

## **The Heterogeneity Testing Calculator for seed lots in multiple containers**

### **Introduction**

The sampling rules and procedures described in the current ISTA Rules and ISTA Handbook on Seed Sampling are based on the assumption that the seed lot is as uniform as practically possible with good seed production practice. If the seed lot is too heterogeneous, there is a risk that the sample drawn does not represent the whole seed lot.

Consequently, if there is evidence of heterogeneity, sampling must be stopped and the sample drawn must be refused. In the case of doubt, if the seed lot is in multiple containers heterogeneity can be tested as described in the ISTA Rules under Chapter 2.9.

The heterogeneity-testing procedure can also be used by seed companies to analyse and improve the function of the seed processing system. Also, national authorities can use heterogeneity testing for monitoring purposes.

The heterogeneity testing described here deals with the variation between the containers. Heterogeneity testing does not cover situations where different components (normal and abnormal seedlings, pure seed and other seed, etc.) are evenly distributed in the whole lot and between each container. In those cases, heterogeneity does not affect the sampling procedure, and the conditions for the issuance of an Orange International Seed Lot Certificate are fulfilled.

The sampling and analysis procedures and the principles and mathematical calculations of heterogeneity testing are described in detail in the ISTA Rules.

### **Presentation of the Heterogeneity Testing Calculator**

The Heterogeneity Testing Calculator is a Microsoft® Excel workbook which can be used to detect heterogeneity which would make a seed lot technically unacceptable for sampling for the issuance of an Orange International Seed Lot Certificate (OIC). The calculations carried out in this workbook are in accordance with the procedures published in the ISTA International Rules for Seed Testing, Chapter 2.9 (Heterogeneity testing for seed lots in multiple containers).

Heterogeneity can be tested by:

- the percentage by weight of any purity component;
- the percentage of any germination test component;
- the total number of seeds or the number of seeds of any single species in the determination of other seeds by number.

There are nine different worksheets in the workbook. They are all used in the same way, although the calculations behind them are different, depending on whether they are intended for testing purity, germination or other seed number.

Purity worksheets:

- Total pure seeds
- Purity-Inert matter
- Purity-Other seeds

Germination worksheets:

- Germ-Normal Seedlings
- Germ-Abnorm Seeds
- Germ-Dead Seeds

Other seed worksheets:

- Total number of other seeds
- Number of species with the highest number
- Number of species with the second-highest number

Purity attributes can be tested by any purity sheet, germination attributes by any germination sheet, and other seed attributes by any other-seed sheet. For example, the percentage of hard seeds can be tested with any germination worksheet.

### **How to use the Testing Calculator**

1. Choose the right worksheet.
2. Enter the total number of containers in the seed lot. The program gives the minimum number of container-samples to be drawn.
3. Draw the minimum number of individual container-samples from the seed lot. Each container-sample must be analysed individually in a seed laboratory.
4. Enter the values of the adopted attribute of each container-sample in the column X. The program gives the mean value.
5. Enter the number of seeds tested from each container-sample (assumption: 1 000 for purity, 100 for germination and 10 000 for other seed number).
6. Enter whether the seed is chaffy (**Y**) or non-chaffy (**N**). Whether a genus is chaffy or not can be found in Table 3B Part 1 in ISTA International Rules for Seed Testing.
7. The program reports V (observed variance of independent container-samples in respect of the adopted attribute), W (acceptable variance of independent container-samples in respect of the adopted attribute) and calculated and critical H values. The program compares the H values. If the calculated H value exceeds the critical H value, the seed lot is classified as significantly heterogeneous. The program tells whether the calculated H value indicates, or does not indicate, significant heterogeneity.  
The H value describes especially in-range heterogeneity, and can be used to decide whether the average quality of the seed lot can be estimated sufficiently by the ISTA sampling procedure.
8. The program reports calculated and critical R values and compares them. If the calculated R value exceeds the critical R value, the seed lot is classified as significantly heterogeneous. The program tells whether the calculated R value indicates, or does not indicate, significant heterogeneity.  
The R value describes off-range heterogeneity, and can be used to estimate whether the OIC can be applied to single containers.

**If either the R value or the H value indicates significant heterogeneity, the seed lot must be declared heterogeneous.**

Note:

When heterogeneity is tested by this calculator, there must be at least five containers in the seed lot.

The H value is not calculated when the mean of a certain attribute is outside the following limits:

- purity components: above 99.8 % or below 0.2 %
- germination: above 99.0 % or below 1.0 %

– number of specified seeds: below two per sample.  
Negative H values are reported as zero.

## Examples

The values in yellow cells can be changed when the worksheets are used.

### Example 1. Purity

**Purity - Total pure seeds (%): Heterogeneity testing for seed lots in multiple containers**  
**ISTA Rules - Chapter 2 - 2.9**

		X	Container sample #
Number of containers in the lot	64	98.2	1
		98.5	2
Minimum number of independent container samples to be drawn	20	97.9	3
		99	4
Number of independent container-samples	20	99.1	5
		97.8	6
Number of seeds tested from each container-sample	1000	99	7
		98.4	8
Chaffy seeds (Y/N)	Y	98.8	9
		98	10
Reported mean value	98.5	99	11
		97.7	12
<b>H-value test</b>		97.8	13
W	0.17	97.9	14
		98	15
V	0.32	98.6	16
		99.3	17
Calculated H value	0.60	99.1	18
		99.2	19
Critical H value	1.09	99.1	20
This H value does not indicate significant heterogeneity.			
<b>R-value test</b>			
R	1.60		
Critical R value	2.4		
This R value does not indicate significant heterogeneity.			
Change any value in a yellow cell			

The H and R values do not indicate significant heterogeneity. The seed lot fulfils the uniformity requirement. Note: the number of seeds tested for purity analysis per container-sample is 1000.

## Example 2. Germination

### Germination - Normal seedlings (%): Heterogeneity testing for seed lots in multiple containers

		X	Container sample #
Number of containers in the lot	12	95	1
Minimum number of independent container samples to be drawn	11	96	2
Number of independent container-samples	12	95	3
Number of seeds tested from each container-sample	100	90	4
Chaffy seeds (Y/N)	Y	85	5
Reported mean value	86	81	6
		92	7
		78	8
		76	9
		77	10
		71	11
		98	12
<b>H-value test</b>			13
W	14.30		14
V	86.70		15
Calculated H value	4.86		16
Critical H value	1.58		17
This H value does indicate significant heterogeneity.			18
<b>R-value test</b>			19
R	27.00		20
Critical R value	20		
This R value does indicate significant heterogeneity.			

Both the H value and the R value indicate significant heterogeneity. The seed lot is declared heterogeneous.

Note: the number of seeds tested for germination analysis per container-sample is 100.

### Example 3. Other seed number

#### Other seeds - Number of species with the second-highest number: Heterogeneity testing for seed lots in multiple containers

Number of containers in the lot	6	X	Container sample #
Minimum number of independent container samples to be drawn	6	1	1
Number of independent container-samples	6	1	2
Number of seeds tested from each container-sample	10000	9	3
Chaffy seeds (Y/N)	Y	12	4
Reported mean value	7	17	5
		1	6

  

H-value test	
W	15.03
V	47.37
Calculated H value	0.95
Critical H value	4.44
This H value does not indicate significant heterogeneity.	

  

R-value test	
R	16.00
Critical R value	19
This R value does not indicate significant heterogeneity.	

The H and R values do not indicate significant heterogeneity. The seed lot fulfils the uniformity requirement. Note: the number of seeds tested for other seed number per container-sample is 10000. The seed is chaffy.

#### Example 4. Other seed number - 2

#### Other seeds - Number of species with the second-highest number: Heterogeneity testing for seed lots in multiple containers

Number of containers in the lot	6	X	Container sample #
		1	1
		1	2
Minimum number of independent container samples to be drawn	6	9	3
		12	4
Number of independent container-samples	6	17	5
		1	6
Number of seeds tested from each container-sample	10000		
Chaffy seeds (Y/N)	N		
Reported mean value	7		
<b>H-value test</b>			
W	9.57		
V	47.37		
Calculated H value	3.55		
Critical H value	2.83		
This H value does indicate significant heterogeneity.			
<b>R-value test</b>			
R	16.00		
Critical R value	15		
This R value does indicate significant heterogeneity.			

Both the H value and the R value indicate significant heterogeneity. The seed lot is declared heterogeneous. Note: The seed is non-chaffy. Chaffiness is the only value that was changed compared to example 3.