

Working Groups

1. Validate new species/methods for the rules.

Leader: Jette Nydam

Members: Mark Bennett, Gary Johnson, Maria-Rosaria Mannino

2. Improve existing rules (orthodox seeds + general improvements).

Leader: Ronald Don

Members: all MOI members

3. Improve the existing rules (non-orthodox seeds).

Leader: Patricia Berjak

Members: Gary Johnson, Joseph Ahenda, Craig McGill, Somboon Wuthipongprasert, Songqvan Song

4. Organise proficiency testing.

Leader: Ronald Don

Members: Doug Ashton (Referee Committee), Martina Roesch (ISTA Secretariat), Harry Nijenstein

5. Improve application of existing rules by means of organising a workshop, and by writing a handbook.

Leader: Jette Nydam

Members: all MOI members

6. Prepare a paper on the future developments in moisture testing, and the consequences for the ISTA Moisture Committee and the ISTA Rules.

Leader: Harry Nijenstein

Members: all MOI members

Activity Report

1. Validate new species/methods for the rules

Most of the 328 species of agricultural and vegetable seeds, listed in part 1 of table 2A of the ISTA Rules were evaluated for oil content, using the website of the Royal Botanic Gardens, Kew (www.kew.org). The results were presented during the moisture workshop in Lyngby in November 2003.

The ISTA methods described are designed to reduce oxidation, decomposition or the loss of volatile substances while ensuring the removal of as much moisture as possible. So a high oil content would lead to the low temperature method, and a low oil content would lead to the high temperature method. The ISTA low temperature method includes species of oil contents as low as 9.9% and as high as 46.9%. On the other hand, the ISTA high temperature method includes species as low as 0.6% and as high as 38.1% oil.

This is another indication that the present classification in the ISTA Rules may not be appropriate. It was suggested to have a split at 20% oil content: higher would mean the low temperature method, lower would mean the high temperature method.

2. Improve existing rules (orthodox seeds + general improvements)

Discussions on the Moisture chapter during the workshop in 2003 revealed many unclaritys and 'mistakes'. This implies that a major revision is necessary. On the other hand the Executive Committee has indicated that no major revisions (reprinting) should occur before 2007.

3. Improve the existing rules (non-orthodox seeds)

The aim of this investigation was to compare the change in whole seed water content with the change in seed component water contents, during desiccation under standardised conditions (as prescribed by the Protocol attached). Essentially, the investigation comprises two stages, viz.;

- determination of seed survival following desiccation to a prescribed range of weight loss by each seed batch during drying (i.e. the preliminary experiment)
- comparison of whole seed and seed component water contents following desiccation to 0, 50 and 10% viability loss (i.e. the main experiment)

The species tested are summarised in Table 1. Of the six species listed, data from only four species are represented in this report. No germination data were available for *Corynocarpus laevigatus*, as the seeds tested were too underdeveloped, and did not germinate.

Table 1. Summary of species tested

| LABORATORY | CONTACT PERSON | SPECIES TESTED | EXPERIMENTS | |
|---|------------------------------|---------------------------------------|--------------------------------|------|
| | | | PRELIMINARY | MAIN |
| Xishaungbanna Tropical Botanical Garden | Songquan Song | <i>Hopea hainanensis</i> | Whole seed data provided only. | |
| Kenya Plant Health Inspectorate Services (KEPHIS) | Joseph Ahenda | <i>No data provided</i> | | |
| | | | | |
| Massey University | Craig McGill | <i>Corynocarpus laevigatus</i> | Yes | Yes |
| University of Natal, Durban | Patricia Berjak & Deon Erdey | <i>Podocarpus henkelii</i> | Yes | No |
| | | <i>Syzygium cuminii</i> | Yes | No |
| | | <i>Trichilia dregeana</i> | Yes | No |
| | | <i>Trichilia emetica</i> | Yes | No |

RESULTS

The results summarised here represent those for *Podocarpus henkelii*, *Syzygium cuminii*, *Trichilia dregeana* and *T. emetica* only.

Initial results

These are summarised in Table 2, and represent the water contents of undried, control material. In all cases, the axis water contents were considerably higher than that for the storage tissues (megagametophyte, in the case of *P. henkelii*, and cotyledons in the other three species) and whole seed water content values.

Table 2. Initial whole seed and seed component water contents, expressed as grams water per gram dry mass (n = 25).

| Species | Water content (g.g ⁻¹) | | |
|----------------------------|------------------------------------|------------------|------------------|
| | Whole seed | Storage tissue | Axis |
| <i>Podocarpus henkelii</i> | 1.04 (± 0.13) | 1.03 (± 0.13) | 1.77 (± 0.11) |
| <i>Syzygium cuminii</i> | 0.74 (± 0.04) | 0.69 (± 0.03) | 1.66 (± 0.31) |
| <i>Trichilia dregeana</i> | 0.80 (± 0.02) | 0.79 (± 0.02) | 2.81 (± 0.04) |
| <i>Trichilia emetica</i> | 0.47 (± 0.02) | 0.46 (± 0.02) | 1.93 (± 0.11) |

Preliminary and main experiment results

The results are represented as % germination (root emergence) versus water content (Figure 1) to emphasise the differences obtained when expressing survival, following desiccation, as a function of whole seed water content, and not as a function of axis water content.

In all cases, viability declined at a higher axis water content, than indicated when the water content was determined on a whole seed basis only. The embryonic axes of these species constitute only a small part of the seed dry weight (10 to 25 %), and retain water contents higher than that of the storage tissues throughout desiccation.

In summary, the main points are:

- The difference in water content values differs greatly between the various seed components
- Determining water content on a whole seed basis only for desiccation sensitive seeds underestimates the desiccation sensitivity of the germinative axis tissues.

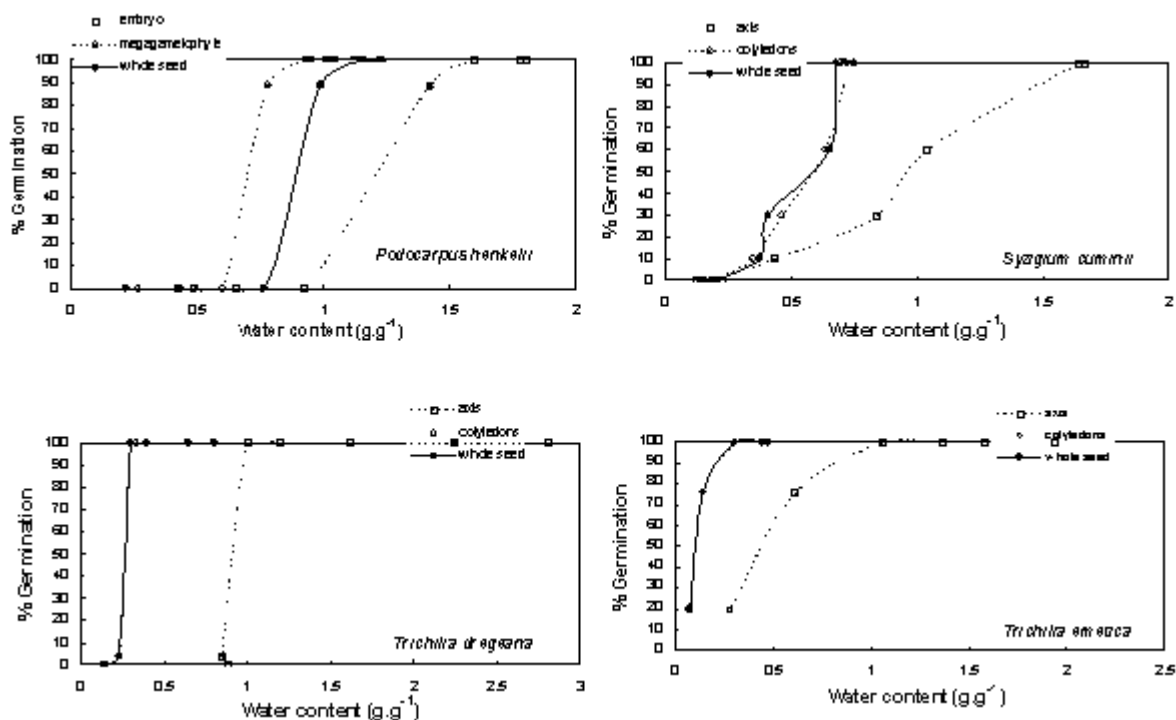


Figure 1. Viability curves (n = 25)

4. First ISTA Moisture Content Proficiency Test

One hundred and ten laboratories took part in the first ISTA Moisture Content proficiency test. Of these 77 were accredited laboratories and 33 volunteer laboratories. The mean results of the Moisture Contents of the *Trifolium* samples that were used in the proficiency test were:

| Sample | Mean of all Laboratories (% Moisture Content) | Mean of Accredited Laboratories (% Moisture Content) |
|--------|---|--|
| 1 | 8.7 | 8.7 |
| 2 | 4.4 | 4.4 |
| 3 | 14.0 | 14.1 |

When reporting the results to ISTA, 90 laboratories did this correctly. Fourteen laboratories reported results to two or more decimal places (the ISTA Rules state that: *moisture content must be reported to the nearest 0.1%*) and 6 laboratories reported replicate results that differed by more than 0.2% (In such cases they should have repeated the determination on another two replicates). An examination of the results show that they were distributed around the mean in a similar way to germination and purity proficiency test results:

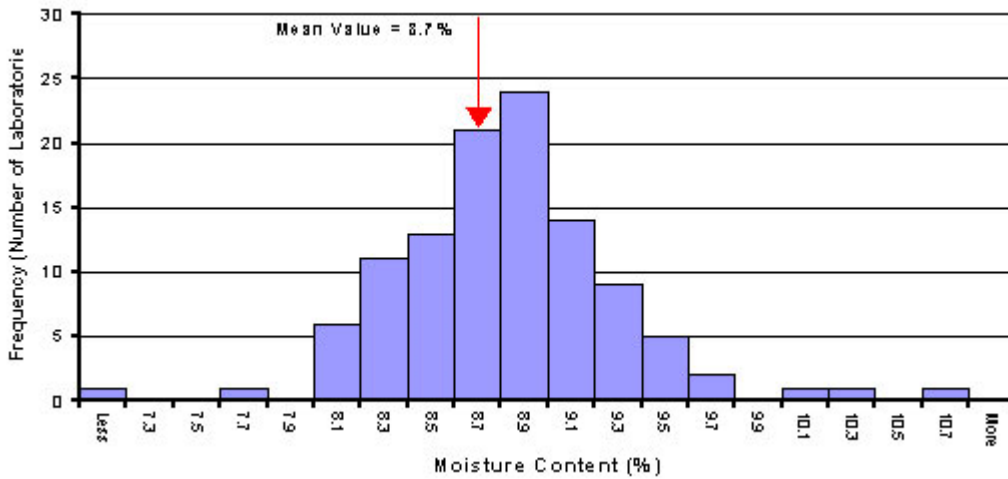


Figure 1. Frequency distribution of results reported on Sample 1 by laboratories participating in the first ISTA Moisture Content proficiency test

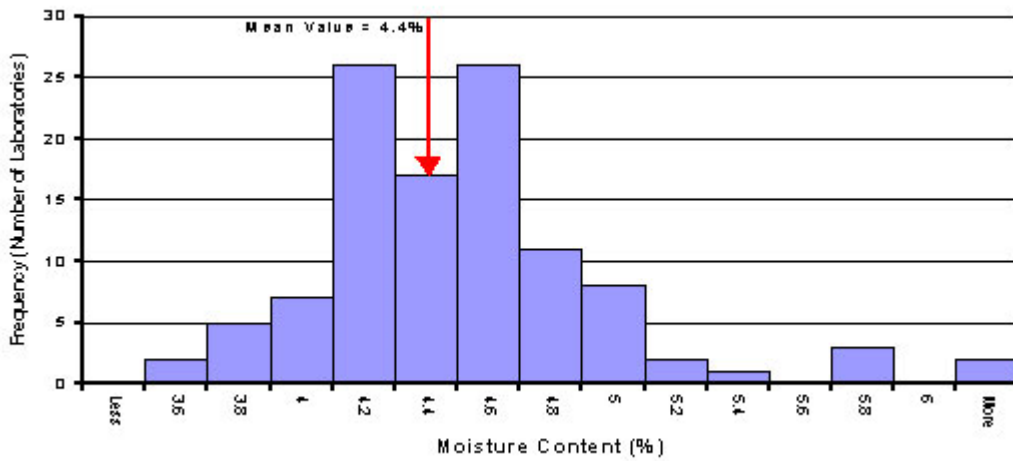


Figure 2. Frequency distribution of results reported on Sample 2 by laboratories participating in the first ISTA Moisture Content proficiency test

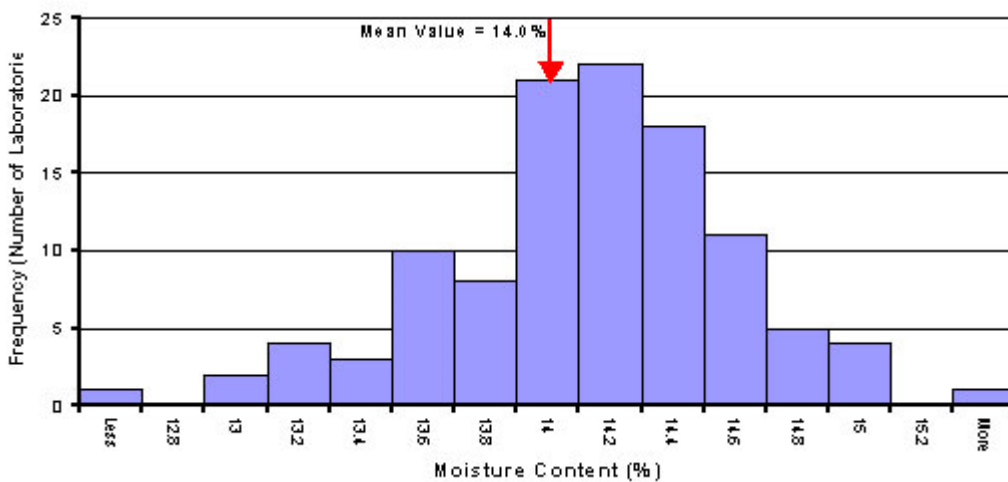


Figure 3. Frequency distribution of results reported on Sample 3 by laboratories participating in the first ISTA Moisture Content proficiency test

Since the moisture content results were distributed in this manner, it was decided to analyse them

using the standard ISTA program that is used to assess the performance of laboratories in ISTA purity and germination proficiency tests. With this program laboratories having normalised or z-score out with the range -2 to $+2$ are considered to have reported a result that is considered questionable/deviant. In such cases the laboratories will be asked to verify its results, examine their procedures and take action necessary to correct any deficiencies that may have contributed towards the deviant. In addition, the test leader may recommend some formal follow-up corrective action that should be reported to the Secretariat.

Because of the nature of seed and its variability, even with a homogenous referee sample, repeated tests in the same laboratory will produce a range of results and 2.5% of these will have z-scores that are less than ($<$) minus 2 and 2.5% will have a z-scores greater than 2 ($>$). It is reassuring to see that less than 5% of ISTA Accredited Laboratories had z-scores achieved an overall z-scores of <-2.0 and $>+2.0$, which is what would be expected by chance. It indicates that there is not any significant problem in moisture content testing in ISTA Accredited Laboratories for species where no grinding is required (Table 1).

Table 1. Details of Analysis of z-scores obtained in moisture content referee test

| | Sample 1 (8.7%MC) | Sample 2 (4.4%MC) | Sample 3 (14.1%MC) | Average |
|--|------------------------------|------------------------------|-------------------------------|----------------|
| Sample Results with z-scores <-2.0 | 3 | 3 | 7 | 4.33 |
| Sample Results with z-scores $>+2.0$ | 5 | 7 | 5 | 5.67 |
| Total of Sample Results with z-scores <-2.0 and $>+2.0$ | 8 | 10 | 12 | 5.00 |
| Proportion of all Laboratories with z-scores <-2.0 and $>+2.0$ | 7% | 9% | 11% | 9% |
| Proportion of Volunteer Laboratories with z-scores <-2.0 and $>+2.0$ | 21% | 18% | 21% | 20% |
| Proportion of Accredited Laboratories with z-scores <-2.0 and $>+2.0$ | 1% | 6% | 7% | 4.67% |

The number of Volunteer Laboratories with scores <-2.0 and $>+2.0$ is however greater than would be expected by chance (20%). It is hoped that the test leader can offer Volunteer Laboratories recommendations and advice that will assist them in achieving moisture content results that are equivalent to those obtained by Accredited ISTA Laboratories.

Overall the distribution of z-scores for all 3 samples is similar to that obtained in purity and germination proficiency tests (figure 4). This is further evidence of the appropriateness of the standard ISTA proficiency test analysis program for moisture content proficiency tests. The results of this proficiency test gives ISTA an assurance of the moisture content results reported on its International Certificates. However, this can not be a one off exercise. Moisture testing must be included in the standard ISTA testing program. The next moisture proficiency test is scheduled to take place in February 2004. It will be more of a challenge as it will involve testing a *Triticum* that requires grinding prior to oven drying.

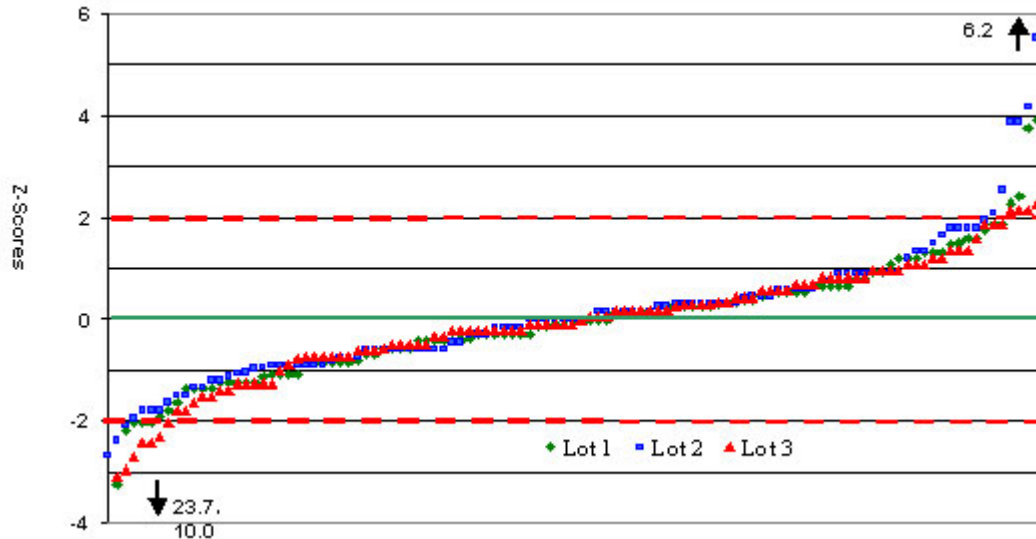


Figure 4. Distribution of z-scores for the three samples tested in the moisture proficiency test

5. Improve application of existing rules by means of organising a workshop, and of writing a handbook

A workshop was successfully organised in Lyngby (Denmark) at the Danish Plant Directorate in November 2003. Nineteen participants from Europe, United States and New Zealand participated. During the workshop a lot of ideas for improving the rules for moisture determination were given. During the workshop the participants worked with quality assurance aspects as well as with calibration of moisture meters. These items were dealt with both by theoretical lessons, practice and exercises.

6. Prepare a paper on the future developments in moisture testing, and the consequences for the ISTA Moisture Committee and the ISTA Rules

The paper was written and sent to all MOI members for comments. The revised paper was discussed during the Extraordinary Meeting in 2003.

One of the subjects discussed was the basic reference method. At present the Karl-Fischer-method is considered to be the basic reference method for moisture. This method has as the main disadvantages that it is expensive, difficult to learn, and not widespread available among moisture committee members. Alternatives have the same or more disadvantages. Above, although being the basic reference methods for the ISTA Rules, the method itself is not described in the ISTA Rules! During our meeting it was suggested to abandon the Karl-Fischer method, and use the oven for validating new species instead in the future.

Future Aims

1. Validate new species/methods for the rules

To validate the split between the high and the low temperature oven method at 20% oil content we will need to organise some comparative testing. This will be organised during the upcoming ISTA Congress.

2. Improve existing rules (orthodox seeds + general improvements)

Three out of four Rules Change Proposal were not accepted by the Extraordinary Meeting in 2003. It appears that there are many parts of the Moisture Chapter that are unclear to users. Although it is an aim to amalgamate the Rules it was not proposed that this should take place next year. It was felt that for the benefit of users there should be a period of stability in the format of the Rules. A complete reprinting and amalgamation is not anticipated until 2007. Between now and then we should aim to clarify and improve the Moisture Chapter in preparation for the amalgamation. It is suggested that we seek the assistance of ISTA auditors to help identify problem areas.

3. Improve the existing rules (non-orthodox seeds)

The working group will continue to gather information provided by the comparative testing. The

information will be used for suggestions for improvement of the existing rules for non-orthodox seeds. There is a need for moisture testing methods for tree seeds. This working group so far concluded that there is a tremendous variation in moisture contents of different parts of non-orthodox seeds, and in seed-to-seed variation of moisture content.

On the other hand, when looking at orthodox seeds, we will probably have similar effects, although to a lesser extent.

A maybe more important difference between orthodox and non-orthodox seeds is seed size.

4. Proficiency testing

It is planned to have a next proficiency test for moisture in February 2004 (extra round, in combination with tetrazolium). Species will be a *Triticum aestivum*, for which grinding will be involved.

This proficiency test round will also be used for obtaining information about the need for grinding and about the state of the art of seed testing. This means that the seeds will be accompanied by a questionnaire. Questions will include type and size of containers, calibration of thermometers and oven, presence of fan in oven, and more.

The Z-scores resulting from proficiency tests in 2003 and 2004 will be the basis for developing a rating system in 2004. The results of both test rounds will also be used for determining the frequency of future proficiency tests.

5. Organise a workshop

We hope to organise a second workshop in Asia or in New Zealand in 2004.

6. Write a handbook

The material used in the workshop will be the basis for the handbook. Also information from working group 6 (paper on future developments) will be incorporated in the handbook. We hope to have the first draft handbook available for discussion during the workshop in 2004.

7. Suggested new working group for 2004-2007

Percentage relative humidity has a direct relationship with seed longevity, whereas absolute moisture content doesn't. In addition the RH-method is non-destructive (important for high-value seeds), and gives relevant results for coated and pelletised seeds (where an oven test gives meaningless results). The possibility for putting the %RH on an ISTA certificate therefore has added value. It was decided to start a working group on this subject. We will have to follow the validation principles.