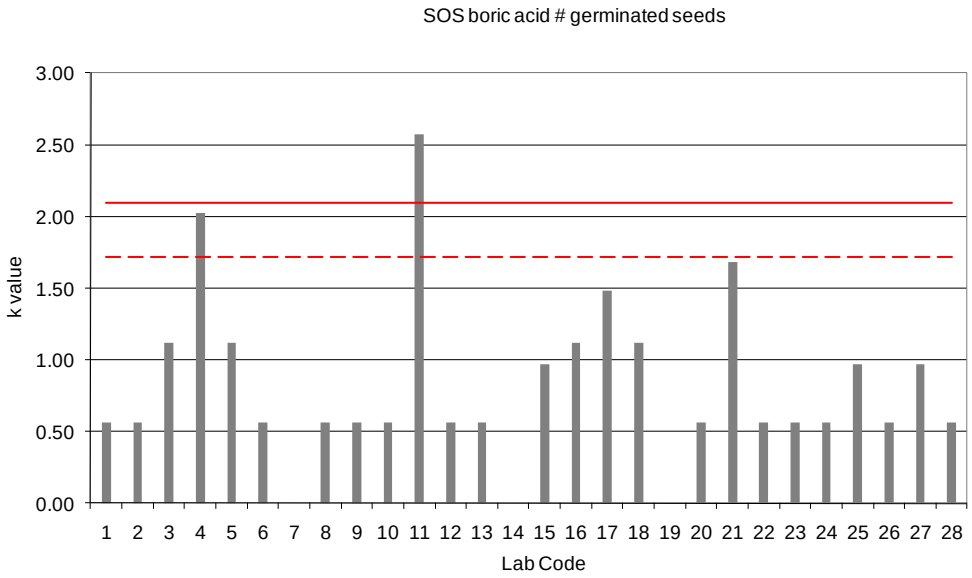


SIA boric acid # germinated seeds						
	All data		**Without k and h outliers**		**Without k and h stragglers**	
N labs	28		23		20	
N repeats	3		3		3	
Mean	9.1		9.6		9.7	
s_r	0.6		0.5		0.4	
CV%	6.9		4.9		4.6	
s_R	1.5		0.6		0.5	
CV%	16.5		6.1		4.7	
h straggler	1		3		0	
h outlier	1		0		0	
k straggler	2		0		0	
k outlier	1		0		0	
95 % Upper limit	12.1		10.7		10.6	
95 % Lower limit	6.2		8.4		8.8	

5 results have been discarded (Labs 21, 4, and 5 as h outliers; labs 1 and 19 as k outliers). The accepted results, based on 23 laboratories (out of the original 28 participating labs), have a mean of 9 germinated seeds, a repeatability of 4.9 % and a reproducibility of 6.1 %.


SOS boric acid
Boric acid # germinated seeds
10
9
8
7
6
5
4
3
2
1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code
Overall mean
95 % Lower limit
max
min
Average

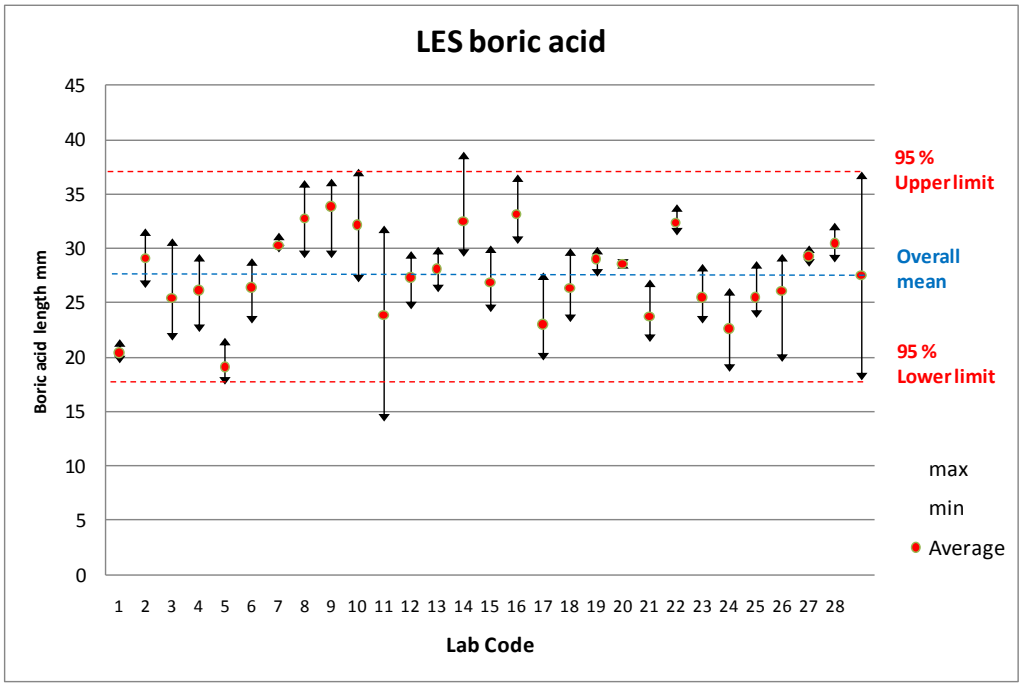

SOS boric acid # germinated seeds
h value
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code



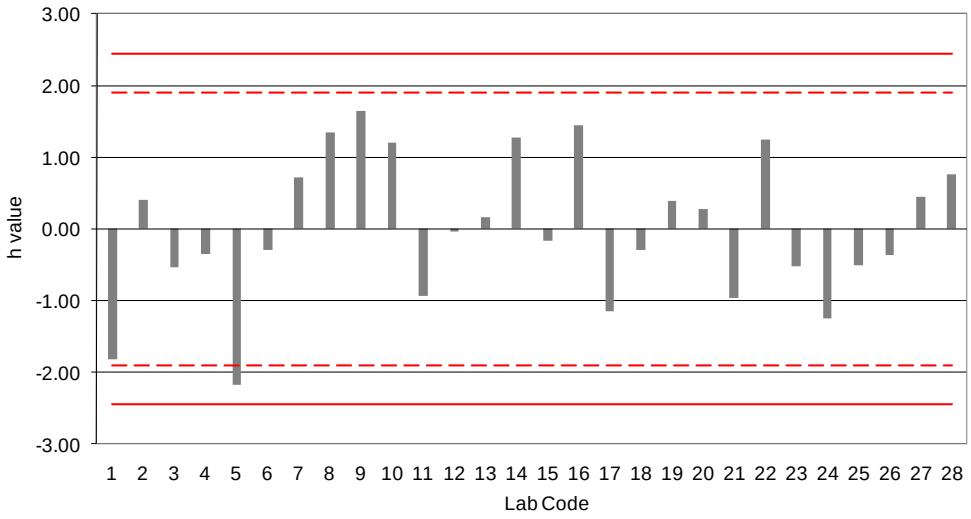
SOS boric acid # germinated seeds						
	All data		**Without k and h outliers**		**Without k and h stragglers**	
N labs	28		25		23	
N repeats	3		3		3	
Mean	8.8		9.0		9.1	
s_r	1.0		0.8		0.8	
CV%	11.6		8.7		8.3	
s_R	1.1		0.8		0.8	
CV%	12.7		8.8		8.3	
h straggler	1		1		0	
h outlier	1		0		0	
k straggler	2		1		0	
k outlier	1		0		0	
95 % Upper limit	11.1		10.6		10.6	
95 % Lower limit	6.6		7.5		7.6	

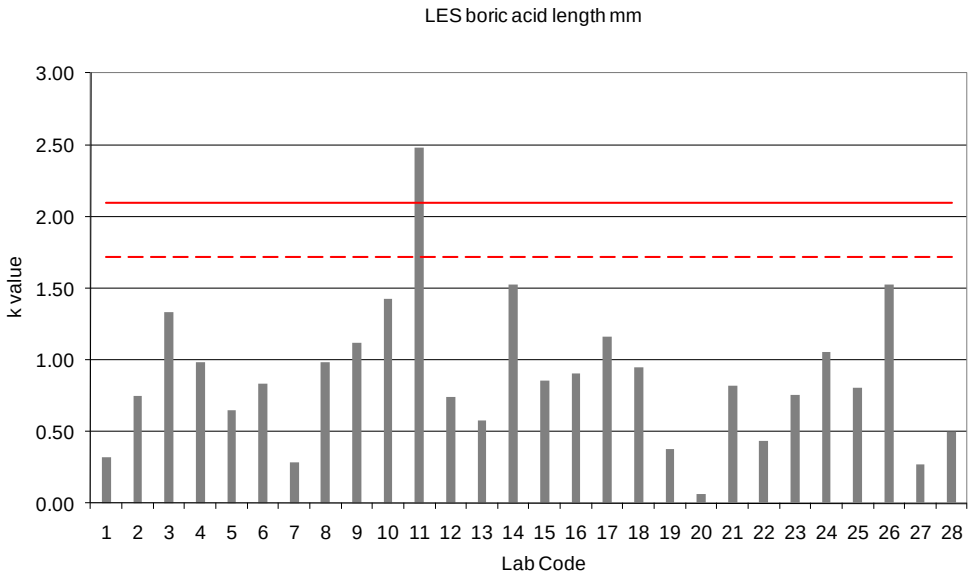
3 results have been discarded (Lab 21 as h outlier, and labs 4 and 11 as k outliers). The accepted results, based on 25 laboratories (out of the original 28 participating labs), have a mean of 9 germinated seeds, a repeatability of 8.7 % and a reproducibility of 8.8 %.

Root length



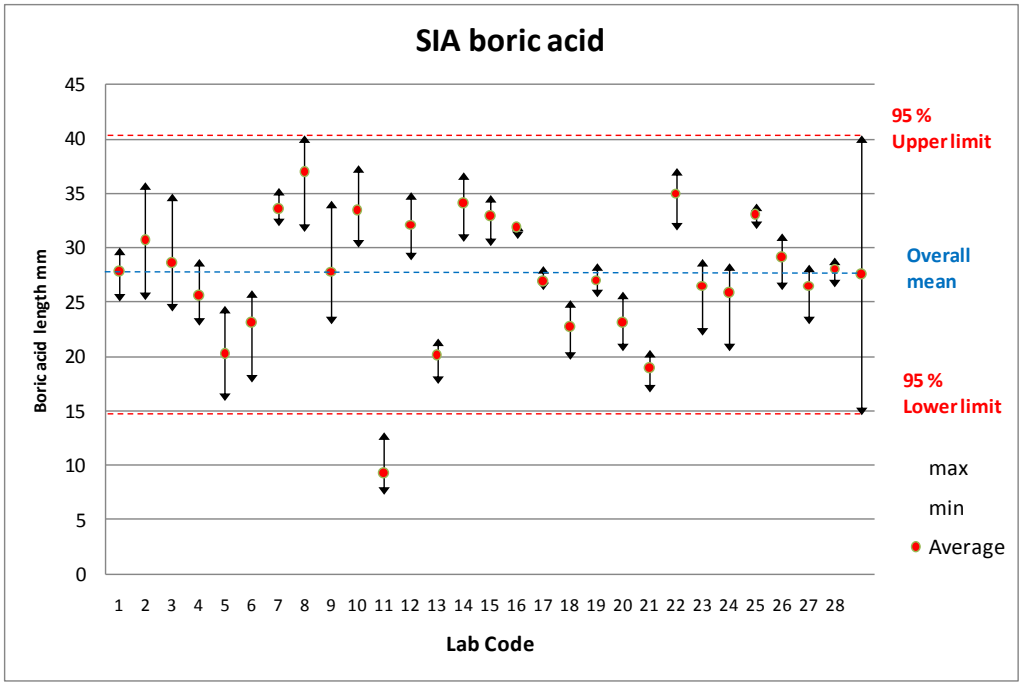
LES boric acid length mm



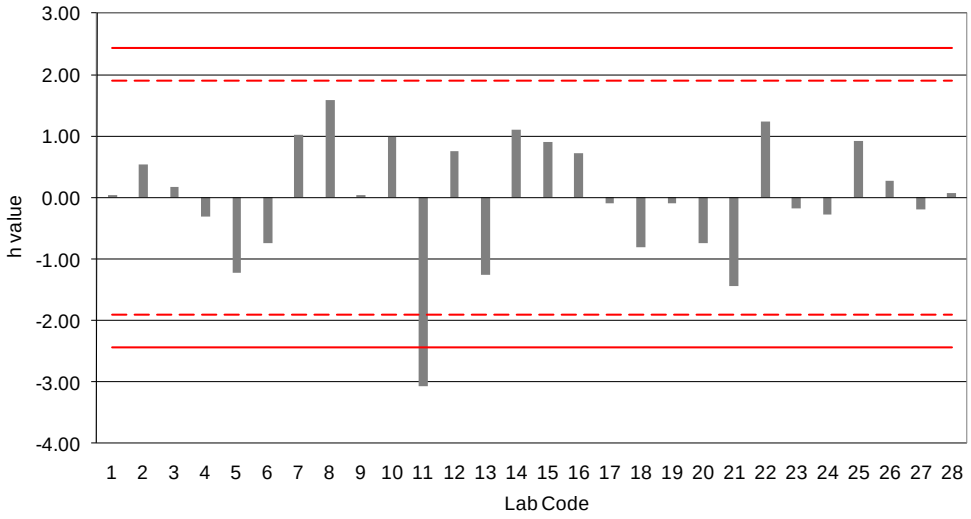


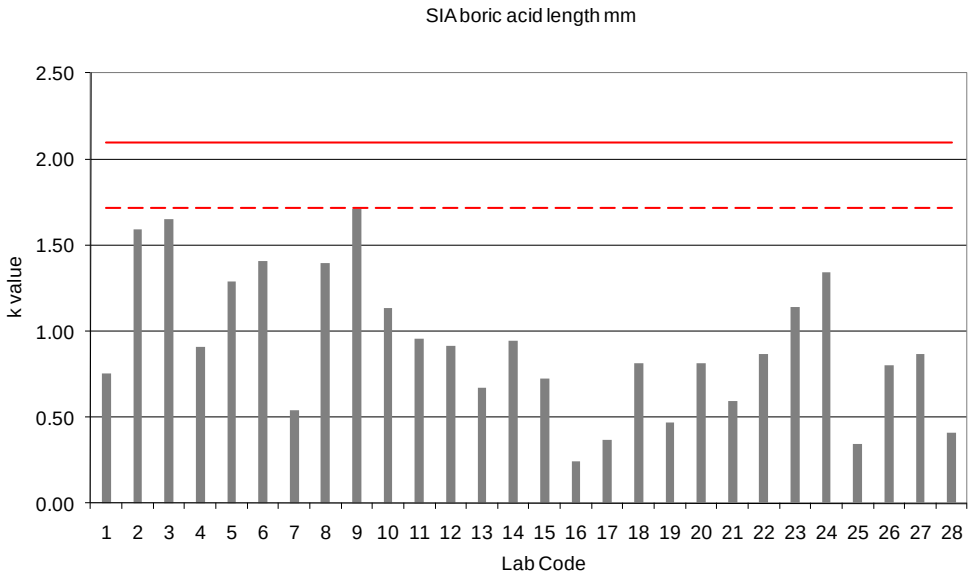
LES boric acid length mm								
	All data		**Without k and h outliers**			**Without k and h stragglers**		
N labs	28		27			25		
N repeats	3		3			3		
Mean	27.5		27.7			28.3		
s_r	3.7		3.3			3.4		
CV%	13.3		11.9			12.0		
s_R	4.9		4.7			4.3		
CV%	17.8		17.1			15.2		
h straggler	1		1			0		
h outlier	0		0			0		
k straggler	1		0			0		
k outlier	1		0			0		
95 % Upper limit	37.1		36.9			36.7		
95 % Lower limit	17.9		18.4			19.9		

1 result has been discarded (Lab 11 as k outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a mean root length of 27 mm, a repeatability of 11.9 % and a reproducibility of 17.1 %.



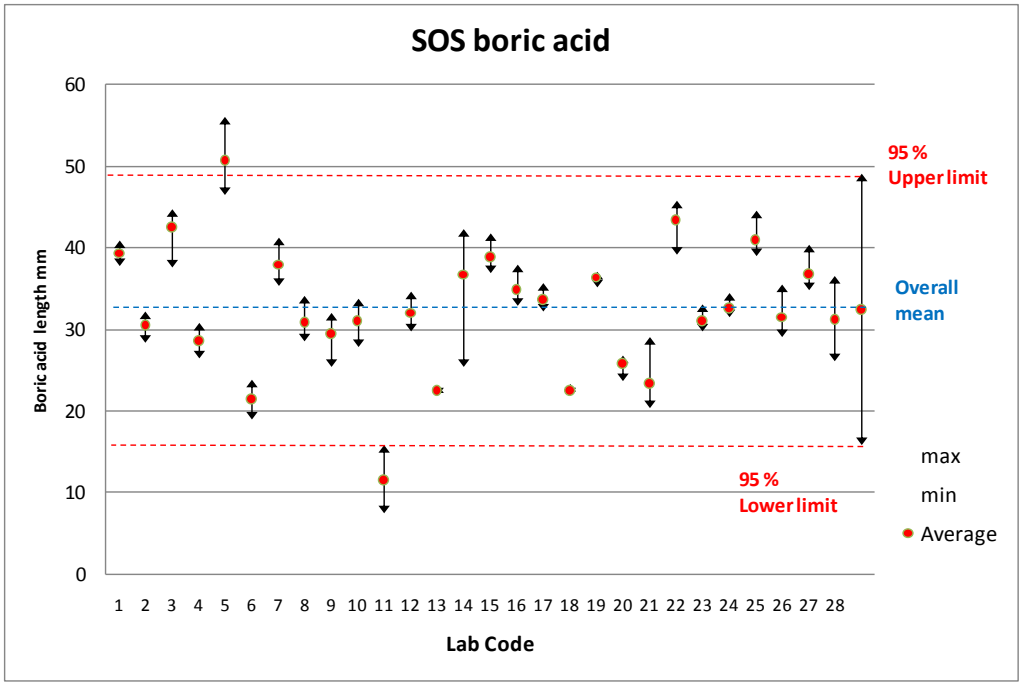
SIA boric acid length mm



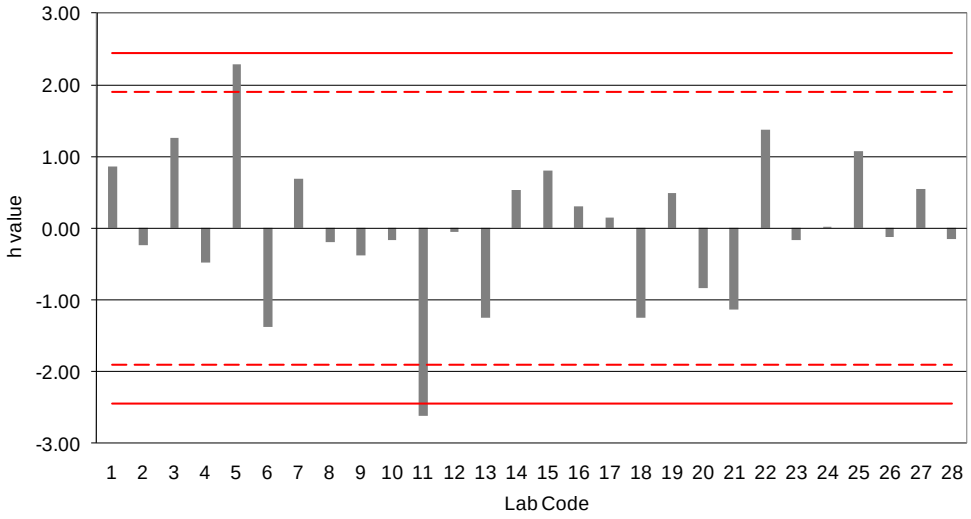


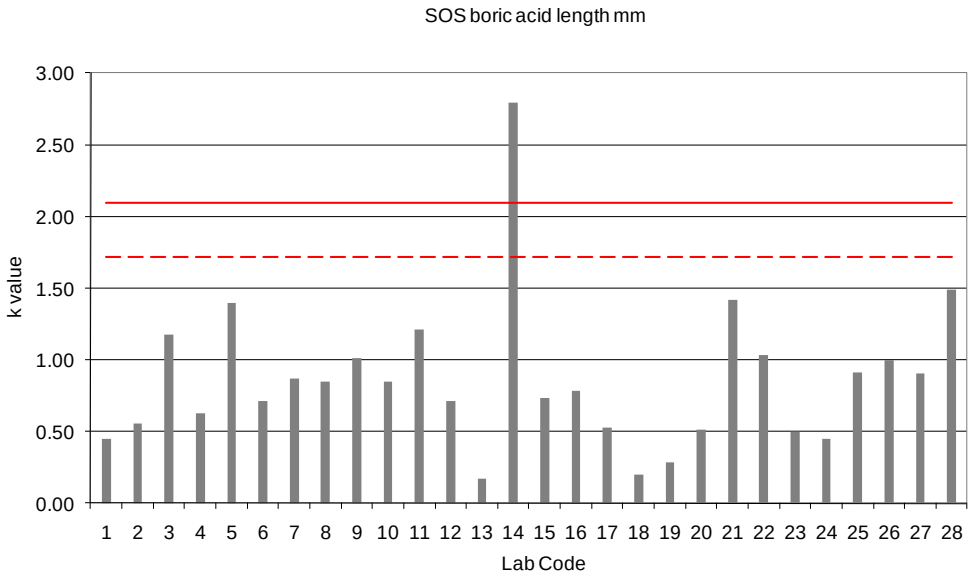
SIA boric acid length mm								
	All data		**Without k and h outliers**			**Without k and h stragglers**		
N labs	28		27			26		
N repeats	3		3			3		
Mean	27.6		28.2			28.6		
s_r	3.4		3.4			3.5		
CV%	12.4		12.1			12.1		
s_R	6.6		5.6			5.4		
CV%	23.9		19.8			18.8		
h straggler	1		1			0		
h outlier	1		0			0		
k straggler	0		0			0		
k outlier	0		0			0		
95 % Upper limit	40.5		39.2			39.2		
95 % Lower limit	14.7		17.3			18.1		

1 result has been discarded (Lab 11 as h outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a mean root length of 28 mm, a repeatability of 12.1 % and a reproducibility of 19.8 %.



SOS boric acid length mm



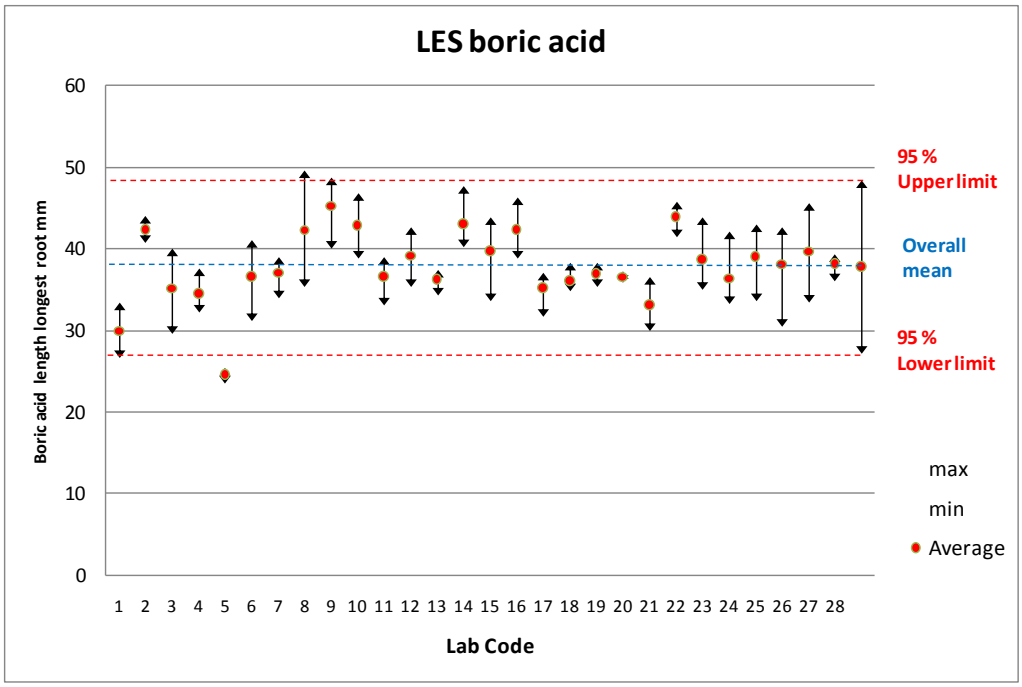


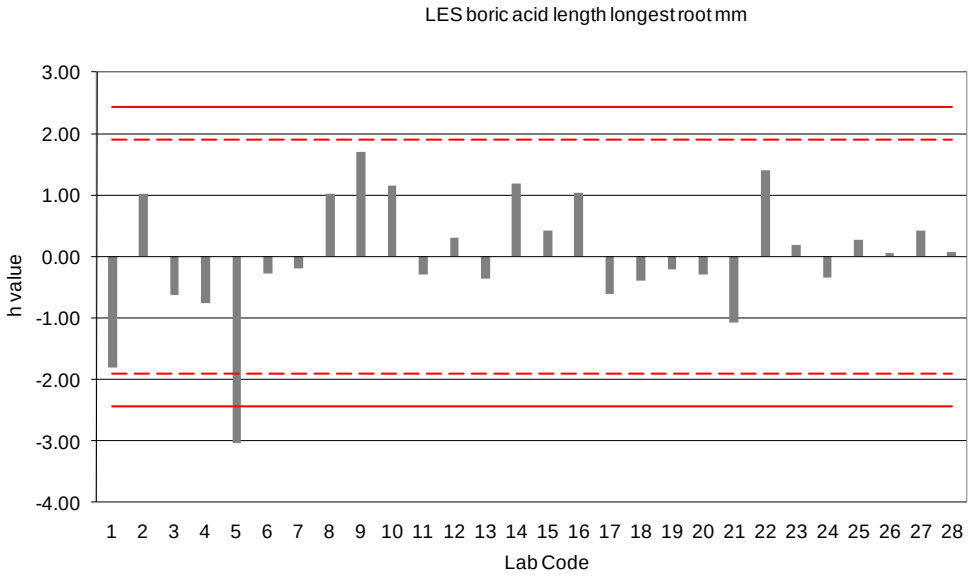
SOS boric acid length mm						
	All data	**Without k and h outliers**		**Without k and h stragglers**		
N labs	28	25		23		
N repeats	3	3		3		
Mean	32.5	32.4		32.8		
s_r	3.5	2.9		2.6		
CV%	10.8	8.8		7.9		
s_R	8.5	6.7		6.6		
CV%	26.2	20.6		20.0		
h straggler	2	0		0		
h outlier	1	0		0		
k straggler	1	2		0		
k outlier	1	0		0		
95 % Upper limit	49.1	45.5		45.7		
95 % Lower limit	15.8	19.3		20.0		

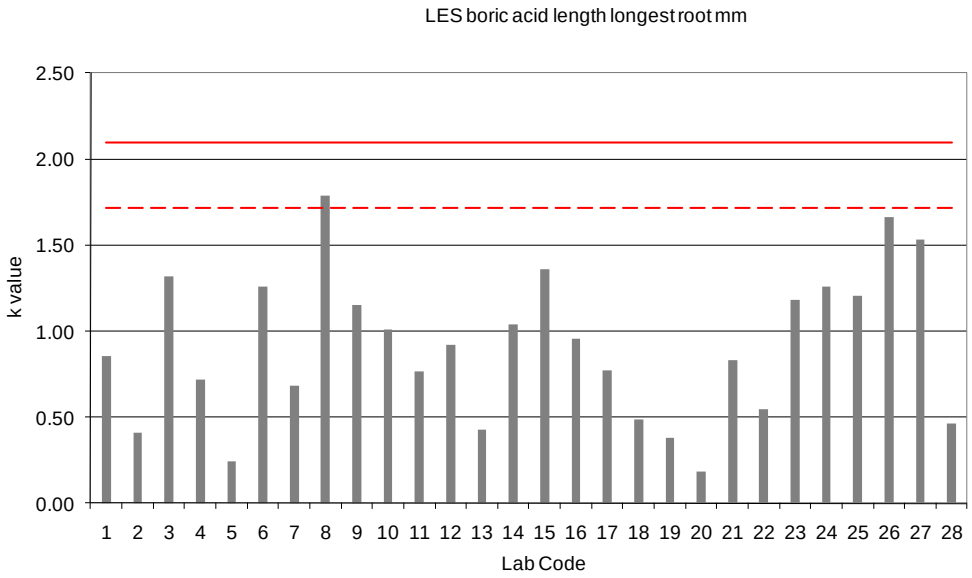
3 results have been discarded (Labs 5 and 11 as h outliers, lab 14 as k outlier). The accepted results, based on 25 laboratories (out of the original 28 participating labs), have a mean root length of 32 mm, a repeatability of 8.8 % and a reproducibility of 20.6 %.

Longest root length

Lepidium sativum (LES)

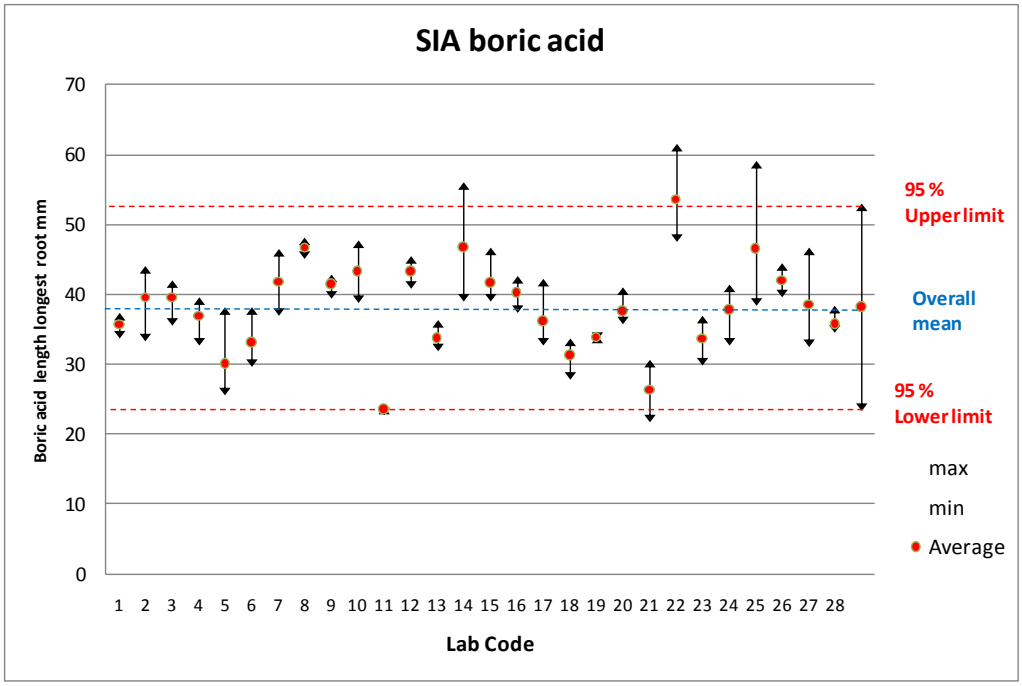


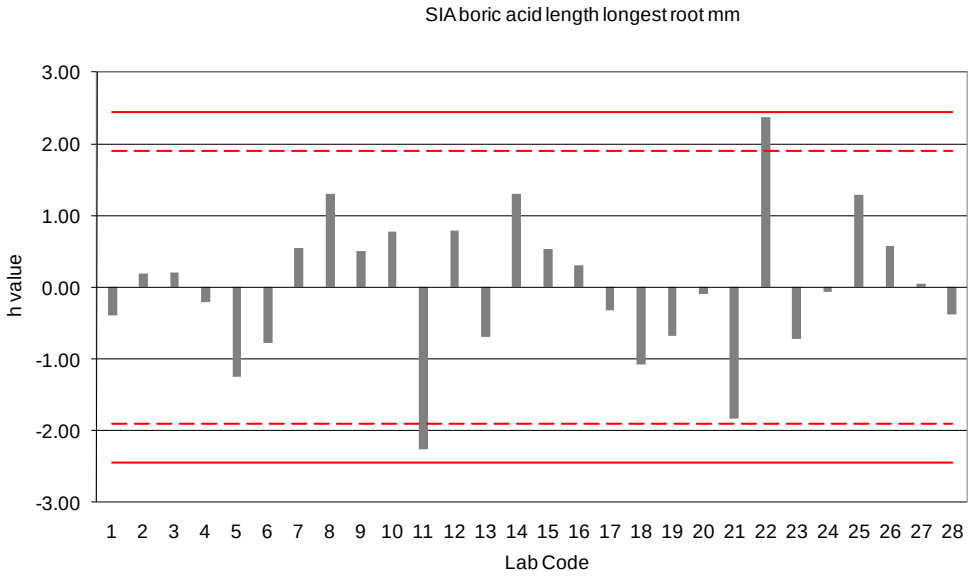




LES boric acid length longest root mm						
	All data		**Without k and h outliers**		**Without k and h stragglers**	
N labs	28		27		23	
N repeats	3		3		3	
Mean	37.9		38.3		38.0	
s_r	4.0		4.1		4.0	
CV%	10.6		10.6		10.4	
s_R	5.5		4.9		4.2	
CV%	14.4		12.8		11.1	
h straggler	1		2		0	
h outlier	1		0		0	
k straggler	1		1		0	
k outlier	0		0		0	
95 % Upper limit	48.6		47.9		46.3	
95 % Lower limit	27.2		28.8		29.7	

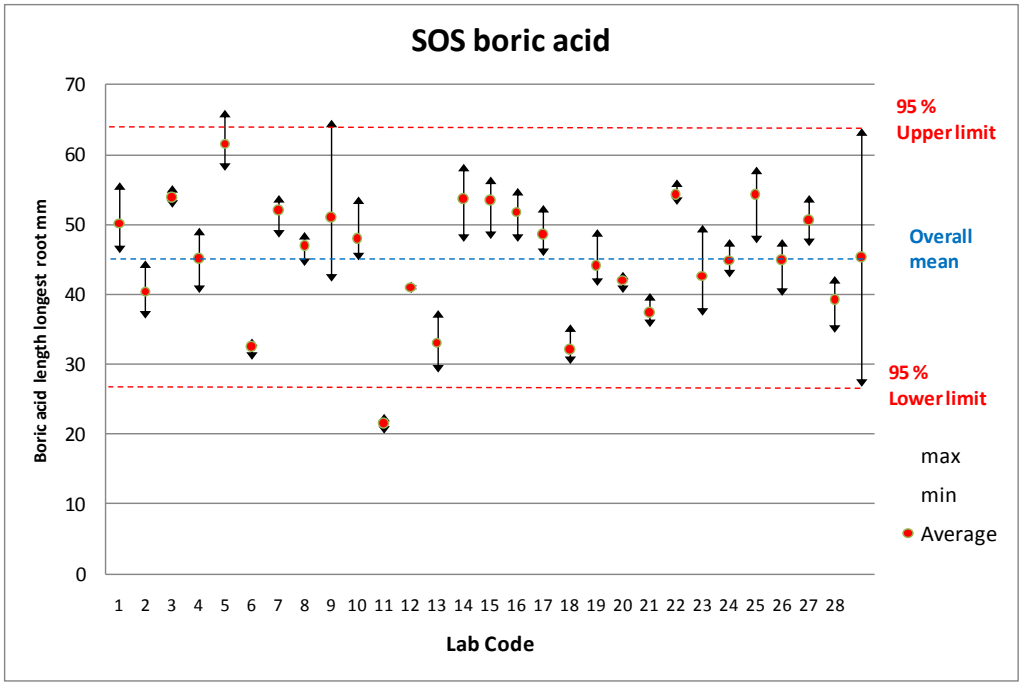
1 result has been discarded (Lab 5 as h outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a mean length of the longest root of 38 mm, a repeatability of 10.6 % and a reproducibility of 12.8 %.

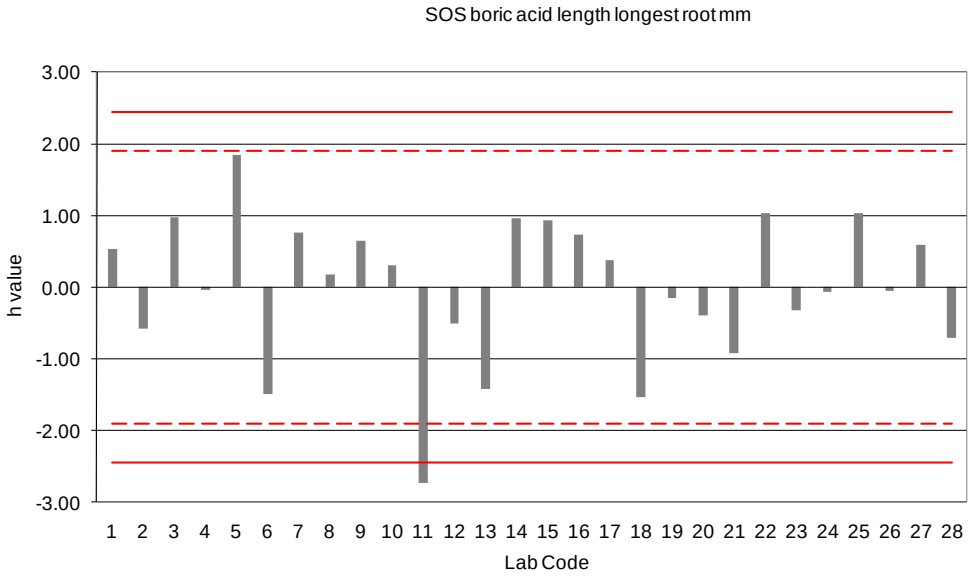

SIA boric acid
Boric acid length longest root mm
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code
95 % Upper limit
Overall mean
95 % Lower limit
max
min
Average

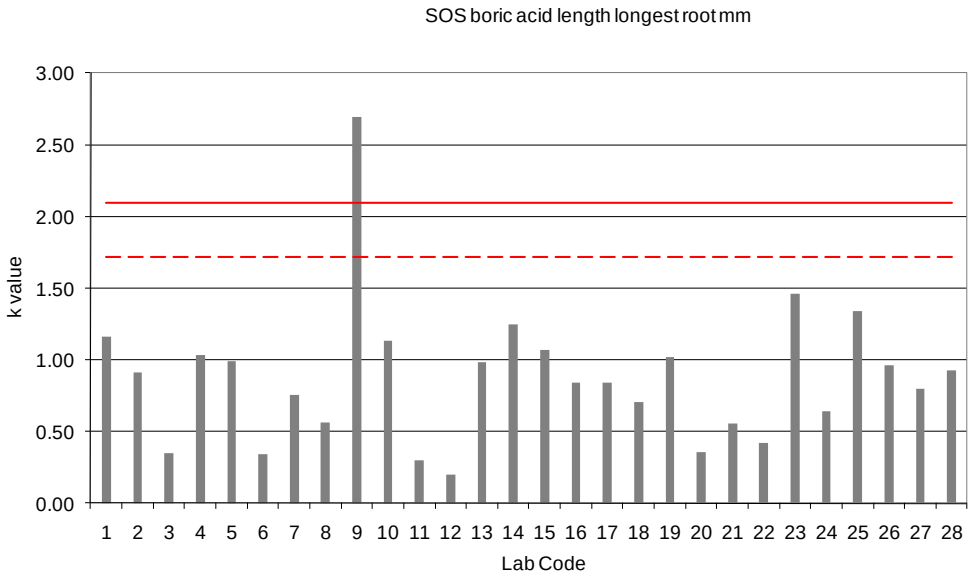

SIA boric acid length longest root mm
h value
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code

SIA boric acid length longest root mm						
	All data		**Without k and h outliers**		**Without k and h stragglers**	
N labs	28		26		20	
N repeats	3		3		3	
Mean	38.3		37.4		38.0	
s_r	4.7		4.2		3.6	
CV%	12.4		11.2		9.4	
s_R	7.5		6.6		4.7	
CV%	19.7		17.7		12.4	
h straggler	2		2		0	
h outlier	0		0		0	
k straggler	2		2		0	
k outlier	1		0		0	
95 % Upper limit	53.1		50.4		47.2	
95 % Lower limit	23.5		24.4		28.8	

2 results have been discarded (Lab 22 as h outlier and lab 25 as k outlier). The accepted results, based on 26 laboratories (out of the original 28 participating labs), have a mean length of the longest root of 37 mm, a repeatability of 11.2 % and a reproducibility of 17.7 %.


SOS boric acid
Boric acid length longest root mm
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code
95 % Upper limit
Overall mean
95 % Lower limit
max
min
Average


SOS boric acid length longest root mm
h value
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code



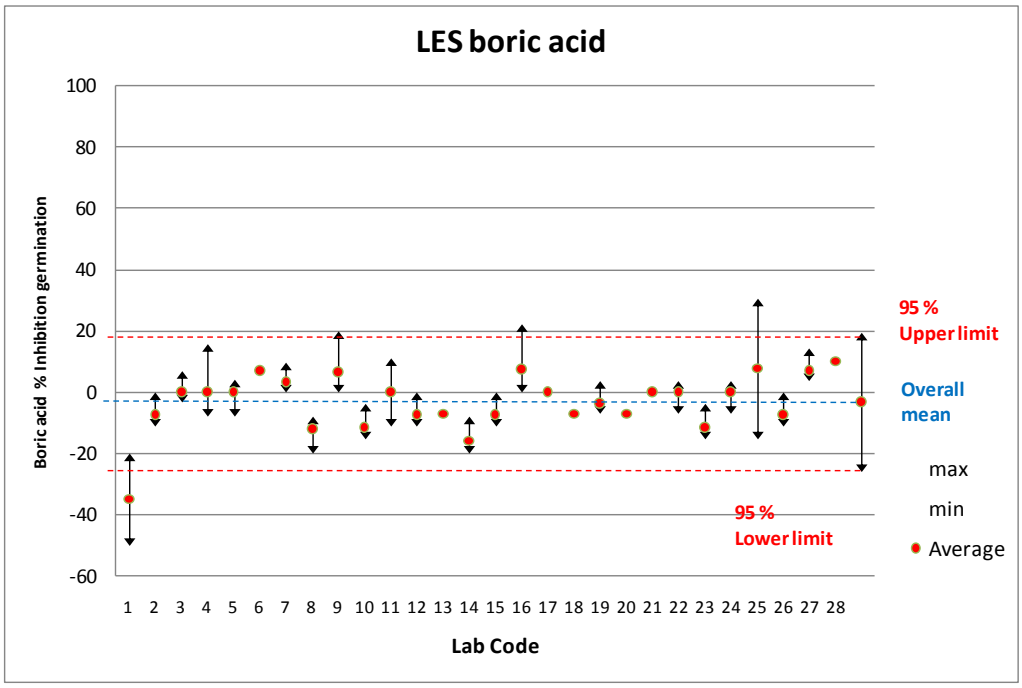
SOS boric acid length longest root mm						
	All data		**Without k and h outliers**		**Without k and h stragglers**	
N labs	28		26		22	
N repeats	3		3		3	
Mean	45.4		46.1		47.3	
s_r	4.6		4.1		4.1	
CV%	10.0		8.8		8.7	
s_R	9.5		8.3		6.4	
CV%	20.8		17.9		13.5	
h straggler	1		1		0	
h outlier	1		0		0	
k straggler	1		0		0	
k outlier	1		0		0	
95 % Upper limit	64.0		62.3		59.8	
95 % Lower limit	26.9		29.9		34.8	

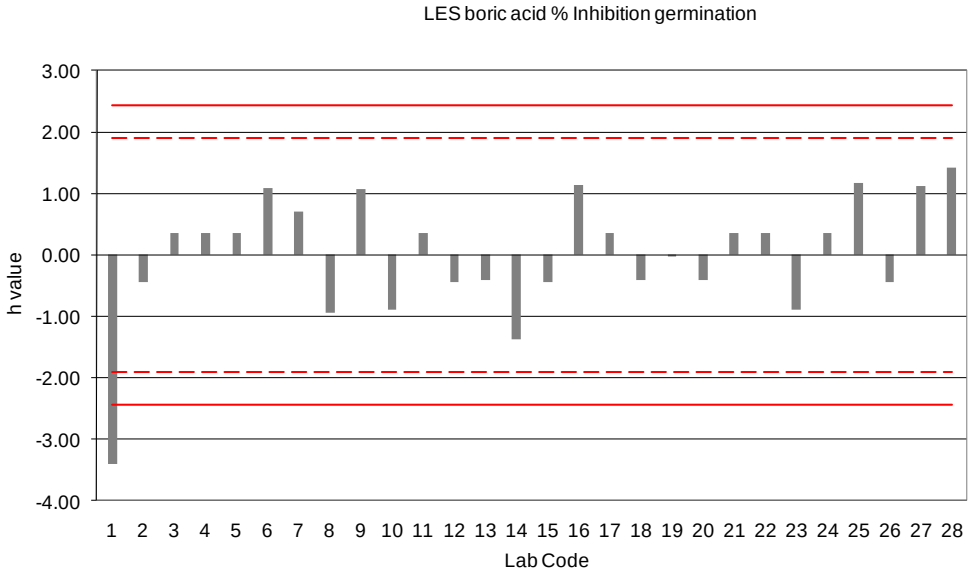
2 results have been discarded (Lab 11 as h outlier, lab 9 as k outlier). The accepted results, based on 26 laboratories (out of the original 28 participating labs), have a mean length of the longest root of 46 mm, a repeatability of 8.8 % and a reproducibility of 17.9 %.

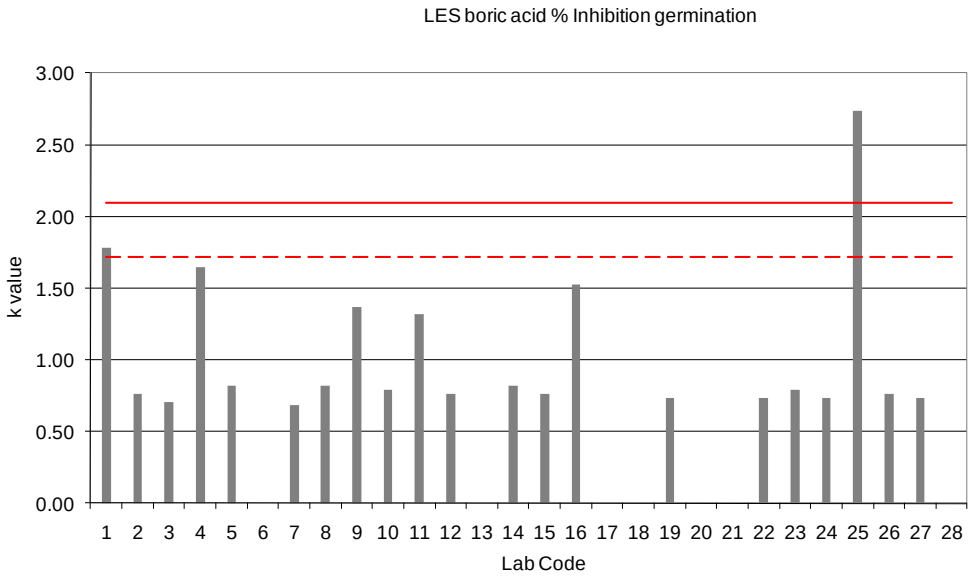
Inhibition calculations

% inhibition seed germination

Lepidium sativum (LES)

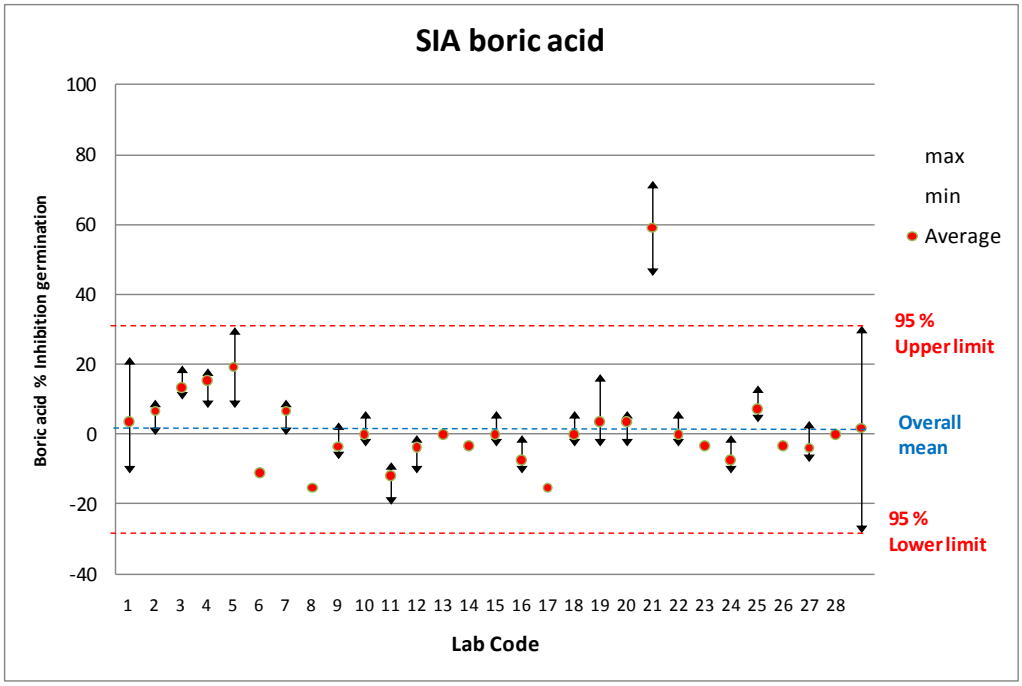


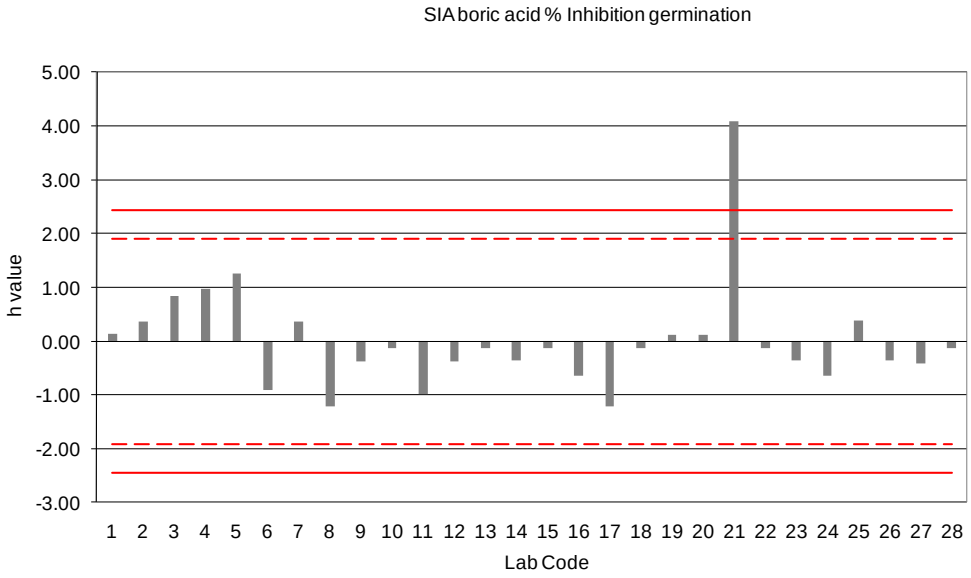


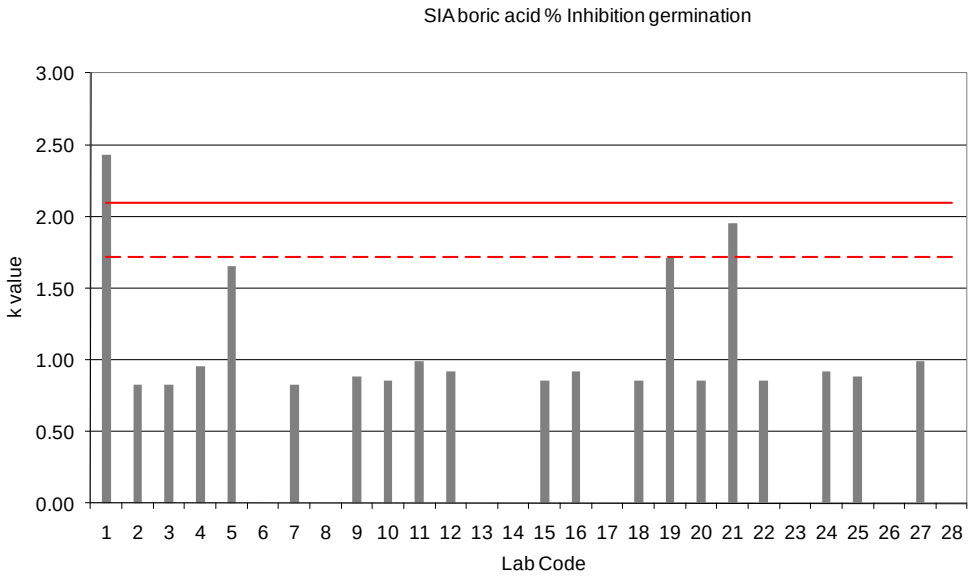


LES boric acid % Inhibition germination			
	All data	**Without k and h outliers**	**Without k and h stragglers**
N labs	28	26	21
N repeats	3	3	3
Mean	-3.3	-2.5	-4.2
s_r	8.4	6.9	5.4
CV%	-257.8	-278.6	-129.1
s_R	11.6	8.9	7.7
CV%	-354.3	-361.2	-182.8
h straggler	1	1	0
h outlier	1	0	0
k straggler	2	2	0
k outlier	1	0	0
95 % Upper limit	19.4	15.0	10.9
95 % Lower limit	-26.0	-20.0	-19.3

2 results have been discarded (Lab 1 as h outlier, lab 25 as k outlier). The accepted results, based on 26 laboratories (out of the original 28 participating labs), have a negative percentage of seed germination inhibition (meaning that seed germination is higher with boric acid than in the negative control), and very poor repeatability and reproducibility.

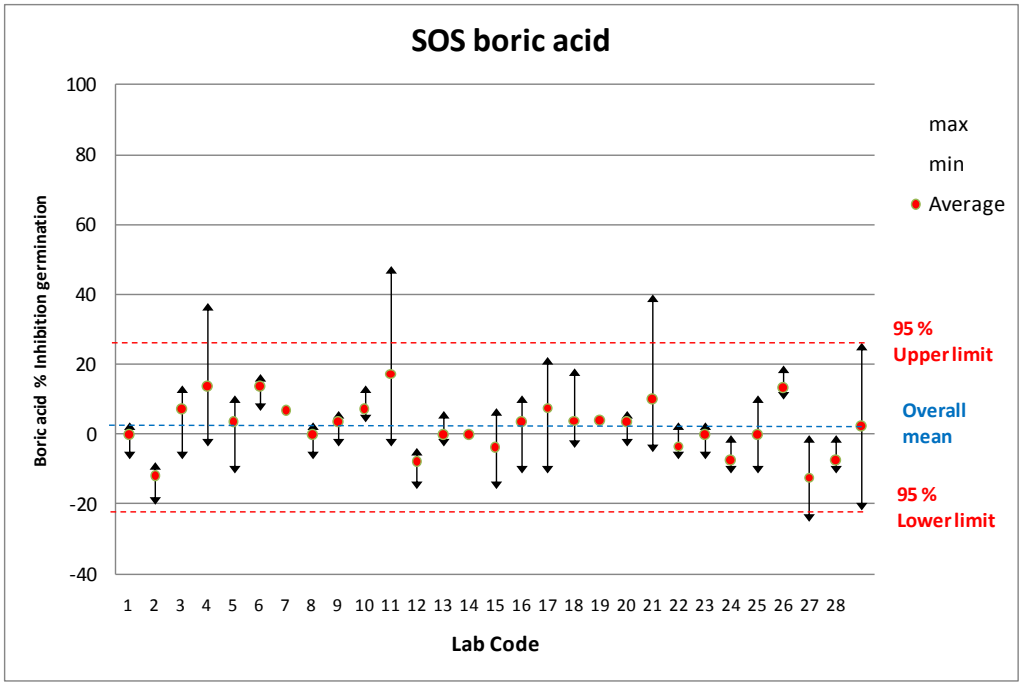

SIA boric acid
Boric acid % Inhibition germination
100
80
60
40
20
0
-20
-40
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code
max
min
Average
95 %
Upper limit
Overall
mean
95 %
Lower limit

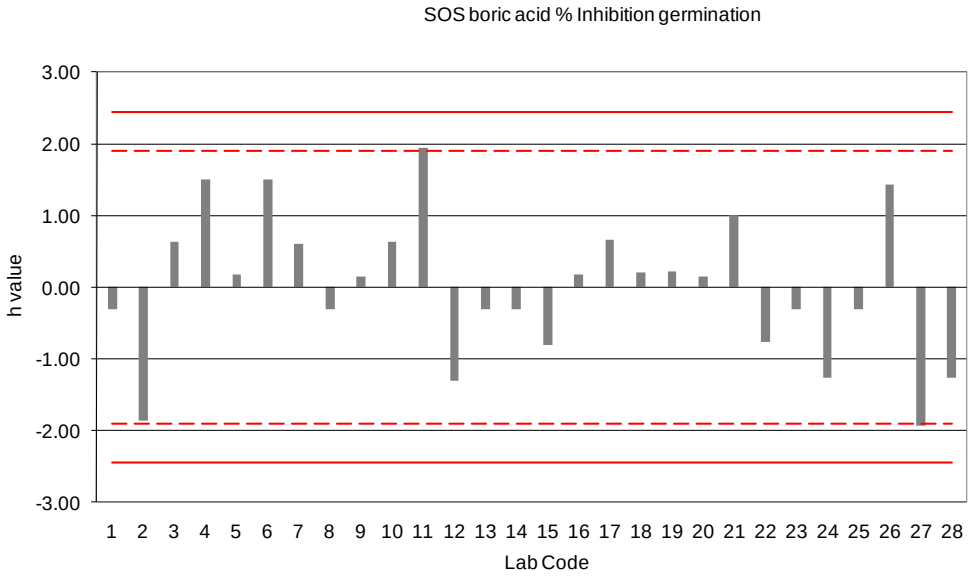

SIA boric acid % Inhibition germination
h value
5.00
4.00
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code

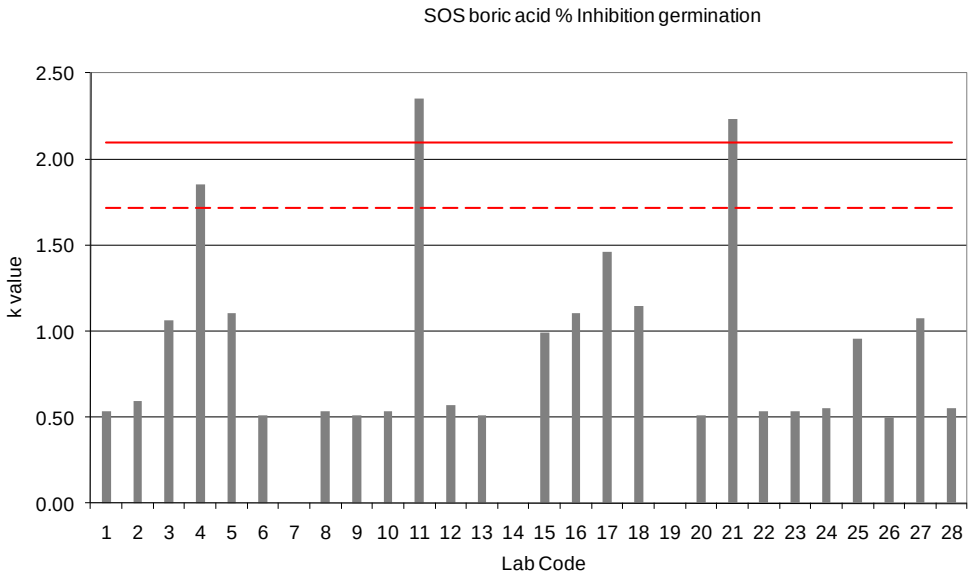


SIA boric acid % Inhibition germination			
	All data	**Without k and h outliers**	**Without k and h stragglers**
N labs	28	26	18
N repeats	3	3	3
Mean	1.7	-0.6	-0.7
s_r	7.0	5.9	5.2
CV%	409.5	-1017.1	-753.0
s_R	15.1	9.9	6.1
CV%	886.2	-1713.6	-883.8
h straggler	1	1	0
h outlier	1	0	0
k straggler	2	2	0
k outlier	1	0	0
95 % Upper limit	31.4	18.8	11.3
95 % Lower limit	-27.9	-19.9	-12.7

2 results have been discarded (Lab 21 as h outlier, lab 1 as k outlier). The accepted results, based on 26 laboratories (out of the original 28 participating labs), have a negative percentage of seed germination inhibition (meaning that seed germination is higher with boric acid than in the negative control), and very poor repeatability and reproducibility.


SOS boric acid
Boric acid % Inhibition germination
100
80
60
40
20
0
-20
-40
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code
max
min
Average
95 %
Upper limit
Overall
mean
95 %
Lower limit


SOS boric acid % Inhibition germination
h value
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code

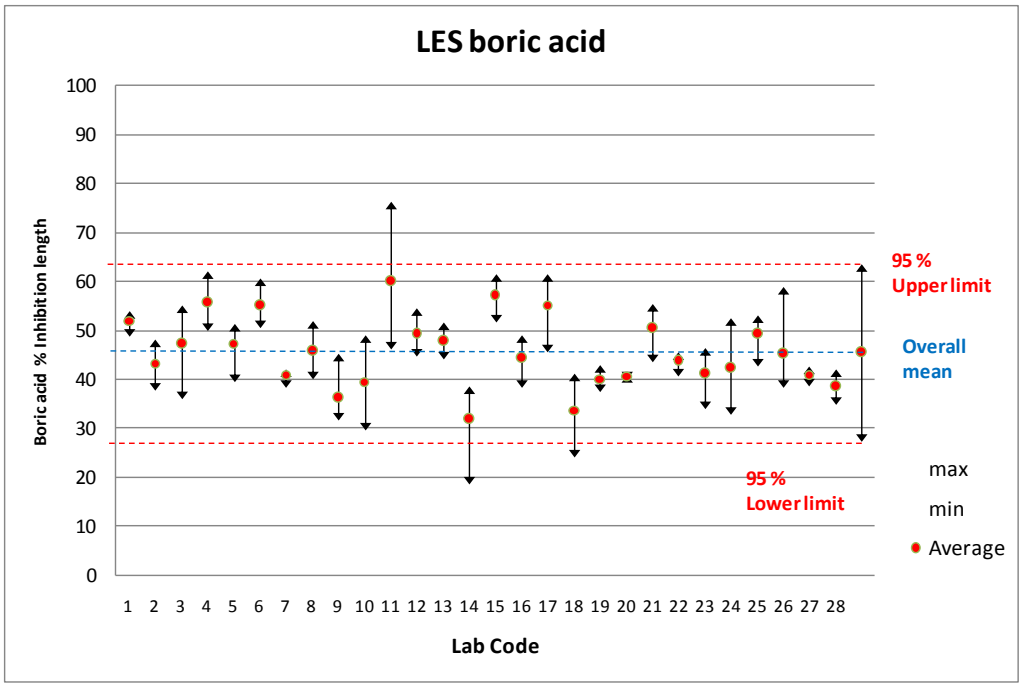


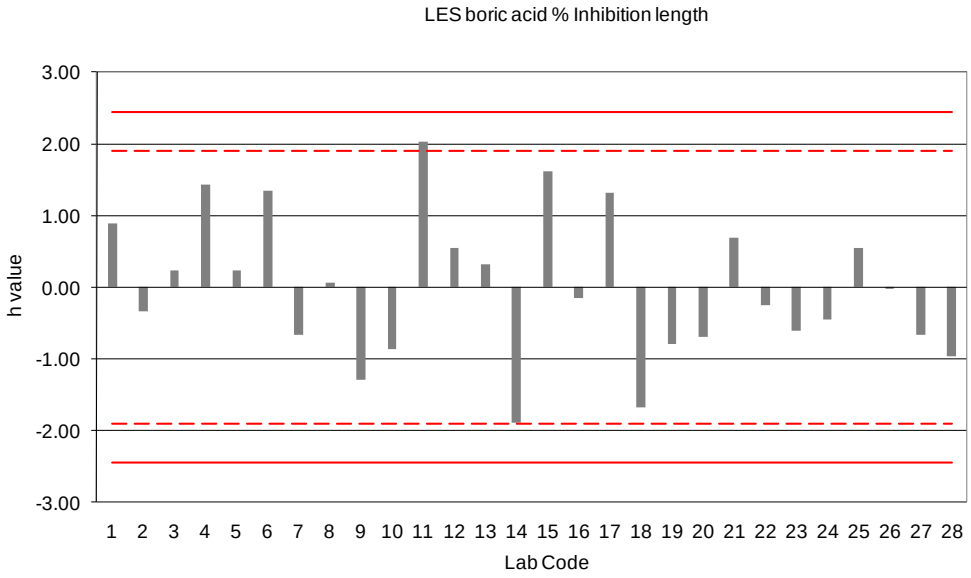
SOS boric acid % Inhibition germination						
	All data		**Without k and h outliers**		**Without k and h stragglers**	
N labs	28		25		22	
N repeats	3		3		3	
Mean	2.3		-1.7		-1.0	
s_r	11.6		8.8		7.7	
CV%	505.3		-523.9		-773.8	
s_R	12.2		14.5		12.9	
CV%	530.4		-859.2		-1297.8	
h straggler	2		1		0	
h outlier	0		0		0	
k straggler	3		1		0	
k outlier	2		0		0	
95 % Upper limit	26.2		26.7		24.3	
95 % Lower limit	-21.6		-30.0		-26.3	

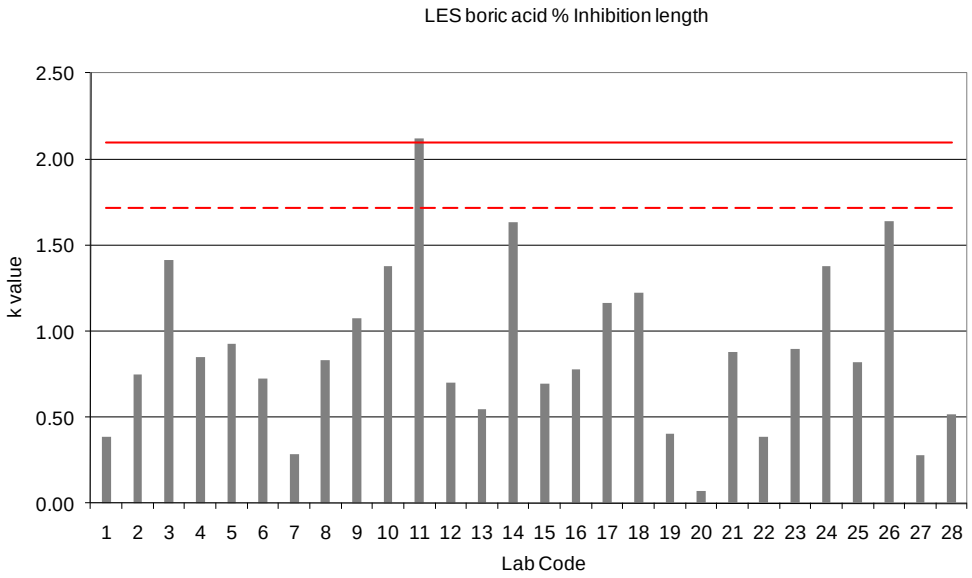
3 results have been discarded (Labs 4, 11, 21 as k outliers). The accepted results, based on 25 laboratories (out of the original 28 participating labs), have a negative percentage of seed germination inhibition (meaning that seed germination is higher with boric acid than in the negative control), and very poor repeatability and reproducibility.

% inhibition mean root length

Lepidium sativum (LES)

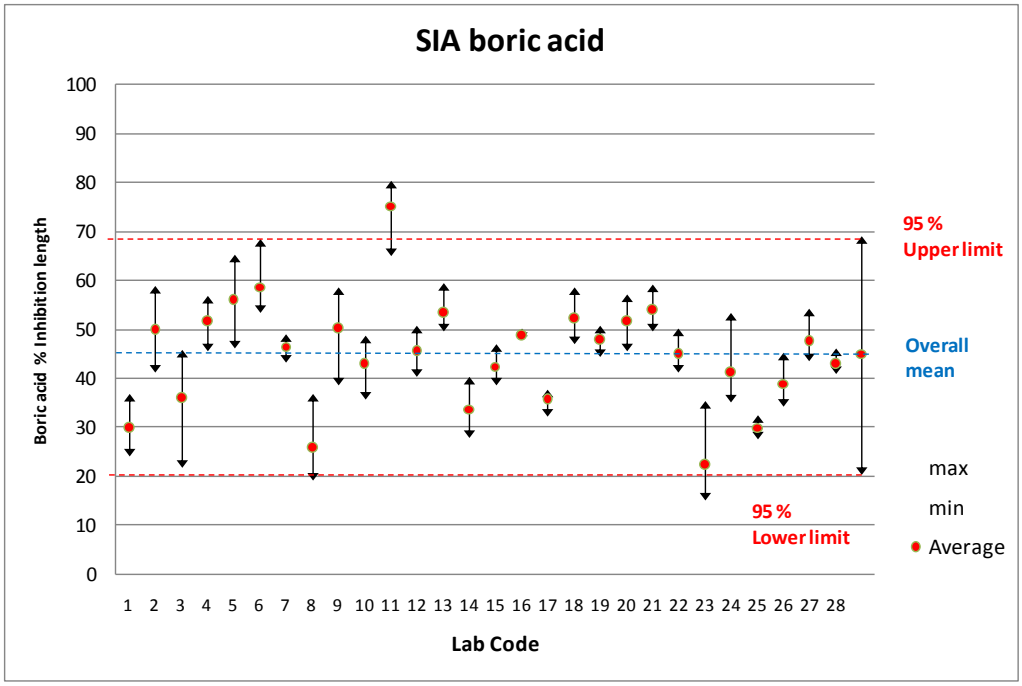


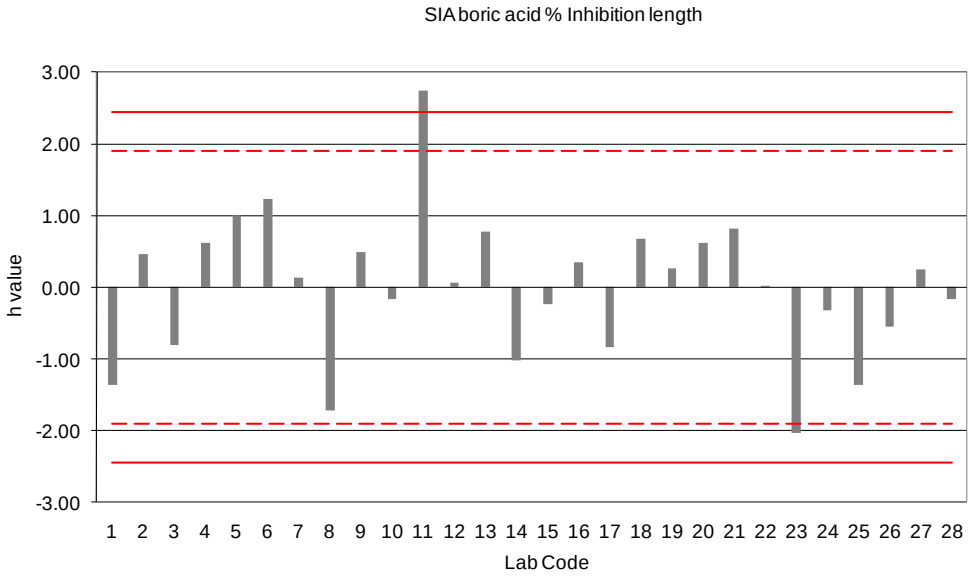


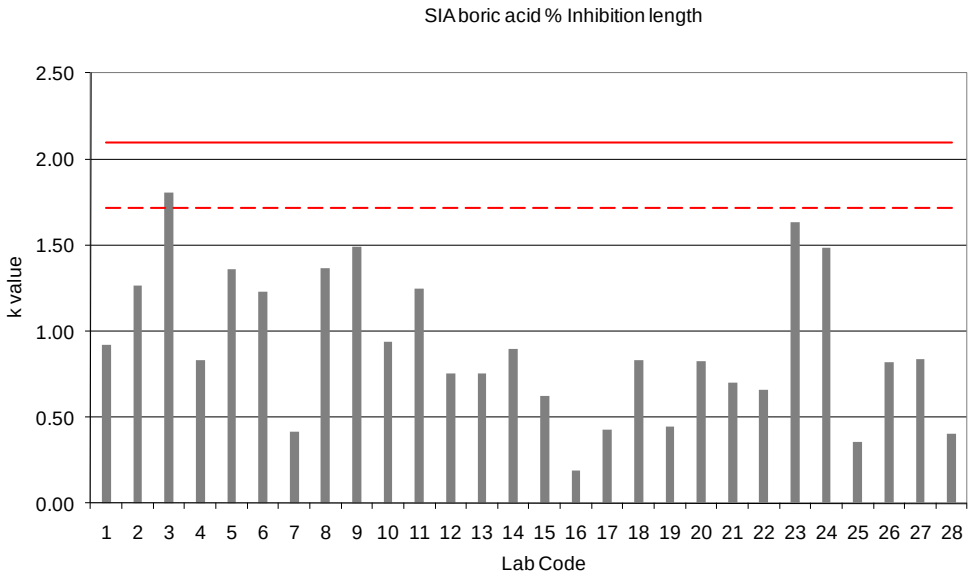


LES boric acid % Inhibition length			
	All data	**Without k and h outliers**	**Without k and h stragglers**
N labs	28	27	24
N repeats	3	3	3
Mean	45.6	45.0	46.1
s_r	7.1	6.7	6.0
CV%	15.7	14.8	12.9
s_R	9.2	8.6	7.8
CV%	20.3	19.1	16.8
h straggler	1	1	0
h outlier	0	0	0
k straggler	1	2	0
k outlier	1	0	0
95 % Upper limit	63.7	61.9	61.2
95 % Lower limit	27.5	28.1	30.9

1 result has been discarded (Lab 11 as k outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a % inhibition of the mean root length of 45 %, a repeatability of 14.8 % and a reproducibility of 19.1 %.

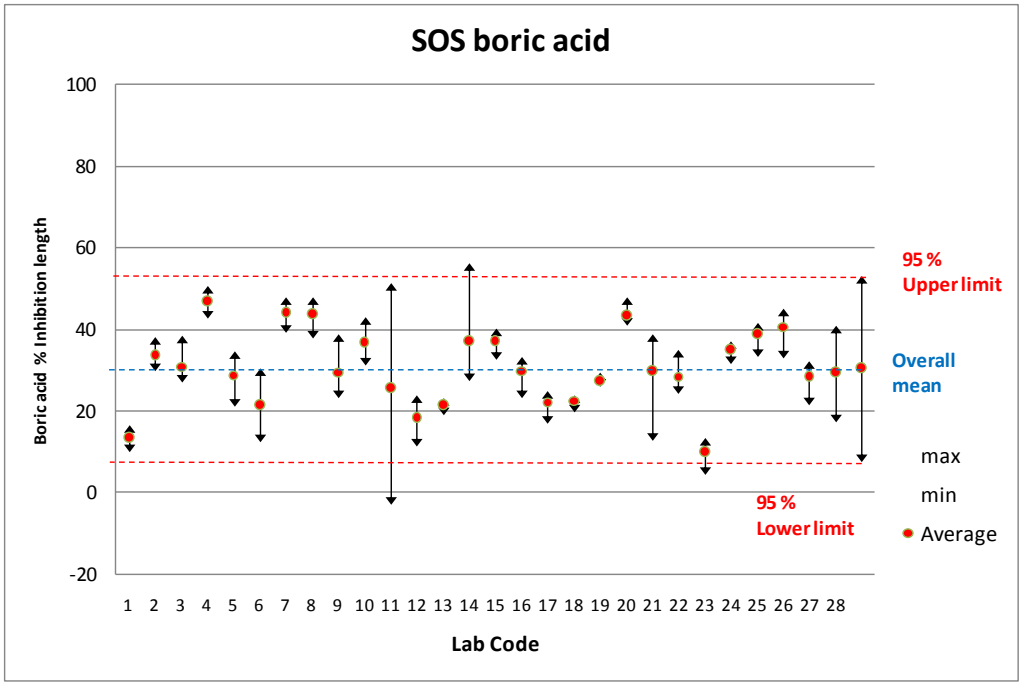

SIA boric acid
Boric acid % Inhibition length
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code
95 %
Upper limit
Overall
mean
95 %
Lower limit
max
min
Average

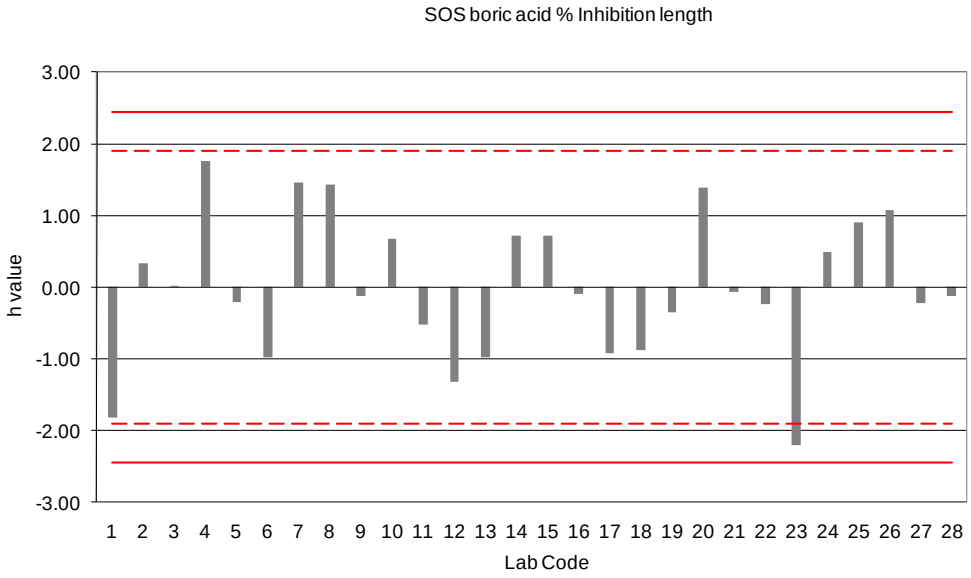

SIA boric acid % Inhibition length
h value
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code

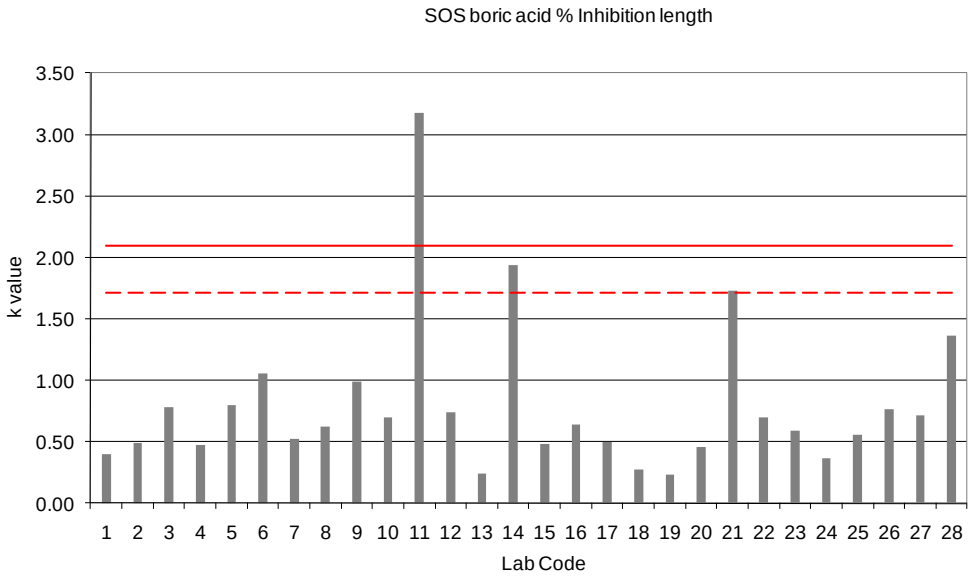


SIA boric acid % Inhibition length						
	All data	**Without k and h outliers**		**Without k and h stragglers**		
N labs	28		27		18	
N repeats	3		3		3	
Mean	44.9		43.8		48.4	
s_r	7.0		6.9		6.3	
CV%	15.6		15.8		13.0	
s_R	12.4		11.1		6.8	
CV%	27.7		25.3		14.0	
h straggler	2		2		0	
h outlier	1		0		0	
k straggler	1		1		0	
k outlier	0		0		0	
95 % Upper limit	69.3		65.5		61.7	
95 % Lower limit	20.5		22.1		35.1	

1 result has been discarded (Lab 11 as h outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a % inhibition of the mean root length of 44 %, a repeatability of 15.8 % and a reproducibility of 25.3 %.


SOS boric acid
Boric acid % Inhibition length
100
80
60
40
20
0
-20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code
95 % Upper limit
Overall mean
95 % Lower limit
max
min
Average


SOS boric acid % Inhibition length
h value
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code

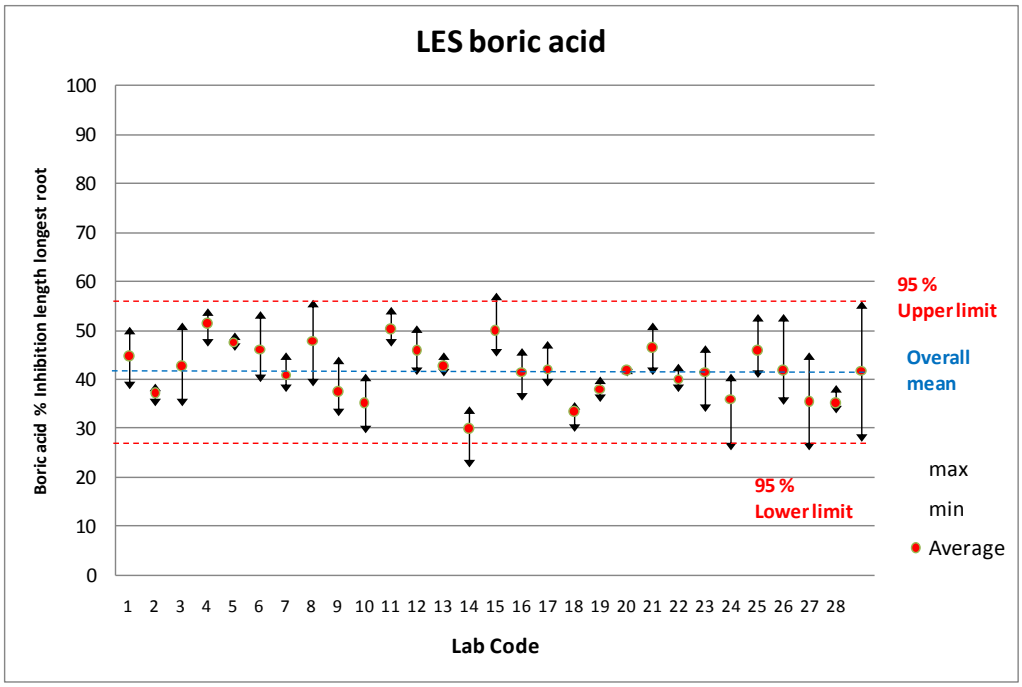


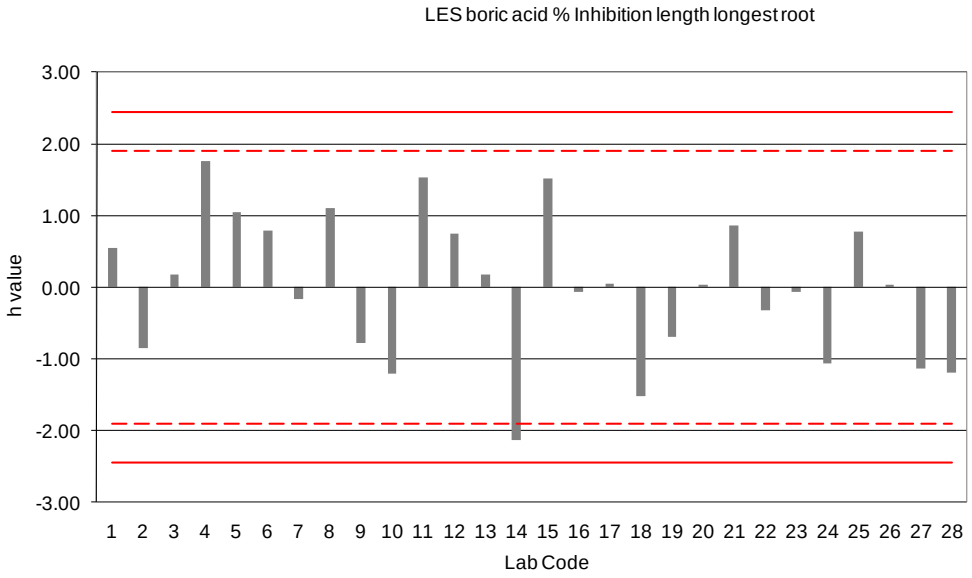
SOS boric acid % Inhibition length			
	All data	**Without k and h outliers**	**Without k and h stragglers**
N labs	28	25	22
N repeats	3	3	3
Mean	30.6	30.5	32.2
s_r	8.6	5.8	5.4
CV%	28.1	18.9	16.9
s_R	11.7	10.8	9.5
CV%	38.1	35.4	29.6
h straggler	1	1	0
h outlier	0	0	0
k straggler	3	1	0
k outlier	1	0	0
95 % Upper limit	53.4	51.7	51.0
95 % Lower limit	7.7	9.4	13.5

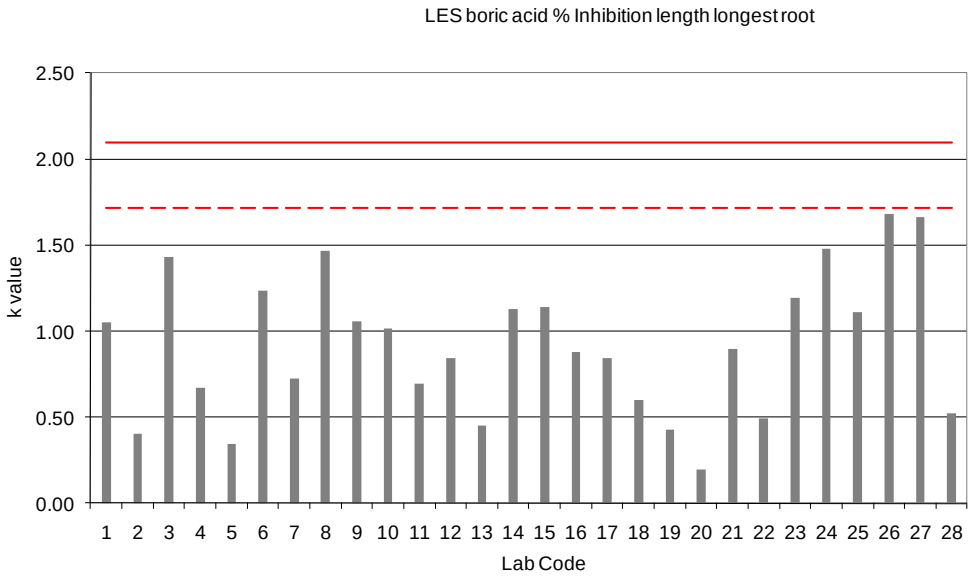
3 results have been discarded (Labs 11, 14, 21 as k outliers). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a % inhibition of the mean root length of 30 %, a repeatability of 18.9 % and a reproducibility of 35.4 %.

% inhibition longest root length

Lepidium sativum (LES)

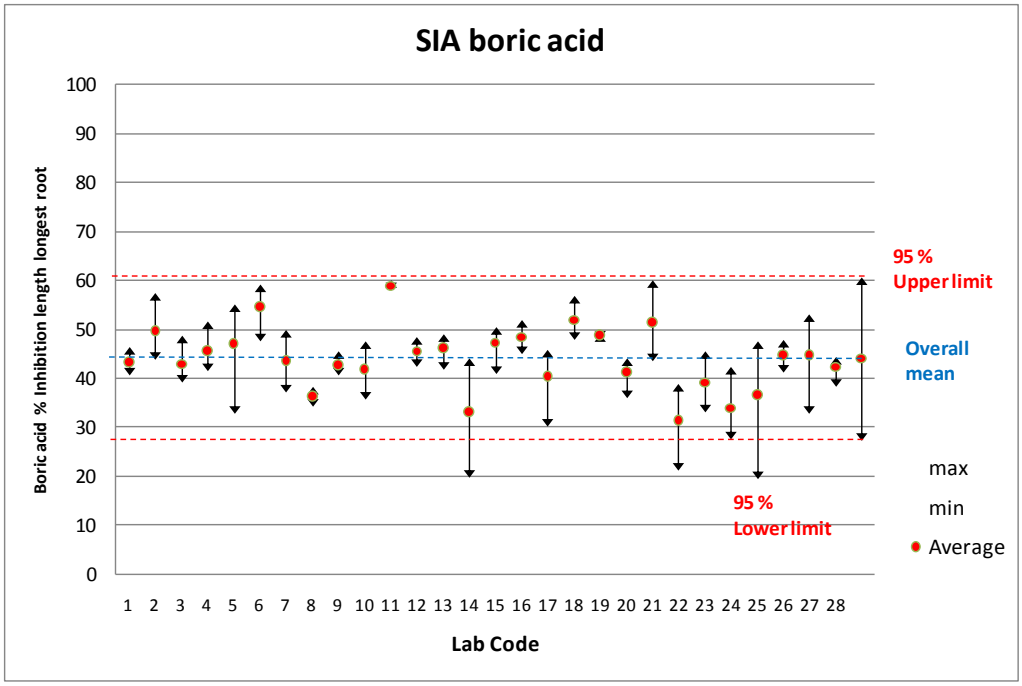


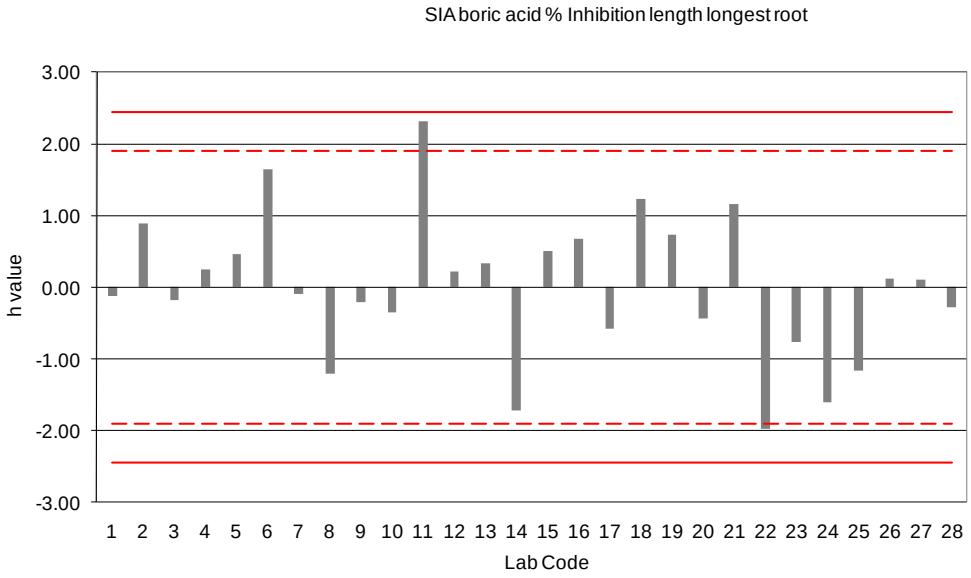


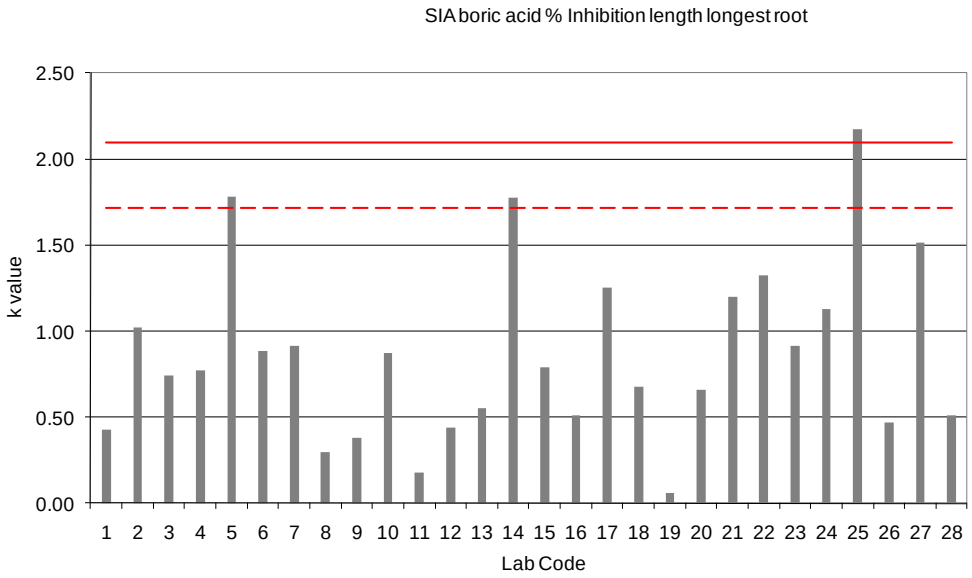


LES boric acid % Inhibition length longest root								
	All data		**Without k and h outliers**			**Without k and h stragglers**		
N labs	28		28			27		
N repeats	3		3			3		
Mean	41.8		41.8			42.2		
s_r	6.0		6.0			6.0		
CV%	14.4		14.4			14.2		
s_R	7.4		7.4			7.1		
CV%	17.6		17.6			16.7		
h straggler	1		1			0		
h outlier	0		0			0		
k straggler	0		0			0		
k outlier	0		0			0		
95 % Upper limit	56.2		56.2			56.0		
95 % Lower limit	27.3		27.3			28.4		

No outlier has been detected. The accepted results, based on all 28 laboratories, have a % inhibition of the longest root length of 42 %, a repeatability of 14.4 % and a reproducibility of 17.6 %.


SIA boric acid
Boric acid % Inhibition length longest root
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code
95 %
Upper limit
Overall
mean
95 %
Lower limit
max
min
Average

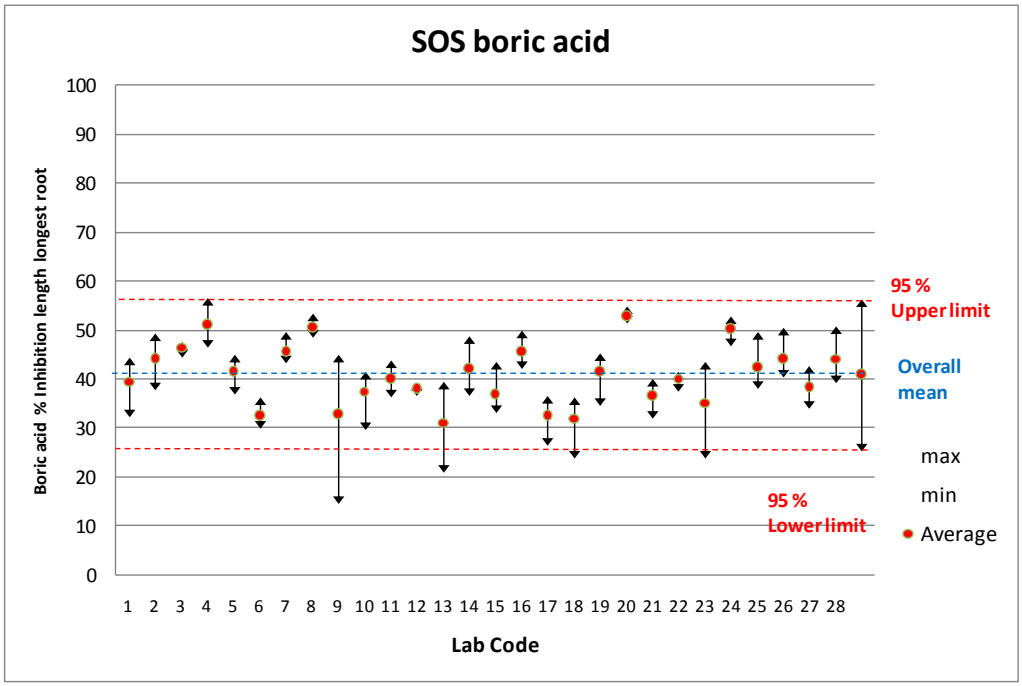

SIA boric acid % Inhibition length longest root
h value
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code

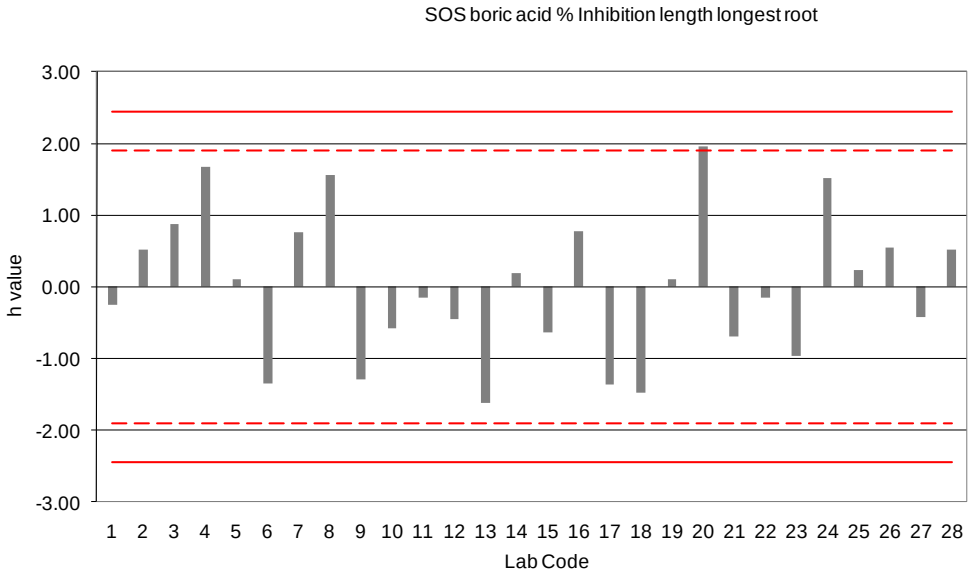


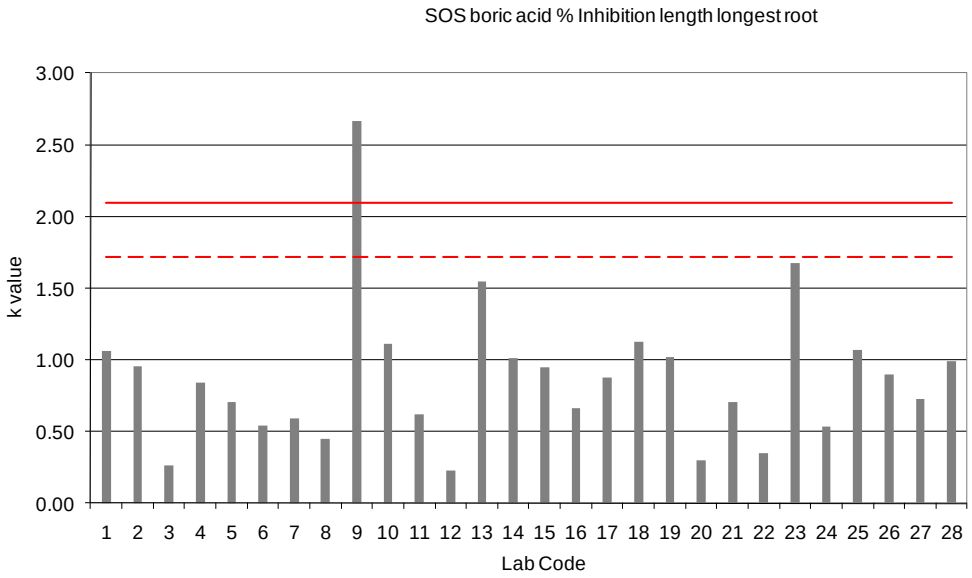
SIA boric acid % Inhibition length longest root						
	All data	**Without k and h outliers**			**Without k and h stragglers**	
N labs	28		27			16
N repeats	3		3			3
Mean	44.1		44.4			44.6
s_r	6.9		6.4			4.7
CV%	15.7		14.5			10.4
s_R	8.5		8.2			4.8
CV%	19.4		18.6			10.8
h straggler	2		2			0
h outlier	0		0			0
k straggler	3		2			0
k outlier	1		0			0
95 % Upper limit	60.8		60.5			54.1
95 % Lower limit	27.3		28.2			35.1

1 result has been discarded (Lab 25 as k outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a % inhibition of the longest root length of 44 %, a repeatability of 14.5 % and a reproducibility of 18.6 %.

Sorghum saccaratum (SOS)


SOS boric acid
Boric acid % Inhibition length longest root
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code
95 % Upper limit
Overall mean
95 % Lower limit
max
min
Average


SOS boric acid % Inhibition length longest root
h value
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code

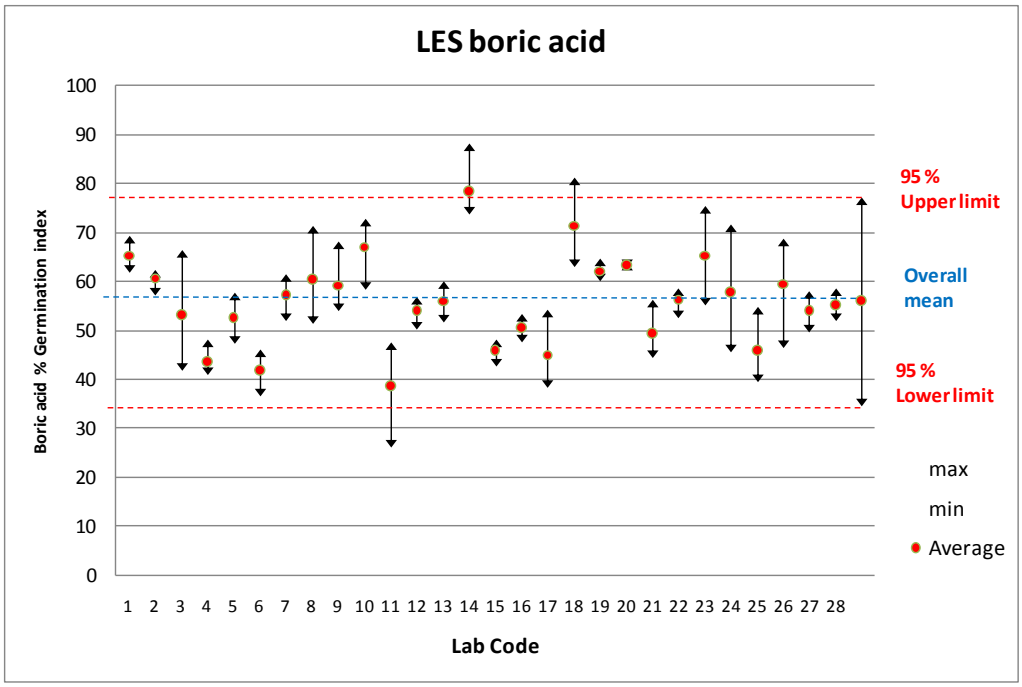


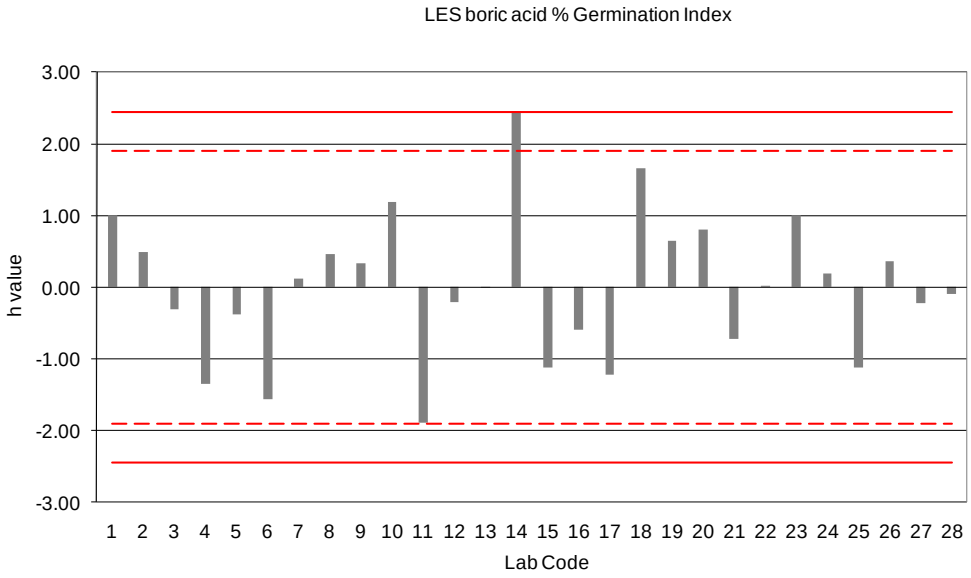
SOS boric acid % Inhibition length longest root			
	All data	**Without k and h outliers**	**Without k and h stragglers**
N labs	28	27	24
N repeats	3	3	3
Mean	40.9	41.2	41.4
s_r	6.0	5.3	4.9
CV%	14.8	12.9	11.8
s_R	7.9	7.5	6.7
CV%	19.3	18.1	16.3
h straggler	1	1	0
h outlier	0	0	0
k straggler	1	2	0
k outlier	1	0	0
95 % Upper limit	56.4	55.9	54.6
95 % Lower limit	25.4	26.6	28.2

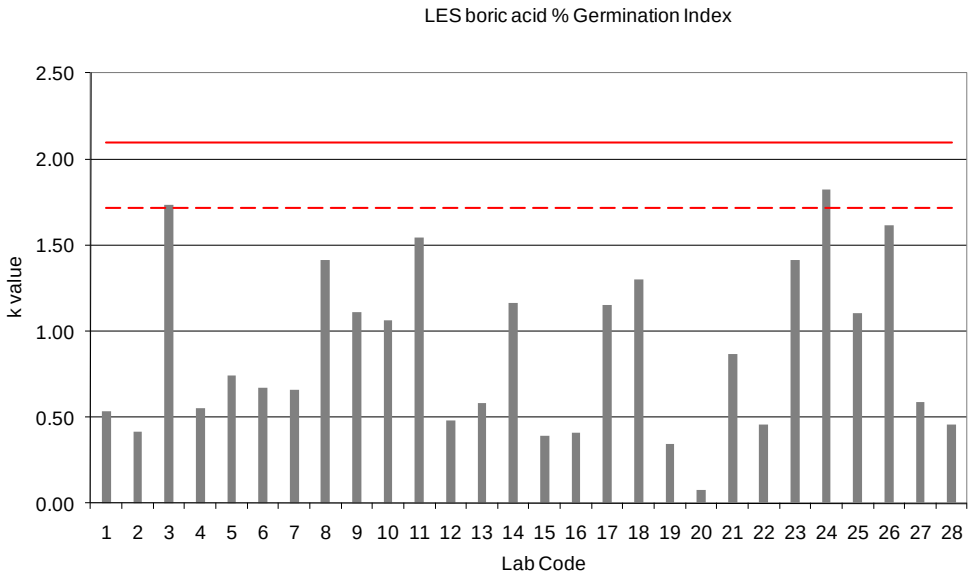
1 result has been discarded (Lab 9 as k outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a % inhibition of the longest root length of 41 %, a repeatability of 12.9 % and a reproducibility of 18.1 %.

% inhibition germination index

Lepidium sativum (LES)

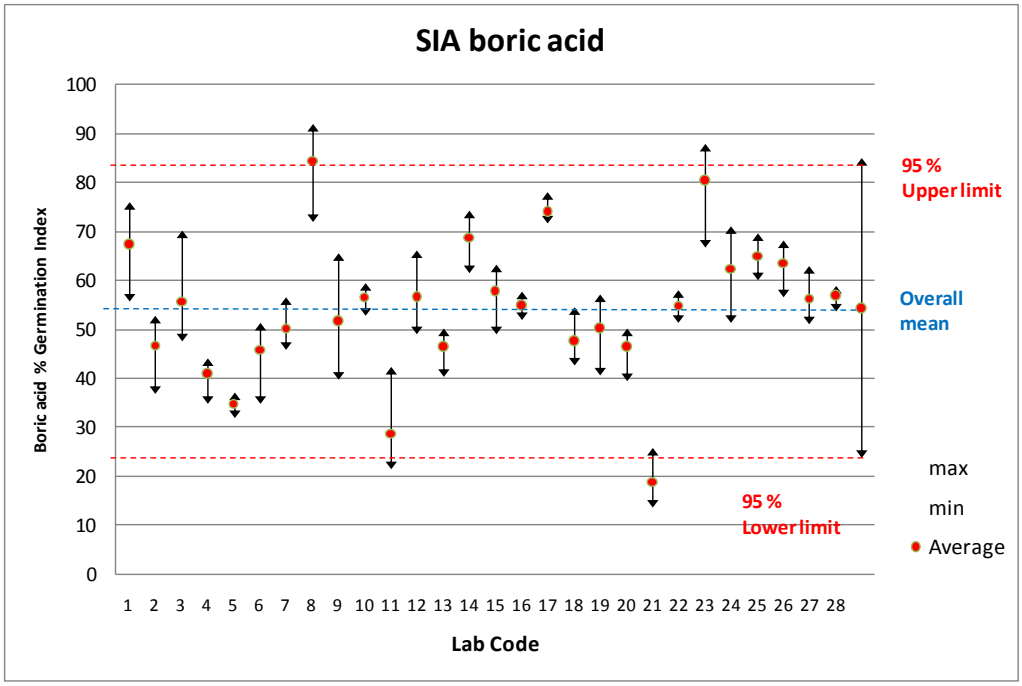


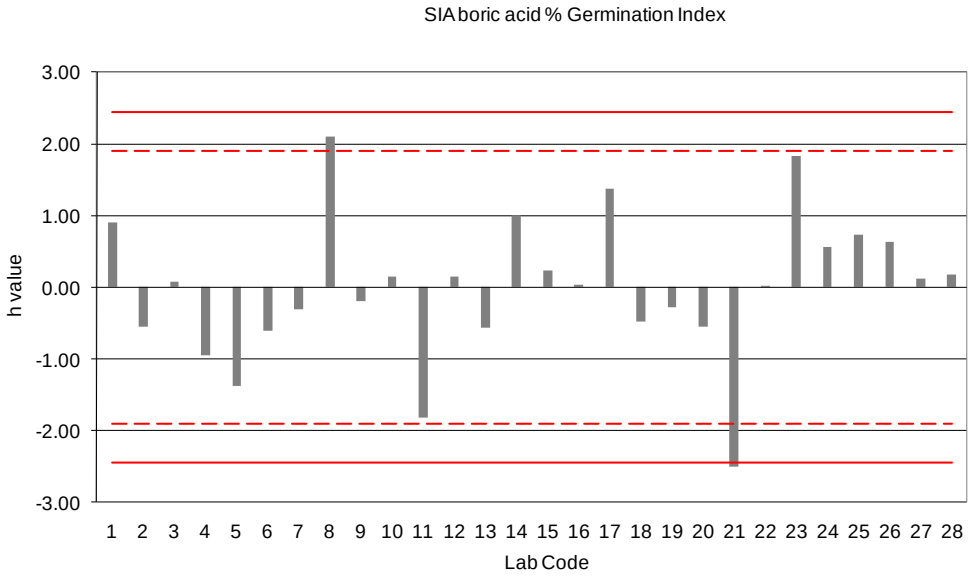


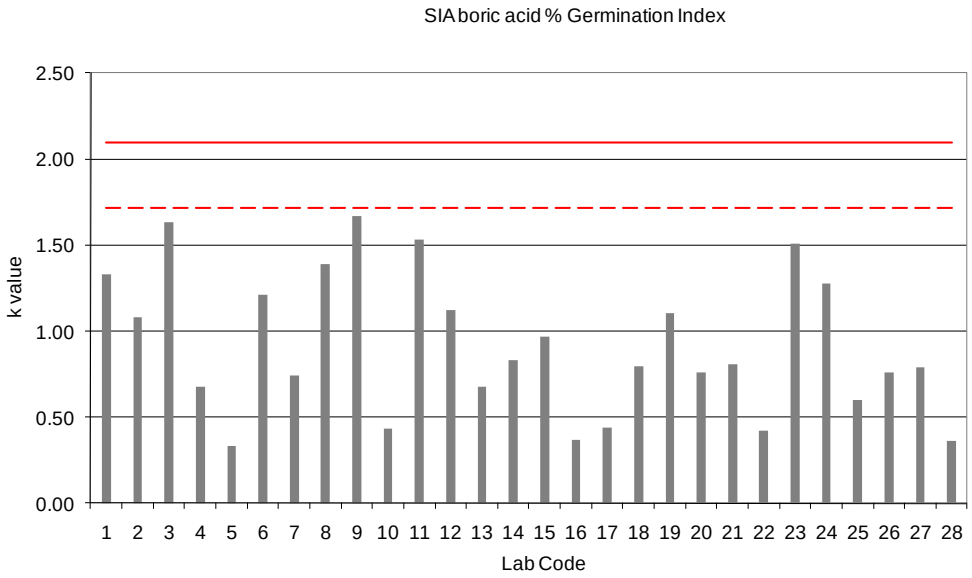


LES boric acid % Germination Index						
	All data		**Without k and h outliers**		**Without k and h stragglers**	
N labs	28		27		20	
N repeats	3		3		3	
Mean	56.1		55.2		54.3	
s_r	7.2		7.2		5.0	
CV%	12.8		13.0		9.2	
s_R	10.9		10.1		8.5	
CV%	19.4		18.2		15.6	
h straggler	1		2		0	
h outlier	1		0		0	
k straggler	2		2		0	
k outlier	0		0		0	
95 % Upper limit	77.4		75.0		70.8	
95 % Lower limit	34.7		35.5		37.7	

1 result has been discarded (Lab 14 as h outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a % Germination Index of 55 %, a repeatability of 13.0 % and a reproducibility of 18.2 %.

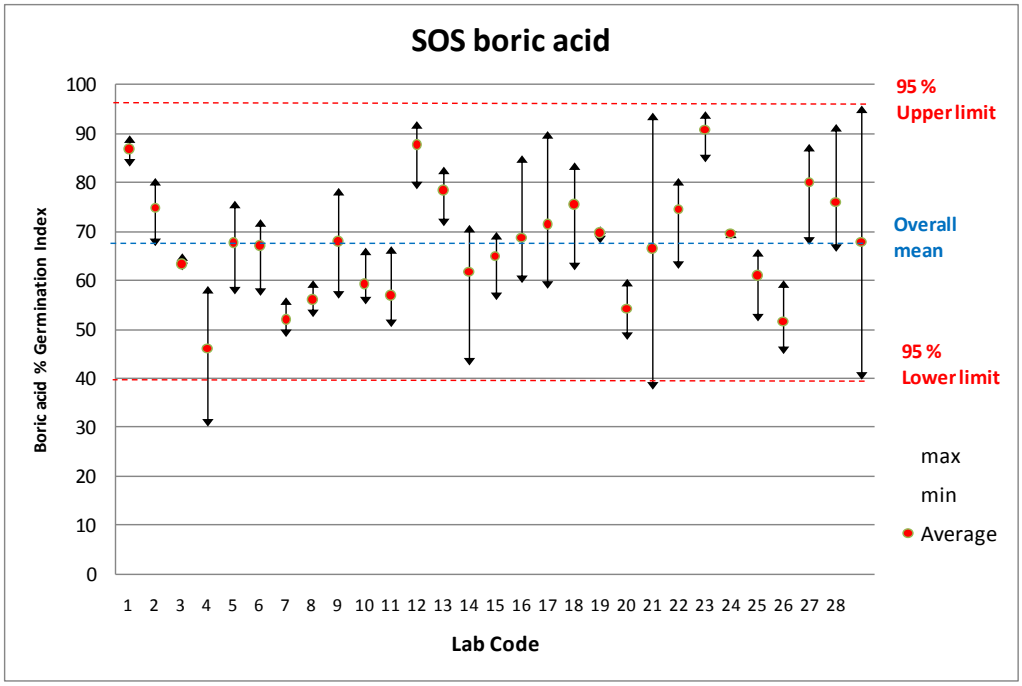

SIA boric acid
Boric acid % Germination Index
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code
95 % Upper limit
Overall mean
95 % Lower limit
max
min
Average

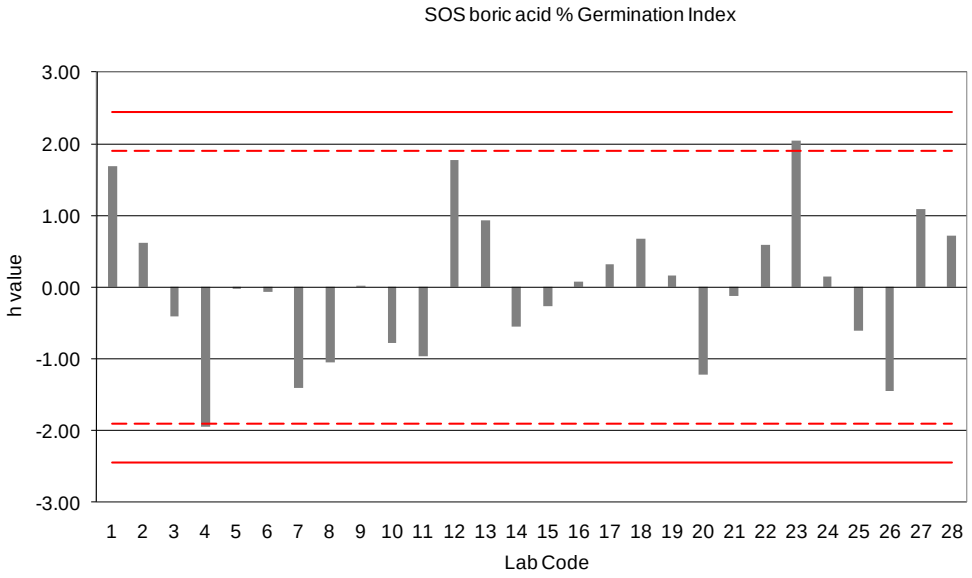

SIA boric acid % Germination Index
h value
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code

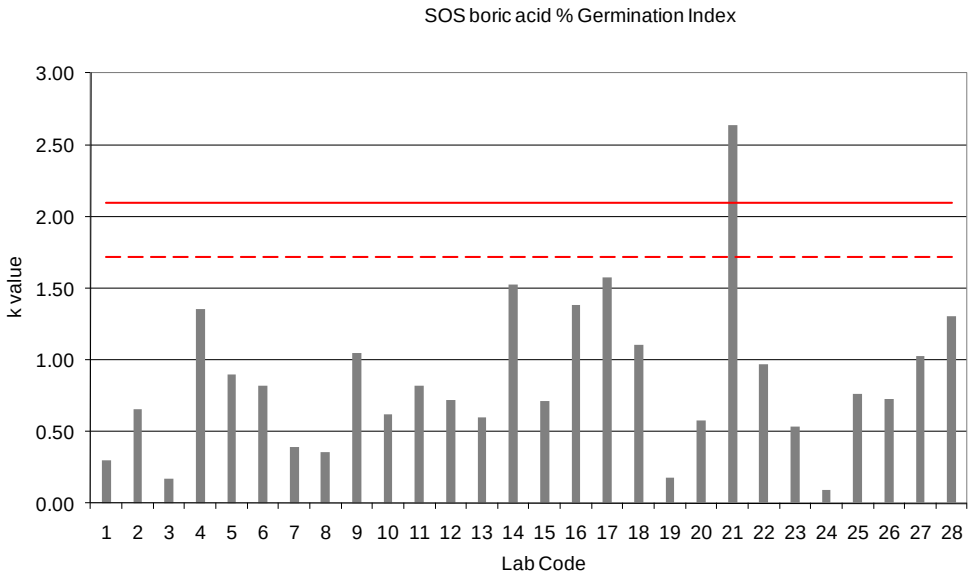


SIA boric acid % Germination Index						
	All data		**Without k and h outliers**		**Without k and h stragglers**	
N labs	28		27		20	
N repeats	3		3		3	
Mean	54.4		55.8		54.8	
s_r	7.8		7.9		6.8	
CV%	14.4		14.2		12.4	
s_R	15.6		14.2		9.6	
CV%	28.7		25.5		17.6	
h straggler	2		3		0	
h outlier	1		0		0	
k straggler	0		0		0	
k outlier	0		0		0	
95 % Upper limit	85.1		83.6		73.7	
95 % Lower limit	23.8		27.9		35.9	

1 result has been discarded (Lab 21 as k outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a % Germination Index of 56 %, a repeatability of 14.2 % and a reproducibility of 25.5 %.


SOS boric acid
Boric acid % Germination Index
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code
95 %
Upper limit
Overall
mean
95 %
Lower limit
max
min
Average


SOS boric acid % Germination Index
h value
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Lab Code



SOS boric acid % Germination Index						
	All data		**Without k and h outliers**		**Without k and h stragglers**	
N labs	28		27		19	
N repeats	3		3		3	
Mean	67.9		68.0		65.6	
s_r	10.8		9.5		7.8	
CV%	15.8		14.0		11.9	
s_R	14.2		13.8		10.9	
CV%	21.0		20.3		16.7	
h straggler	2		2		0	
h outlier	0		0		0	
k straggler	1		2		0	
k outlier	1		0		0	
95 % Upper limit	95.8		95.0		86.9	
95 % Lower limit	40.0		40.9		44.2	

1 result has been discarded (Lab 21 as k outlier). The accepted results, based on 27 laboratories (out of the original 28 participating labs), have a % Germination Index of 68 %, a repeatability of 14.0 % and a reproducibility of 20.3 %.