



SeedCalculator 2.1

User's Guide

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Report 10



SeedCalculator 2.2 User's Guide

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What is SeedCalculator?

Analysis of germination tests

SeedCalculator is a dedicated software program for the seed industry developed by Plant Research International. Cumulative germination data and seedling scores are presented in the form of a spreadsheet. Germination parameters and seedling scores, including standard error, can be quickly calculated, then viewed either graphically or in table format. The complete test can be stored and printed, including details on which germination protocol was used. Different tests can be compared using student's t-Test.

The basis of SeedCalculator

SeedCalculator uses a mathematical equation with three variables to fit the cumulative germination data. The equation starts at zero value at time $T=0$, and ends at the maximum germination for T approaching infinity. The shape of the curve is very flexible. Depending on the values of the three variables, the shape can be symmetric to anti-symmetric. Having calculated the best fit, SeedCalculator calculates ten germination parameters of the replicates and the mean germination data (including standard errors, s.e.). The standard errors of the parameters of the mean germination are calculated from values of the replicates. There is an option to calculate the parameters based on the maximum germination (G_{max}) or the number of seeds (N) used. G_{max} is given by the maximum germination of each replicate, or by the averaged maximum germination of the mean germination. For the calculation towards N , the number of each replicate is used to calculate the parameters, or the averaged total seeds used for the mean germination.

Definitions of the parameters

- **T₁**: time to 1% of G_{max} or N ; defines the onset of germination.
- **T₁₀**: time to 10% of G_{max} or N .
- **T₂₅**: time to 25% of G_{max} or N .
- **T₅₀**: time to 50% of G_{max} or N .
- **T₇₅**: time to 75% of G_{max} or N .
- **T₉₀**: time to 90% of G_{max} or N .
- **Uniformity I (T₇₅-T₂₅)**:
 - time between 25 and 75% of G_{max} or N .
- **Uniformity II (T₉₀-T₁₀)**:
 - time between 10 and 90% of G_{max} or N .
- **MGT (Mean Germination Time)**:
 - calculated by integration of the fitted curve and proper normalisation.
- **Skewness (MGT/T₅₀)**:
 - a measure for the anti-symmetry of the curve. Normally the value of MGT is between 0 and 1. A value of 1 means that the curve is symmetric and a value between 0 and 1 means that the curve is anti-symmetric. The closer the curve is to 0, the more anti-symmetric it is. A value of 0.9 means that the rate of germination before T_{50} is larger than the rate of germination after T_{50} (normally the case). A smaller value of MGT, i.e. 0.8, means that the relative difference between the rate of germination before T_{50} and the rate of germination after T_{50} is larger, as in the case of MGT=0.9. In some cases MGT>1 can occur, when the rate of germination before T_{50} is smaller than the rate of germination after T_{50} .

Features

- User-friendly graphical interface.
- Quick and standardised evaluation of germination tests.
- Calculation of valuable germination parameters including s.e..
- Calculation based on the maximum germination or on total seeds used.
- Provides final seedling score of the germination test: normal and abnormal seedlings (three categories), and dead and fresh seeds can be presented in a spreadsheet.
- Multiple germination tests can be displayed in graphics and table form.
- Statistical analysis by a student's t-Test to find significant differences (four levels of significance, p-value: 0.1 – 0.05 – 0.01 – 0.001).
- Import of cumulative germination data in ASCII format.
- Export of calculated parameters in ASCII format.
- The complete test can be stored and printed in a SeedCalculator print Report.
- Runs under Windows 95, 98 or NT 3.5, 4.0 (minimum screen resolution 800x600).

Getting started

System requirements

SeedCalculator requires the following hardware and software:

Hardware

- An 80486-based or pentium IBM or compatible computer.
- At least 8 megabytes of memory.
- A monitor and SVGA graphics card compatible with Windows.
- Minimum screen resolution: 800x600.
- A Windows compatible mouse.
- Any printer supported by Windows.
- Dongle delivered with SeedCalculator software plugged into parallel printer port.

Software

- Microsoft® Windows™ version 95, 98 or NT 3.5, 4.0.

Installation

SeedCalculator is delivered on a 3½" diskette. The files are compressed and should not be copied directly. SeedCalculator comes with an installation program which creates the necessary directories, expands the files and places them into the appropriate directories. An uninstall program will also be copied onto the hard disk - run this to correctly remove SeedCalculator.

Installing SeedCalculator

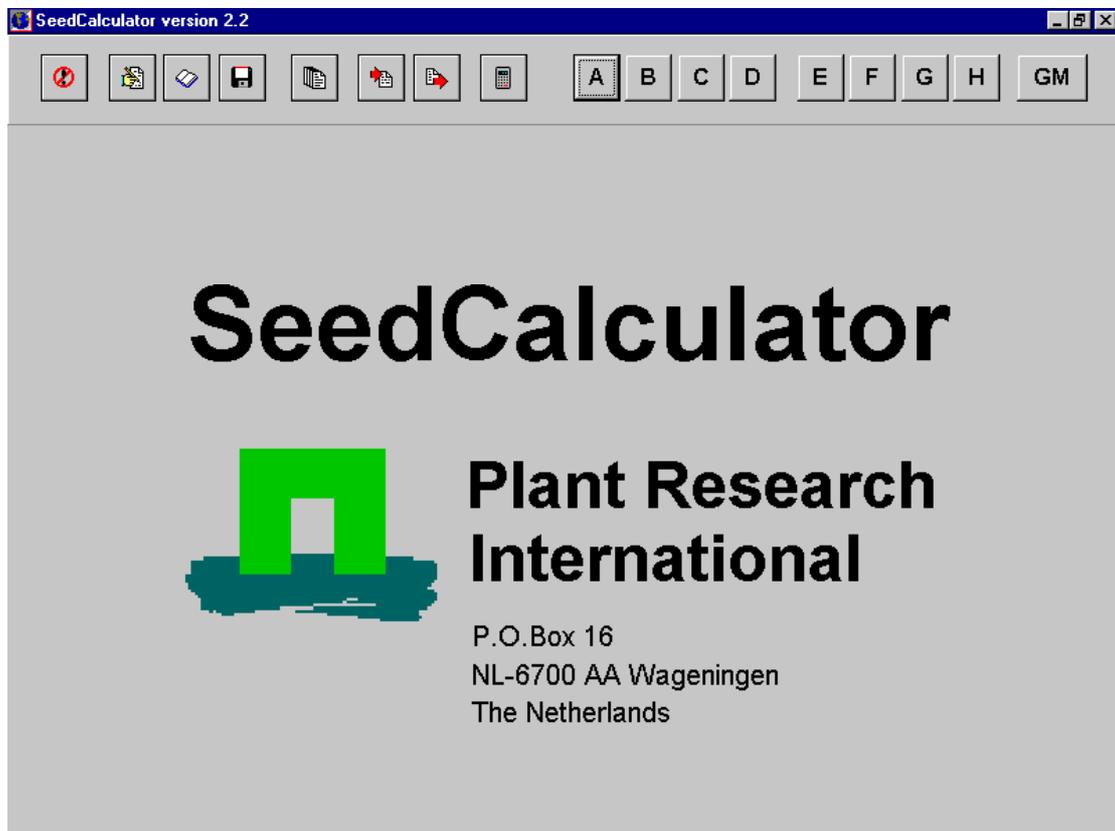
- Turn off the computer before connecting the dongle and/or the printer cable to the computer or serious damages may result to the parallel printer port.
- Plug the included dongle in the parallel printer port of the computer (LPT1-port). If a printer was connected to the computer connect the printer cable to the dongle.
- Place the SeedCalculator disk in the floppy disk drive.
- Open *My computer* and double click *Configuration*, select *Software* and click on the button *Installation*. Select the installation file: *a:\setup.exe* and click on *Finish*. The installation wizard of SeedCalculator will be started which will guide you through the installation.

SeedCalculator files

SeedCalculator contains the following files:

- SEEDCALC.EXE SeedCalculator executable file.
- SEEDCALC.SET Stored settings of SeedCalculator.
- BIVBX11.DLL Dynamic link library used by SeedCalculator.
- _ISREG32.DLL Dynamic link library used by the installation program.
- DELSL1.ISU Used to uninstall SeedCalculator.
- PICT.VBX File containing icons used by SeedCalculator.
- SPECIES.TXT File containing the list of crops.
- BRAS1NO.SC Example data file.
- BRAS2NO.SC Example data file.
- BRAS3NO.SC Example data file.
- BRAS3NO.ASC Example of file BRAS3.SC exported to ASCII format.

SeedCalculator Main Menu



(SeedCalculator Main Menu)

Stop SeedCalculator

Use this button to leave the program. Should data not have been saved, the program will ask if you want to proceed. Ignoring this message will lead to loss of all newly entered or changed data.

Input window

Germination data can be entered or modified in this window. Four types of data can be distinguished:

- A. General information about the seed lot**
- B. The germination protocol**
- C. Germination data**
- D. Final seedling score**

A. General information about the seed lot

Species: Use the down arrow to open the dropdown list, which contains more than 600 crops. Use the up and down arrow to scroll through the list. Select a crop by clicking on the name.

Note: When the dropdown list is open, enter the first letter of a crop. The program will jump to the first crop beginning with that letter.

Adding new crops:

The list of crops used by SeedCalculator is stored in the SPECIES.TXT file in the C:\PROGRAM FILES\PRI\SEEDCALC folder. A plain text editor can be used to add new crops to the bottom of the list: the program will sort these automatically.

Cultivar: Enter the crop cultivar name.

Id: An extra identification code can be entered - the seed lot number, for example.

Treatment: Enter the treatments given to the seeds.

Experiment date: Enter the experiment date.

The screenshot shows the 'BRASNO3.SC' window with the following fields and values:

- Species: Brassica oleracea
- Cultivar: B
- Id: 12345
- Treatment used: Protocol 3
- Date of experiment: 29-12-1998
- Substrate: TP (selected)
- Temperature °C: 20
- Light hours/day: 8
- Dormancy breaking: Dry storage
- Data input: germination (selected)
- Time: days (selected)
- Score: seeds (selected)
- Replicates: 4
- Individual Seeds per repl: 50

Time	A	B	C	D	-	-	-	-	Total (%)
0.750	0	0	0	0					0.0
1.010	0	0	0	0					0.0
1.930	5	11	6	7					14.5
2.060	6	13	8	9					18.0
2.710	26	30	29	26					55.5

Buttons at the bottom: Current row 1, Ins row, Del row, Del all, Exclude, Clear, Cancel, Ready.

(SeedCalculator Input window)

B. The germination protocol

Part of the input window for entering the germination protocol used for the germination test. All protocols described by the ISTA in the International Rules for Seed Testing 1996, Seed Science and Technology volume 24 can be selected.

Substrate: Select the substrate used during the germination test from the following:

1. TP Top of paper (= default option)
2. BP Between paper (folded envelopes)
3. PP Pleated paper (accordion-like paper strip)
4. S Sand (covered with 10-20 mm uncompressed sand)
5. TS Top of sand (pressed into the surface of the sand)

Temperature: Use the dropdown list box and click on the temperature used during the germination test. Alternating temperatures are also available at the bottom of the list (default = 20°C).

Light: Set the hours of illumination per day using the up and down arrows (default = eight hours).

Dormancy breaking: For species where dormancy is naturally of short duration, it is often sufficient to store the seeds in a dry place for a short period. If there is still physiological dormancy and a method was used for breaking it, select this by opening the dropdown list and clicking on the method used (default = dry storage).

C. Germination data

Select the **Germination** (= default) option in the **Data input box**. Once the data is entered the mean germination values are calculated online in percentage terms and displayed in the last column of the spreadsheet - **Total (%)**.

Use the **Time** box to set the time data dimension in **days** or in **hours**. Data already entered is recalculated to the new dimension when switching from hours to days or vice versa.

Use the **score** box to select between data input in **seeds** (number of seeds or seedlings) or in **percentage** (germination or seedling percentage). Switching between these two input options will recalculate the data already entered.

Use the **replicate** box to set the number of replicates used in the germination experiment. The maximum number allowed is eight replicates from A to H in the column header. If less replicates are selected, the letters in the column header not used will be replaced by a dash. The column must be empty before it can be deselected (default number of replicates = eight).

Use the **seeds per repl** box to set the number of seeds per replicate. Use the up and down arrow to change this value, ranging from 10 to 200 with a step size of five. If different number of seeds per replicate have been used or some seeds lost during a treatment, the exact number of seeds can be corrected by clicking on **individual**. A new window is opened for entering (only by the valid replicates) the exact number of seeds used. The percentages in the spreadsheet are recalculated when the window is closed (default = 50 seeds per replicate). The number of seeds will, when **seeds per repl** are used, be shown in the column header of each replicate.

Buttons:

Use **Ins row** to insert a blank row and **Del row** to delete the active row. Use **Del all** to delete all the rows of germination data and seedling score in both spreadsheets. If the option **Delete time data** is selected also the time data is deleted. (**Current row** indicates which is the active row.)

Clear will empty the input form and set all options to their default values, including the protocol information and the general information.

Cancel will exit the input form. All changes made since the last time **Ready** was clicked are ignored. Exceptions are the **Del row** and **Del all** buttons; removed data cannot be restored.

Ready will close the input window when the following checks are valid.

These checks ensure data input errors are kept to a minimum:

1. At least four rows of data must be entered.
2. No empty rows or cells between the data are allowed.
3. If the input mode is in number of seeds, data can only be an integer and must be smaller or equal to the selected number of seeds per replicate.
4. If the input mode is in percentage terms, data cannot be larger than 100%.
5. Time data must be increasing.
6. Germination data must be cumulative.

If the **Ready** button is pressed and no warning appears, the data satisfies the above criteria.

Exclude: after the data has been analysed deviate replicates can be excluded. Click on **Exclude** and mark the replicate(s) to exclude from the calculations. The letter in the column header of the spreadsheet will be replaced by **Excl** to show that a replicate is excluded from the calculations. Perform the calculations again.

D. Final seedling score

Final seedling score can be entered by selecting the **Seedlings** option in the **data input** box. A new spreadsheet will appear with the same replicates in the spreadsheet header, but with scoring classes on the left side of the spreadsheet instead of the time column.

Click on **Info** and the following category recommendation will be presented in a window.

A **normal** seedling conforms to one of the following categories:

1. Intact seedlings: all essential structures well developed, complete and healthy.
2. Seedlings with a slight defect: showing an otherwise satisfactory and balanced development compared to the intact seedlings of the same test.
3. Seedlings with secondary infection: conforming to above categories but affected by fungi or bacteria from sources other than the parent seed.

Abnormal seedlings: not showing the potential to develop in a normal plant under favourable conditions. The following categories are classified as abnormal:

1. Damaged seedlings: missing or badly damaged essential structures so that balanced development cannot be expected.
2. Deformed or unbalanced seedlings: weak development, physiological disturbances, deformed or essential structures out of proportion.
3. Decayed seedlings: diseased or decayed essential structures due to primary infection to the extent that normal development is prevented.

Dead: ungerminated seeds which can no longer produce a part of a seedling. Includes rotten seeds, empty seeds or seeds without an embryo.

Fresh: seeds which have failed to germinated but remain clean and firm and still have the potential to develop into a normal seedling.

Data can be entered in percentage terms or in number of seeds. Data already entered will be recalculated (for both spreadsheets).

Once input is completed, select **germination** in the **data input** box. Again, the input is checked and will only be accepted if the following checks are not valid:

1. If the input mode is in **seeds**, data can only be integer and smaller or equal to the selected number of seeds per replicate.
2. If the input mode is in percentage terms, data cannot be larger than 100%.
3. Total per column must be 100%.

Open SeedCalculator data file

Press this to open a window to select a data file. First select the drive where your file is located, open the correct folder and set 'List file of type' to the extension of your choice (default extension = 'SC'). Highlight the file of your interest by clicking on its name and click on OK. The input window will now be opened and the data becomes available.

The default dimension in which the data is shown, can be set by pressing the **SeedCalculator defaults** button (see page 9).

Save data

Use this to save modified or new data. A window will be opened where the drive, folder and the name of the file can be specified. The file extension 'SC' is recommended. If the filename entered already exists, the program will warn that an existing file will be overwritten.

Compare SeedCalculator data files

Comparing data files is a powerful feature of SeedCalculator. The set of data files must satisfy the following criteria:

Data must be of the same crop.

1. Time parameters must be calculated in the same mode, based on the total germination or total number of seeds.
2. All data (replicates and mean germination) must have been fitted.
3. A maximum number of eight files can be compared.

Click on **compare germination tests** to open a window where the files to be compared can be selected. After selecting the required files click on **Compare**. Multiple files can be selected using the Shift or Ctrl key in combination with the left mouse button.

The output window will be opened and the germination curves of the selected files displayed in a graph. From the curve which is highlighted (in red) the parameters and the filename are displayed and the corresponding treatment is also highlighted. Use **Next** to scroll through the treatments to view the corresponding curve, parameters and filename. Click on **Final** to activate the final seedling score. Use **Next** to view the final seedling score of every treatment in numbers. Click on **Table** to see an overview in table form of all the parameters of each file.

To see if there are significant differences between the parameters, use **t-Test** to start Student's t-Test. A window will be opened in which to set the calculation criterion:

- | | |
|-------------------------------|-----------------------------|
| 1. Indication for differences | p-value: $0.05 < P < 0.10$ |
| 2. Significant | p-value: $0.01 < P < 0.05$ |
| 3. Strongly significant | p-value: $0.001 < P < 0.01$ |
| 4. Very strongly significant | p-value: $P < 0.001$ |

Mark the file for which the calculation must be performed (i.e. the control seeds) and press **Calculate**. A window with a table of all the parameters will appear with the parameters of the marked file in blue and the parameters which satisfy the calculation criteria in red.

Use **Print** to get a print report of the comparison (page 13 contains an example). By clicking on **Print** after a t-Test has been completed, the results of this test will also be displayed in the print report.

Use **Close** to leave the output window and return to the main menu.

Import ASCII data

Germination data in ASCII format can be imported by SeedCalculator if it satisfies the following criteria:

1. The first column contains the time data in hours or in days.
2. The other columns contain the germination data in number of seeds or in percentage terms.
3. Data can be cumulative or in increase per counting period.
4. Maximum number of eight columns containing germination data.
5. Columns are separated by space, decimal point, tab or by semicolon.
6. The columns may contain up to 23 data points.

If these criteria are satisfied, open the **Import window**. Set the parameters to the appropriate values and select the filename. Click on **Import** and the data will be imported and placed in the input window, which will then be opened.

Export SeedCalculator data to ASCII

SeedCalculator can export data to an ASCII file. The categories to be exported have to be marked. The following categories are available:

1. Germination data
2. Seedling score
3. Calculated parameters

Enter a filename (default extension = 'ASC') and click on **Export**. In the appendix an example of exported data is shown.

SeedCalculator defaults

Before using SeedCalculator, set the calculation mode for all the time parameters to the that of your choice (SeedCalculator will then store this so it only has to be done once). Be aware that files calculated in different modes cannot be compared. The modes are:

1. Calculations based on the total number of seeds per replicate.
2. Calculations based on the total germination of a replicate.

For example: when a seed lot germinates for 80% the T_{50} is the time when 40% of the seed lot has germinated when the calculation mode to total germination. If the total number of seeds mode is used, T_{50} will be the time when 50% of the total number of seeds have germinated.

Also the default dimensions in which the input window will be opened can be set. Choose **Days** or **Hours** for the time axis and **Seeds** or **Percentage** for the germination data.

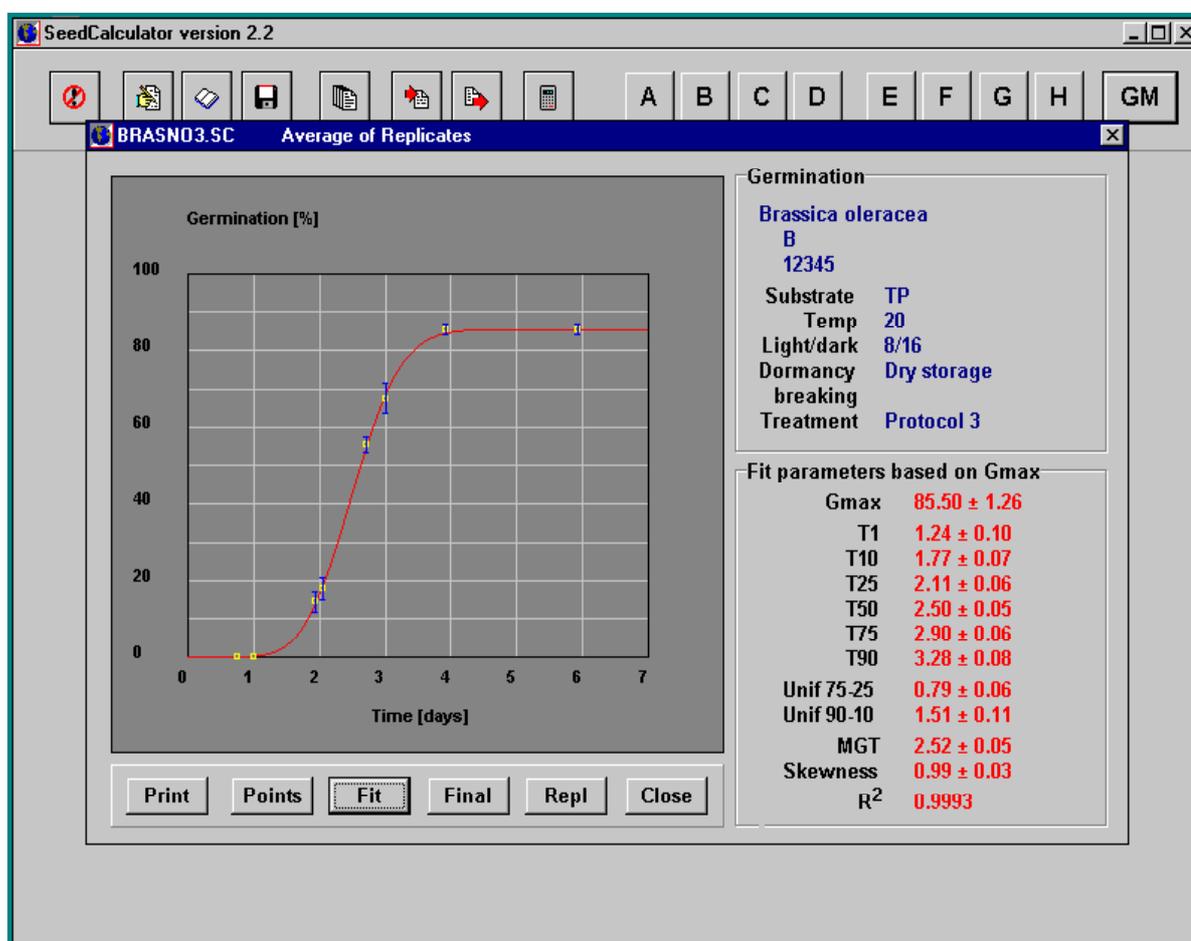
A..H Show replicate data (output window)

Once germination data has been entered or a SeedCalculator file opened and the input window is closed, the output window can be opened by clicking on a replicate button.. Now the data points per replicate A to H can be viewed and fitted (click on **Fit**). The program checks the data points and generates a warning if the following criteria are not satisfied:

- At least five different data points.
- The first non-zero data point must be below 20%.
- The step size between two successive points should not exceed 50%.

Should the data points not satisfy these criteria, decide whether the calculated parameters are meaningful by checking the fit through the data points. If the fit proves incorrect, exclude the replicate from further calculations.

Parameters will be calculated and displayed automatically. Click on **Final** to view the final seedling score graphically and in numbers (if entered in the input window). Click on **Points** to view the data points. **Print** gives a print report of the replicate (see appendix). The fits of the replicates and the calculated parameters will be stored in a file when the data is saved.



(SeedCalculator Output window)

GM Show mean germination data (output window)

Use this to view the mean germination data in the output window. The standard error of the mean germination data points is also shown in the graph, as error bars through the data points. Use **Fit** to fit the data points. If the replicates have already been fitted, the standard error in the parameters is also calculated and displayed. **Final** will show the mean seedling score (if seedling data is available) including standard error. Click on **Points** to view the data points and click on **Repl** to get a graphical overview of the replicates, including the most important parameters. **Print** will print a report (see appendix).

Appendix I.

Print example ASCII data

Example of exported data of file: BRAS3NO.ASC

Time	A	B	C	D	Mean	S.E.
0.75	0.0	0.0	0.0	0.0	0.0	-
1.01	0.0	0.0	0.0	0.0	0.0	-
1.93	10.0	22.0	12.0	14.0	14.5	-
2.06	12.0	26.0	16.0	18.0	18.0	-
2.71	52.0	60.0	58.0	52.0	55.5	-
3.01	62.0	72.0	76.0	60.0	67.5	-
3.92	82.0	86.0	88.0	86.0	85.5	-
5.92	82.0	86.0	88.0	86.0	85.5	-
Norm	82.0	86.0	88.0	86.0	85.5	1.3
Abn1	16.0	12.0	12.0	12.0	13.0	1.0
Abn2	0.0	0.0	0.0	0.0	0.0	0.0
Abn3	0.0	0.0	0.0	0.0	0.0	0.0
Dead	0.0	0.0	0.0	0.0	0.0	0.0
Fresh	2.0	2.0	0.0	2.0	1.5	0.5
Gmax	82.0	86.0	88.0	86.0	85.5	1.26
T1	1.45	1.03	1.37	1.14	1.24	0.10
T10	1.91	1.59	1.87	1.75	1.77	0.07
T25	2.20	1.95	2.17	2.13	2.11	0.06
T50	2.55	2.37	2.50	2.58	2.50	0.05
T75	2.94	2.80	2.82	3.04	2.90	0.06
T90	3.33	3.21	3.11	3.47	3.28	0.08
U75-25	0.74	0.86	0.66	0.91	0.79	0.06
U90-10	1.42	1.63	1.25	1.72	1.50	0.11
MGT	2.60	2.39	2.49	2.60	2.52	0.05
Skewness	0.98	0.99	1.00	0.99	0.99	0.03
R2	0.9968	0.9992	0.9995	0.9956	0.9991	

Appendix II.

SeedCalculator Results: Repl B

Appendix III.

SeedCalculator Results: Mean

Appendix IV.

SeedCalculator Results: Overview

